PHYSIOLOGICAL, COGNITIVE, AND EXPECTANCY EFFECTS OF AROMATHERAPY FOLLOWING ACUTE STRESS

By

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This is to certify that the PhD dissertation of

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Abstract

Objective. Aromatherapy is commonly used for stress relief, yet the evidence for its effectiveness is weak due to the poor quality of previous studies and lack of understanding for the aromatherapy mechanism. The main goal of this work was to use a rigorous randomized control trial (RCT) design to assess effects of a popular stress-reducing aromatherapy on subjective, physiologic, and cognitive measures sensitive to stress. Additionally, the study evaluated the role of a psychological mechanism (expectancy) in aromatherapy actions.

Methods. Ninety-two healthy adults were randomized to 3 groups based on aroma type (experimental lavender aroma, detectable coconut placebo aroma, and undetectable water placebo aroma) to evaluate efficacy of lavender aromatherapy compared to the placebo groups. Detectable placebo aroma was utilized to test the role of aroma-mediated expectancy in aromatherapy actions. Additionally, participants in each group were randomized to one of two subgroups: either receiving a suggestion that the assigned aroma is a powerful stress-reducing agent (prime) or receiving a neutral statement about the aroma qualities (no prime). This manipulation was used to assess the role of verbally-mediated expectancy in aromatherapy actions. To reach the study goals, participants underwent a stress battery and completed baseline and post-stress assessments during which participants' physiological, endocrine, and cognitive function were assessed with objective stress markers. Subjects' perceived stress, affect, anxiety, and expectancy of improvement were also evaluated.

Results. Beneficial effects of lavender aromatherapy during stress exposure were shown for cognitive function (performance on working memory task) as well as physiological (respiration rate) outcome measures. Next, the results suggested that both aroma- and verbally-mediated expectancies contribute to aromatherapy actions. Aroma-mediated expectancy associated with the presence of a detectable aroma affected EEG frontal asymmetry and chromogranin A after stress induction. Hedonic qualities of aroma, including aroma intensity and pleasantness, were linked to aroma-mediated expectancy and were likely critical for producing such effects.

The role of verbally-mediated expectancy, enhanced by the suggestion of stress-reducing properties of the assigned aroma, was also supported for aromatherapy effects on cognitive performance and function. Specifically, priming aromatherapy recipients on expected stress-reducing aromatherapy effects produced beneficial effects on cognitive function evident in ERP changes and behavioral performance on cognitive tests of processing speed and executive functioning. Curiously, the changes in objective measures were not paralleled by changes in subjective stress, anxiety, or affect.

Conclusions. In addition to showing specific effects of lavender aromatherapy on physiologic and cognitive measures, current findings indicated that expectancy effects play a major role in aromatherapy actions. Overall, the results indicate that objectively measured aromatherapy effects are produced by a combination of pharmacological and psychological mechanisms that are probably independent.

Chapter 1: General Introduction

Effects of stress exposure on brain, body, and health

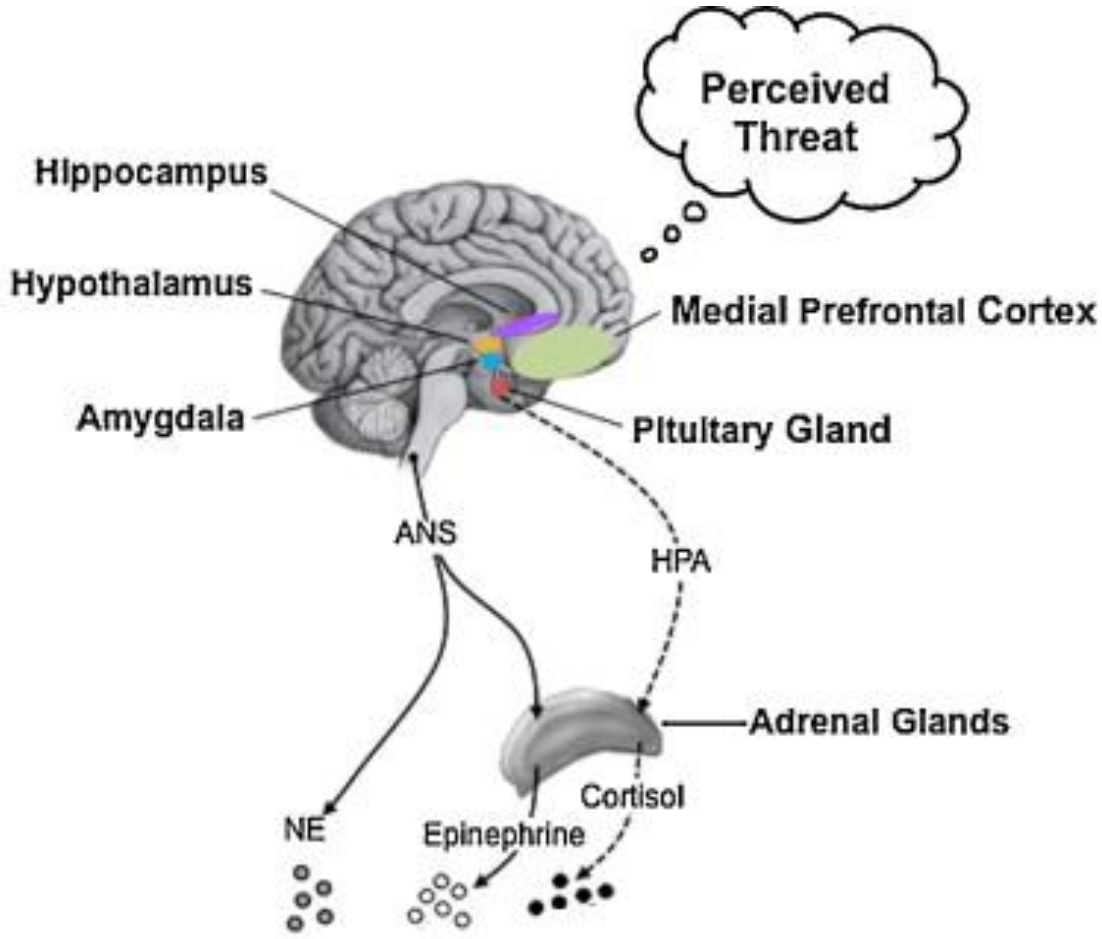
Stress, broadly defined as a real or anticipated threat to homeostasis (Ulrich-Lai & Herman, 2009), is inevitable in life of any organism. In humans, stressful experiences can be physiological or psychological in nature, real or imagined; and they can be consequences of positive (e.g. job promotion) or negative (e.g. car accident) events. Regardless of the origin of a stressor, exposure to stress triggers a highly conserved and coordinated response of multiple body systems in order to minimize the net cost to the organism.

Stress response involves a range of changes in autonomic and endocrine systems, psychological state, and behavior (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; McEwen, 2008; Ulrich-Lai & Herman, 2009). While the stress response is adaptive and critical to survival, when called upon too often, it can become dysregulated leading to a broad range of health problems. Stress-related health problems are diverse and include an increased risk for depression, immune dysregulation, sleep disturbance, cardiovascular problems, diabetes, cancer, cognitive impairment, and neurodegenerative diseases (Bremner, 1999; Chrousos, 2009; de Kloet, 2000; Graham, Christian, & Kiecolt-Glaser, 2006; McEwen, 2008; McEwen et al., 1997).

For a brief and simplified overview of stress response, acute stress activates the neural and endocrine systems after stressor-related sensory information is relayed to the brain (Figure 1). The autonomic nervous system (ANS) is the first to respond with its sympatho-adrenomedullary arm enabling rapid but short-lived

alterations in physiological states, including increase in heart rate, breathing rate, and blood pressure that might facilitate escape from a threatening stimulus (Ulrich-Lai & Herman, 2009). Exposure to stress is also accompanied by increase of central levels of catecholamines and other neurotransmitters and neuromodulators that have the ability to alter neuronal functioning of widely distributed brain regions, and particularly prefrontal cortex (PFC), leading to increase in vigilance, alertness, attention, and allowing the organism to select the best response strategy in the face of challenge (Arnsten, 2009; de Kloet, Joels, & Holsboer, 2005; Joels & Baram, 2009; Qin, Cousijn et al., 2012). Hypothalamic-pituitary-adrenocortical (HPA) axis is activated next leading to elevations in circulating glucocorticoid hormones peaking about 30 minutes after exposure to a stressor in humans (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). The HPA axis activation ensures a lasting endocrine response promoting energy storage and other effects involving sympathetic system and multiple brain structures (Joels & Baram, 2009; Ulrich-Lai & Herman, 2009). The two major systems involved in a stress response (neural and endocrine) are believed to have a substantial degree of independence and provide complementary actions through the body (Ulrich-Lai & Herman, 2009).

Figure 1. Outline of the body response to a perceived threat (stress).



Threat is processed by the brain and leads to activation of the autonomic nervous system (ANS) associated with rapid increase in ANS measures (heart rate, breathing rate, blood pressure, and catecholamines (norepinephrine (NE) and epinephrine) as well as activation of the hypothalamic-pituitary-adrenocortical (HPA) axis leading to elevations in circulating glucocorticoid hormone cortisol. The two major systems involved in a stress response (neural and endocrine) act individually and provide complementary actions throughout the body. Adapted from O'Donovan, Slavich, Epel, & Neylan (2013).

Frequent exposure to stress leads to reorganization of stress-control circuitry producing long-term changes in many functions and often is viewed as a catalyst of accelerated aging and of disease including cardiovascular, mental, immune, and neurodegenerative diseases, as well as cognitive decline (Arnsten, 2009; Caswell et al., 2003; Esch & Stefano, 2010; Marin et al., 2011; Vitaliano, Zhang, & Scanlan, 2003). However, stress does not need to happen often and become chronic to produce negative effects on health and function: even mild uncontrollable acute stress may lead to obvious impairments and cause deficits in higher order cognitive functions requiring complex, flexible thinking and sub-served by the prefrontal cortex (PFC) (Arnsten, 2009; Esch & Stefano, 2010; Marin et al., 2011).

Because stress is a natural part of life and impossible to avoid, investigating optimal ways to manage stress and combat its negative consequences is an active area of research (Esch & Stefano, 2010). In recent decades many promising and popular stress management approaches came from the field of complementary and alternative medicine (CAM).

Complementary and alternative medicine (CAM) for well-being

CAM is a broad and changing field defined by the National Center for Complementary and Alternative Medicine as "*a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Complementary medicine is used together with conventional medicine, and alternative medicine is used in place of conventional medicine."* (The National Center for Complementary and Alternative Medicine,

2011). Another term often involving CAM is “integrative medicine” or combining treatments from conventional medicine and CAM approaches that were shown safe and efficacious (The National Center for Complementary and Alternative Medicine, 2011). Because specific CAM practices may become widely accepted with sufficient evidence for their safety and effectiveness, the boundaries between specific CAM modalities and conventional medicine might become less distinct with time.

CAM approaches have long historical roots and have been practiced in many civilizations for centuries (Norton, 1995; Pirotta, Cohen, Kotsirilos, & Farish, 2000; Whitmore & Leake, 1996). More recently CAM has been gaining the favorable reputation in many parts of Western world as well (Herman, Poindexter, Witt, & Eisenberg, 2012). In the United States, the 2007 National Health Interview Survey indicated that approximately 38% of adults were using one or more CAM modalities (The National Center for Complementary and Alternative Medicine, 2011). Accumulating evidence points to diverse health benefits of many CAM practices for variety of conditions from general improvements in physical function, flexibility, and well-being to helping with pain relief and ameliorating chronic conditions (Diamond et al., 2003; Ernst, Rand, & Stevinson, 1998; Hemming & Maher, 2005; Keegan, 2000; Oken, 2004; Patricia, 2004; Pilkington, Rampes, & Richardson, 2006; Smith, Collins, Cyna, & Crowther, 2003; van der Watt, Laugharne, & Janca, 2008; Wahbeh, Elsas, & Oken, 2008). Systematic reviews also indicate that CAM can be cost-effective for some conditions (Herman, Craig, & Caspi, 2005; Herman et al., 2012).

CAM approaches for relaxation, stress and anxiety reduction, and general well-being are gaining popularity and receiving much attention from researchers and healthcare providers. One of the CAM approaches for stress reduction growing in popularity among diverse populations and health practitioners in the United States and around the world is aromatherapy (Butje, Repede, & Shattell, 2008; d' Angelo, 2002).

Aromatherapy: an overview of a popular CAM approach

Aromatherapy involves the use of essential oils from fragrant plants to help improve physical and psychological health. It is an ancient practice dating back centuries that was used in ancient China (as incense), in ancient Egypt (along with beauty treatments), and in Roman Empire (with baths) (Herz, 2009; Lis-Balchin, 1997). In the Western countries aromatherapy began developing as a serious discipline in the 1980s stimulated by the interest in mind-body healing and psychoneuroimmunology (Butje et al., 2008). Contemporary aromatherapy is a part of phytotherapy, or the use of plants for medicinal purposes.

Aromatherapy essential oils, volatile non-oily, highly fragrant essences extracted from plant by distillation (Wildwood, 1996), might contain a wide range of different compounds that contribute to the therapeutic benefits of the oils. The exact chemical profile can be identified by analytical methods such gas chromatography and mass spectroscopy (Horowitz, 2011). Essential oils can be applied in several ways: they are commonly used in oil burners or aroma diffusers, with bath, or during massage so that the aroma of essential oil could evaporate and stimulate the olfactory sense (Butje et al., 2008). Some essential

oils prior to application need to be diluted in inert carrier or base oils such as vegetable oils or unscented lotions (Wildwood, 1996). The effects of aromatherapy are believed to be almost instantaneous, and some evidence suggests that aromatherapy can work beyond the level of conscious awareness: aromatherapy presented below detection threshold to unsuspecting subjects might affect emotions, cognition, daytime behaviors, and sleep (Moss, Cook, Wesnes, & Duckett, 2003; Wildwood, 1996). The most effective application for relieving emotional distress is, reportedly, by inhalation (Butje et al., 2008).

Aromatherapy is becoming a popular and accepted practice across the globe: for example, aromatherapy is formally taught in French medical schools, prescribed by many European physicians, reimbursed by health insurance in some European countries; it is also present in over half of children hospitals in Australia, and utilized in Japan to enhance productivity and prevent spread of airborne infections (Butje et al., 2008; Horowitz, 2011). Due to some of the attractive aromatherapy features such as ease of use, reasonable cost, and low incidence of side effects when used as directed, aromatherapy has been evaluated for use with diverse populations from children to elderly (Butje et al., 2008; Horowitz, 2011). In the United States the use of aromatherapy is not regulated and not officially prescribed by conventional medical practitioners; however surveys show that aromatherapy, especially aromatherapy for reducing stress and anxiety is increasingly used by CAM and conventional medicine practitioners (Buckle, 2002; Cawthorn & Carter, 2000; d' Angelo, 2002; Hicks, 1998; Horowitz, 2011; La Torre, 2003).

Lavender aromatherapy: therapeutic effects and mechanisms

English lavender (*Lavandula angustifolia*) is one of the most popular aromatherapy essential oils used for reduction of stress and anxiety (Cavanagh & Wilkinson, 2002; Perry, Terry, Watson, & Ernst, 2012). Not only is English lavender (further referred to as lavender) widely used for relaxation purposes, but it is also one of the most researched essential oils.

Lavender essential oil, typically produced by steam distillation of the plant flower heads and foliage, has over 100 constituents, including linalool, linalyl acetate, 1,8, cineole, β-ocimene, terpinen-4-ol, and camphor (Cavanagh & Wilkinson, 2002). Linalool and linalyl acetate (ranging between 26-49% and 18-53% in lavender essential oil, respectively) are major contributors to the volatile component (or aroma) of the essential oil, and they are also thought to be the active ingredients responsible for producing relaxing, anxiolytic, antidepressant, anti-inflammatory, and sedative effects commonly attributable to lavender (Denner, 2009; Perry & Perry, 2006; Perry et al., 2012). Lavender aromatherapy, similarly to any type of aromatherapy, is believed to start affecting the person almost immediately following inhalation; however the data on the rate of absorption of lavender essential oil or detecting levels of lavender or its constituents after inhalation in bodily fluids in human subjects is lacking (Herz, 2009). In rodents, peak concentrations of the main aroma constituents are usually detected in plasma at 60-90 min following inhalation administration (Buchbauer, Jirovetz, Jager, Dietrich, & Plank, 1991).

Current research data demonstrates that lavender is a safe essential oil when used as directed. The most often reported side effects of using lavender in previous clinical trials (with the mode of application ranging from injecting to inhalation) include infections, gastrointestinal problems, and unspecified nervous systems disorders, none of which described as serious (Perry et al., 2012). Lavender is considered a potential allergen if applied directly to the skin or injected, and currently also is recommended to be avoided during pregnancy and breastfeeding (Perry et al., 2012). Despite these precautions, when inhaled, lavender is considered gentle and safe to use with a wide range of populations including infants and clinical populations such as patients suffering from depression, cancer, or dementia (Perry et al., 2012; Wildwood, 1996).

The mechanism of lavender action has not been clearly established, but the current belief is that it involves major lavender constituents influencing several neurotransmitter systems potentially affecting cholinergic, dopaminergic, glutamate, opioidergic, and gamma-aminobutyric acid (GABA) transmission (Denner, 2009). Animal and molecular studies evaluated several pathways involving lavender essential oil and its major constituents including linalool, linalyl acetate, 1,8-cineole, perillyl alcohol, and aromatic phenol. Most relevant to current work, research demonstrated dose-related binding of linalool to the glutamate receptors, potentially antagonizing cyclic adenosine monophosphate (cAMP) regulatory mechanism activated during stress-induced mobilization of the sympathetic nervous system (Elisabetsky, Marschner, & Souza, 1995). Such modifying effect of linalool on the glutamatergic system is comparable to the

mode of action of a known anticonvulsant phenobarbital (Elisabetsky et al., 1995). Previous research also suggested that linalool affects $GABA_A$ receptor binding producing central nervous system (CNS)-depressant actions similar to anxiolytic drugs benzodiazepines, that act by enhancing GABA effects (Cavanagh & Wilkinson, 2002). Lavender has also been shown to act post-synaptically to modulate the cAMP activity that is decreased in sedation (Lis-Balchin & Hart, 1999). Additionally, studies found a dose-dependent inhibitory effect of linalool and linalyl acetate on acetylcholine and channel function showing a local anesthetic action (Ghelardini, Galeotti, Salvatore, & Mazzanti, 1999; Re et al., 2000). In general linalool and linalyl acetate are believed to cause CNS depression, with linalyl acetate producing narcotic actions and linalool producing sedative effects (Cavanagh & Wilkinson, 2002). The described above actions of lavender constituents observed in previous studies are consistent with lavender sedative, anxiolytic, and anticonvulsant effects that might also contribute to relaxation and stress reduction (Brum, Elisabetsky, & Souza, 2001; Elisabetsky et al., 1995; Hossain, Aoshima, Koda, & Kiso, 2004).

Unfortunately, relevance of the animal studies for understanding lavender effects on humans might be limited due to potentially different exposure routes and doses used in animal subjects. The studies exploring mechanism of action associated with lavender in humans are scarce. There is a view that calming effects of lavender might potentially arise from psychological and emotional responses to aroma (Denner, 2009). Previous neuroimaging studies have produced the evidence for involvement of the olfactory- trigeminal nerves and

amygdala pathway in emotionally significant response to odor (Royet et al., 2000; Zald & Pardo, 2000). However, the mechanism of action related to generating emotional response to aromas is not yet elucidated (Denner, 2009).

The current lack of clear understanding of lavender mechanism and effect on human health and well-being is surprising because a vast number of research studies have been devoted to this topic. For example, over 400 studies have been conducted to assess the effect of lavender just on anxiety symptoms (Perry et al., 2012). A brief review of current literature focused on the effects of lavender aromatherapy on systems activated by stress, including nervous system and endocrine system, elucidates some of the issues with the research to date.

Most of the evidence for beneficial lavender effects comes from earlier research suffering from questionable methodology that included small sample sizes, lack of objective assessments, and limited transparency and standardization in the essential oil preparation (Denner, 2009). Several research studies assessing effects of lavender aromatherapy on subjective measures of mood, well-being, and general health outcomes found positive changes due to aromatherapy in both healthy and clinical populations (Denner, 2009; Perry et al., 2012; Vickers, 1997). In studies with healthy subjects lavender aroma has been found beneficial for reducing exam and pre-procedural anxiety, relieving stress, enhancing perception of well-being, and speeding the recovery from exercise (Hudson, 1996; Kutlu, Yilmaz, & Cecen, 2008; Morris, 2002; Muzzarelli, Force, & Sebold, 2006; Romine, Bush, & Geist, 1999). Taking baths with lavender essential oil has resulted in less crying and an increased amount of sleep in

infants, as well as in increased sense of relaxation in the infants' mothers (Field et al., 2008).

Similar calming effects of lavender have been indicated when used in hospitals and with clinical populations. When used in hospital wards, lavender odor has been found pleasant and useful in combating unpleasant hospital smells; additionally, the pleasantness of lavender aroma was correlated with changes in autonomic system including decreased duration of electrodermal and skin blood flow responses and a decreased heart rate (Alaoui-Ismaili, Vernet-Maury, Dittmar, Delhomme, & Chanel, 1997; Tysoe, 2000). Using lavender aromatherapy in long-stay psychogeriatric ward for people with severe dementia has been associated with reduction in agitative behaviors of the ward patients (Holmes et al., 2002). Presence of lavender aroma in a hospital during hemodialysis has been related to decreased anxiety scores in the patients (Itai et al., 2000). Exposing people with terminal cancer in a hospice setting to lavender aromatherapy has been associated with small changes in vital signs, pain, anxiety level, and general sense of well-being (Louis & Kowalski, 2002). Such studies paved the road for more research evaluating aromatherapy in general and lavender aromatherapy in particular in health care settings: in hospital wards for creating a pleasant ambience for improved well-being, and in clinics for reducing preoperative anxiety, as well as for minimizing distress and pain during labor (Ching, 1999; Edge, 2003; Graham, Browne, Cox, & Graham, 2003; Holmes et al., 2002; Kim, Nam, & Paik, 2005; Lehrner, Marwinski, Lehr, Johren,

& Deecke, 2005; Louis & Kowalski, 2002; Snow, Hovanec, & Brandt, 2004; Soden, Vincent, Craske, Lucas, & Ashley, 2004).

In contrast to predominantly positive lavender effects reported by the studies utilizing only subjective outcome measures, some conflicting evidence has resulted from the studies using objective outcome measures. For example, when measures of autonomic nervous system (ANS) have been included as study outcomes, some researchers demonstrated no physiological effects attributable to lavender presence that were not due to expectancies (Howard & Hughes, 2008; Kiecolt-Glaser et al., 2008; Shiina et al., 2008). However, others indicated changes in measures of autonomic function such as heart rate and several vital signs (Kuroda et al., 2005; Louis & Kowalski, 2002). Further, to date research evaluating influence of lavender aromatherapy on salivary stress markers has been inconclusive. On one hand, lavender aromatherapy has been shown to buffer changes in levels of stress-related markers cortisol, chromogranin A, and free radical scavenging activity after stress induction (Atsumi & Tonosaki, 2007; Shiina et al., 2008; Toda & Morimoto, 2008). On the other hand, no effect of lavender aromatherapy has been observed on such a well-established stress marker as cortisol in some studies (Kiecolt-Glaser et al., 2008; Toda & Morimoto, 2008). Additionally, other studies with less frequently used objective measures sensitive to stress including coronary flow velocity, changes in vocal pitch, polysomnography, and encephalography (EEG) have reported beneficial lavender effects (Diego et al., 1998; Goel, Kim, & Lao, 2005; Shiina et al., 2008).

Among the studies utilizing objective outcomes, several assessed how lavender aromatherapy might affect measures of brain function and cognitive performance. Such studies are relevant to the assessment of lavender aromatherapy not just because of the sixteenth century belief that lavender skullcaps could enhance intelligence (Cavanagh & Wilkinson, 2002) but because of the proposed lavender stress-reducing effects and a known link between stress and cognitive function (Arnsten, 2009). Analyses of brain activity patterns using EEG have been used to evaluate effects of lavender aroma on mood, attention, and alertness. Specifically, presence of lavender aroma has been shown to be associated with changes in EEG activity patterns and has resulted in producing EEG patterns indicative of more positive mood and approach behaviors in subjects who show patterns indicative of more negative mood at baseline (Sanders et al., 2002). Studies also have shown decreased beta frequency power in frontal sites indicative of decreased alertness following lavender aromatherapy (Diego et al., 1998) and changes in alpha 1 frequency power linked to “feeling comfortable” (Masago et al., 2000). Furthermore, lavender aroma inhalation has influenced cognitive performance. A facilitative effect of lavender on cognitive performance has been suggested when participants demonstrated faster and more accurate performance on math computations after exposure to lavender essential oil (Diego et al., 1998). However, in another study decrements in performance on working memory task accompanied by impaired reaction times on memory and attention tasks have been detected in lavender but not control group (Moss et al., 2003). Overall, with

the increase of the number of studies using objective outcome measures to test effects of lavender aromatherapy on different aspects of health, there seems to be a decrease in confidence among the researchers about true beneficial lavender effects.

Interestingly, positive subjective effects of lavender aromatherapy are not always observed in the presence of objectively measured changes, just as objective changes are not always detected along with subjectively reported improvements: for example, in some studies lavender effects on physiological measures have not been paralleled by changes in measures of anxiety (Toda & Morimoto, 2008) or mood (Goel et al., 2005). In another study subjective mood enhancement and reduction in anxiety levels and mental stress due to lavender exposure were not associated with any changes in physiological measures (Motomura, Sakurai, & Yotsuya, 2001). Therefore there might be some dissociation between changes in subjective and objective measures assessed in the same study.

Recently studies utilizing more rigorous study designs such as randomized control trials (RCTs) have been published on the topic of lavender aromatherapy efficacy. The number of RCTs is still very small compared to the overall literature on the topic, however it is growing. A recent systematic review on anxiolytic effects of lavender found 440 studies potentially assessing effects of lavender on anxiety symptoms with only 15 utilizing RCT design, and only 8 evaluating lavender effects through inhalation (Lee, Wu, Tsang, Leung, & Cheung, 2011). In this systematic review, four RCTs investigating lavender oil inhalation have

reported significant improvement due to aromatherapy on at least one anxiety-related outcome measure (Braden, Reichow, & Halm, 2009; Kritsidima, Newton, & Asimakopoulou, 2010; Kutlu et al., 2008; Motomura et al., 2001), while the other four RCTs have not demonstrated any anxiolytic effects of lavender (Howard & Hughes, 2008; Muzzarelli et al., 2006; Sgoutas-Emch, Fox, Preston, Brooks, & Serber, 2001; Toda & Morimoto, 2008). In most of the RCTs stress exposure was used prior to using aromatherapy, and stress was induced by a mental or arithmetic task, arousal task, or exam (Perry et al., 2012).

One of these RCTs included a strong odor condition as a comparison group, in addition to a no odor comparison, and utilized different cognitive primes (suggesting that the odor might assist with relaxation, inhibit relaxation, or providing no information) to assess expectancy effects of aromatherapy (Howard & Hughes, 2008). The findings indicated that the type of prime rather than lavender aromatherapy was responsible for producing effects on the study outcome measures (Howard & Hughes, 2008).

Overall the trend in research evaluating lavender aromatherapy effects is such that investigations using objective measures and more rigorous study designs such as RCT produce evidence that is often less encouraging about the efficacy of lavender aromatherapy than earlier lower quality studies.

Due to this trend, a recent review of 10 systematic reviews evaluating use of aromatherapy for healthcare (with the majority of studies using lavender aromatherapy) have stated that though aromatherapy may induce relaxation and positively affect psychological health, the overall available “evidence fails to be

convincingly positive" (Lee, Choi, Posadzki, & Ernst, 2012). The main problem marring previous aromatherapy research and preventing reviewers from accepting any evidence for aromatherapy efficacy as convincing is predominance of poorly designed and inadequately controlled studies. Though the limitations in previous research on lavender aromatherapy prevent the research community from making a definitive conclusion about the efficacy of lavender aromatherapy for stress and anxiety reduction, they also present opportunities for more studies that can evaluate the intervention and its effects more effectively by addressing the problems observed in previous research. Below there is a brief overview of major problems inherent in aromatherapy studies that might be particularly relevant to evaluation of lavender aromatherapy for stress reduction.

Major limitations in previous research: lessons learned for future studies

Several systematic reviews evaluating use of aromatherapy for several conditions indicated that the majority of the studies that assessed aromatherapy mechanisms or efficacy have been lacking scientific rigor (Herz, 2009; Lee et al., 2012; Lee et al.). Specific criticisms and commonly reported weaknesses of previous research included small sample sizes and lack of adequate control groups (Lee et al., 2012). Specifically, many published aromatherapy studies drew conclusions from samples that included fewer than 10 participants, and majority of these studies utilized either no control group or single control group (Yim, Ng, Tsang, & Leung, 2009). Another common criticism of aromatherapy literature is that many of the previous studies assessed aromatherapy effects using only subjective measures of anxiety, perceived stress, or depressive

symptoms (Perry et al., 2012). Though more recently published studies began using more rigorous designs including randomized control trials (RCT) and utilizing objective measures as study outcomes (Lee et al., 2012; Perry et al., 2012; Yim et al., 2009), a few methodological problems still remain.

First, a number of studies assessing effects of aromatherapy on different conditions utilized aromatherapy massage rather than using aromatherapy alone (Cooke & Ernst, 2000; Cooke, Holzhauser, Jones, Davis, & Finucane, 2007; Herz, 2009; Lee et al., 2012; Lee et al., 2011; Yim et al., 2009). Tactile and somatosensory stimulation intrinsic to massage therapy might produce separate effects or interact with the olfactory stimulation due to aromatherapy (Cooke & Ernst, 2000; Cooke et al., 2007; Herz, 2009; Lee et al., 2012; Lee et al., 2011; Yim et al., 2009). Similar effects of interventions using massage with and without essential oils have been previously shown (Soden et al., 2004), so it is possible that the observed positive effects were solely due to massage.

Next, the majority of the studies using control groups utilized either no odor control or a different aroma as a control, but not both. Some research shows that aromatherapy might not show any benefit when it is compared to other condition with perceptible smell, for example pleasant smelling hair conditioner (Wiebe, 2000). However, if pleasant smelling control is the only control used it is unclear whether aromatherapy acts purely due to the presence of a pleasant smell or whether there is no benefit to using aromatherapy at all (Bent, 2000). Therefore, controls must include both no-odor and scented conditions to differentiate

between the effects arising specifically from the experimental aroma and effects arising due to presence of any aroma.

Another important issue in aromatherapy research is how study goals and study aromas are presented to participants. Several studies indicated that priming participants about expected effects of study aromatherapy (e.g. sedating vs. stimulating) might influence the results and produce specific effects congruent with the received prime in the absence of any aroma or produce differential effects congruent with different primes for the same aroma (Campenni, Crawley, & Meier, 2004; Howard & Hughes, 2008; Knasko, 1995). This evidence highlights the importance of participants' expectancies about aromatherapy in general and specific aromas in particular. Assessing participants' expectancies and describing how aromas are presented is important for interpreting the aromatherapy study results.

Furthermore, another important consideration in research is blinding that was lacking in earlier aromatherapy studies (Perry et al., 2012). In aromatherapy studies blinding of the participants might be unrealistic because participants are likely to distinguish absence or presence of the aroma and potentially recognize familiar and commonly used aromas. However, blinding of the individual assessing the study outcome is essential to reduce any bias due to assessor's beliefs about aromatherapy actions.

The combined recommendations for future researchers from available systematic reviews include strengthening study designs by using groups of adequate sample size to detect difference, including necessary control groups

such as perceptible smell controls along with no aroma control, utilizing both subjective and objective measures of change, assessing aromatherapy separately from other therapies like massage, assessing relevant variables related to aroma hedonics and expectancy, as well as blinding assessors and study participants to different study conditions when possible (Herz, 2009; Lee et al., 2012; Perry & Perry, 2006). Some other lessons from previous aromatherapy studies include using a more stress-provoking task to induce stress and employing longer inhalation times (over 5 minutes) for more prominent aromatherapy effects (Perry et al., 2012).

Based on the recommendations from previous literature, the current work has been designed to provide a rigorous test of efficacy of lavender aromatherapy for stress reduction by utilizing RCT design, assessing aromatherapy effects through inhalation only, including placebo groups featuring detectable and undetectable aromas, inducing stress with a comprehensive stress battery, exposing participants to aromatherapy for the duration of the stressors and post-stress assessments, and blinding outcome assessor to the aromas. Additionally, the current work has been designed to evaluate the role of expectancy as a potential mechanism underlying effects of aromatherapy.

Hypotheses of aromatherapy mechanism

Lack of understanding of the mechanisms responsible for aromatherapy actions is a major problem in aromatherapy research in addition to prevalence of inadequately designed studies (Herz, 2009). To date, investigations designed to elucidate mechanism of aromatherapy actions have resulted in formulating two

primary hypotheses: the two are pharmacological hypothesis and psychological hypothesis reviewed below as previously proposed (Herz, 2009).

Pharmacological hypothesis. Pharmacological hypothesis of aromatherapy action proposes that the effects on mood, physiology, and behavior attributable to aromas result from direct and intrinsic aroma ability to affect the autonomic nervous system, central nervous system, or endocrine system (Herz, 2009). Supporters of the pharmacological hypothesis propose olfactory stimulation as the likely mechanism by which aromatherapy acts. Specifically, they believe that olfactory stimulation with aromas could result in direct, immediate, and unconscious interaction with neural substances producing changes in autonomic nervous system and brain wave patterns, as well as in sleep and arousal states (d' Angelo, 2002; Herz, 2009; Kuroda et al., 2005). According to this view, aroma molecules can bind to receptors and activate the release of neurotransmitters including serotonin, endorphins, and catecholamines, affect HPA axis, modulate immune system neuroreceptors causing alterations in mood, relieving anxiety, and interrupting the stress response (d' Angelo, 2002). Some evidence pointing to the pharmacological properties of the aroma comes from animal studies in which exposure to specific aromas has resulted in physiologic and behavioral changes congruent with expected aroma effects (Komiya, Takeuchi, & Harada, 2006; Kovar, Gropper, Friess, & Ammon, 1987). Some evidence for this mechanism from human studies comes from observing physiological changes congruent with suggested aroma properties in studies where aromas were presented to participants below detection thresholds, with participants unaware

of aroma presence (Kuroda et al., 2005), studies conducted with babies who had not developed expectations about particular aromas (Sanders et al., 2002), research with anosmic patients, or investigations in which aroma was inhaled by sleeping people who have little olfactory awareness (Goel et al., 2005; Holmes et al., 2002).

Psychological hypothesis. The second proposed mechanism for aromatherapy actions, *psychological*, suggests that aromas can produce effects through expectations, conscious perceptions, or emotional learning. According to this hypothesis any effects due to aroma exposure, including physiological effects, are the consequences of the psychological-emotional responses elicited by the aroma (Herz, 2009). Associative and emotionally evocative properties of aromas are consistent with the anatomy of olfactory and limbic system: olfactory efferents can access the neural substrates of emotional and memory processing (amygdala and hippocampus, respectively) facilitating associative learning and classical conditioning (Cahill, Babinsky, Markowitsch, & McGaugh, 1995). Much of olfactory processing is localized to the orbitofrontal cortex and amygdala, which play an important role in stimulus reinforcement association learning (Otto, Cousens, & Herzog, 2000).

Perceptual experience of the aroma might also play a critical role in shaping responses to aromatherapy. It has been shown that presence of ambient aroma can affect mood, which in turn can influence behavior (Baron, 1990, 1997; Rotton, 1983). Attitude to aroma (liking or disliking) is related to the influence of

the aroma on mood and physiologic and behavioral outcomes (Villemure, Slotnick, & Bushnell, 2003).

Other significant influences from aroma exposure might arise due to beliefs and expectations. Research shows that verbal suggestion of specific aroma effects can lead to experiencing such effects (both psychological and physiological) in the absence of aroma or to producing effects congruent to verbal suggestion in the presence of aroma with expected effects that are opposite to the suggestion (Campenni et al., 2004; Knasko, 1995). Such studies demonstrate that expectation of the aroma effect might override the chemical essence of the aroma (Herz, 2009). The current study aims to address the role of different types of expectancy in producing aromatherapy actions.

Overall, the evidence is available to support both pharmacological and psychological mechanisms for producing therapeutic effects of aromatherapy. It is likely that the therapeutic effect of aroma arises from combined effects of the described mechanisms. It is likely that different effects of aromatherapy are achieved through different mechanisms.

In addition to the two major hypotheses explaining aromatherapy actions described above, some other views on how aromatherapy might produce its effects are available. For example, there is a separate set of potential mechanisms proposed specifically for producing effects of aroma on cognitive function (Jellinek, 1997) that will be reviewed in Chapter 4 of this thesis.

Goals of this work

The goals of this work have been defined based on the major problems in previously published research on stress-reducing aromatherapy reviewed above. Specifically, the goals of the current work included: 1) evaluating efficacy of stress-reducing aromatherapy using English lavender essential oil and 2) elucidating its mechanism of action by exploring the role of verbally-mediated expectancy and aroma-mediated expectancy.

The current work has been designed to provide a rigorous test of aromatherapy efficacy by comparing the effects of experimental stress-reducing lavender aroma to the effects of two control aromas on subjective and objective measures. The objective measures evaluated in the current work included physiologic markers sensitive to stress (addressed in Chapter 3), cognitive tests evaluating primarily high-order cognitive functions vulnerable to stress (addressed in Chapter 4 and Chapter 5), and brain responses to a cognitive task assessing attention, a higher-order cognitive function (addressed in Chapter 5). Additionally, to further understanding of the mechanisms associated with aromatherapy, the study has included evaluation of the role of expectancy effects in stress-reducing aromatherapy. Better understanding of efficacy and mechanisms of a popular and practical stress-reducing approach, aromatherapy, might benefit millions of people suffering from stress and stress-related conditions who need safe and effective stress management therapies.

Chapter 2: General approach

RCT overview and specific aims

To achieve the study goals, a single-blind randomized controlled trial (RCT) was conducted with generally healthy adults exposed during a laboratory visit to either: 1) putative stress-reducing aroma (lavender), 2) detectable placebo aroma (coconut), or 3) non-detectable placebo aroma (water). Exposure to the assigned aroma started 5 minutes before laboratory stressors (challenges) and lasted for the duration of the study. Participants' subjective experiences, physiologic stress markers, and cognitive function were assessed before and after laboratory challenges (Refer to Diagram 2 for visit activities). By comparing magnitude of changes in objective and subjective measures in response to challenges between the aroma groups, efficacy of stress-reducing lavender aromatherapy was assessed.

Further, half of the participants in each group were assigned to receive a *prime* suggesting they are inhaling a powerful stress-reducing aroma. The mechanism of aromatherapy action, more specifically the role of expectancy, was evaluated by comparing differences in stress response and cognitive performance during aromatherapy between: 1) the primed and non-primed participants and 2) participants exposed to detectable placebo aroma and those exposed to undetectable aroma. The main prediction was that efficacy of lavender aromatherapy would be demonstrated, and both aroma-specific pharmacological and psychological (expectancy) effects of aromatherapy would

be shown to contribute to the aromatherapy mechanism of action. The following specific aims were addressed:

Specific Aim 1: Assess effects of lavender aroma on physiological function

Hypothesis: Inhaling lavender aroma will result in attenuated stress response to laboratory challenges compared to placebo aromas.

Chapter 3 devoted to this specific aim describes stress-related changes due to exposure to different aromas in several physiological measures previously shown to be sensitive to stress.

Specific Aim 2: Assess effects of lavender aroma on cognitive function

Hypothesis: Inhaling lavender aroma will result in improved performance on cognitive tests after laboratory challenges compared to placebo aromas.

Chapters 4 and 5 devoted to this specific aim describe effects of different study aromas on cognitive performance on tasks vulnerable to stress and present changes in brain function using event related potentials (ERP) due to exposure to different study aromas.

Specific Aim 3: Assess the role of expectancies in aromatherapy actions

Specific Aim 3a: Investigate the role of verbally-mediated expectancy

Hypothesis: The subgroups provided with a verbal prime suggesting efficacy of the assigned aroma for stress reduction will demonstrate greater stress reduction and fewer consequences of stress in response to laboratory challenges compared to the subgroups not provided with such a prime.

Specific Aim 3b: Investigate the role of aroma-mediated expectancy

Hypothesis: The group inhaling detectable placebo aroma will demonstrate greater stress reduction and fewer consequences of stress in response to laboratory challenges than the group inhaling an undetectable placebo aroma. Specific aims 3a and 3b are addressed in chapters 3, 4, and 5 in relation to different types of outcome measures (physiological, cognitive, ERP-related).

Methods and procedures

The flow of the RCT is presented in Diagram 1. This chapter reviews the methods and procedures that are general to the study and applicable to all subsequent chapters. The methods and procedures associated with specific outcome measures are described in the chapters focused on those specific outcomes.

Participants

To evaluate study specific aims, generally healthy adults were recruited from the community using advertisements and media announcements. The eligibility criteria for study participation were as follows: 1) age at least 50 years; 2) good physical and cognitive health indicated by screening and score of ≥25 on the Modified Telephone Interview for Cognitive Status (Welsh, Breitner, & Magruder-Habib, 1993); 3) score ≥ 9 on the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983) indicating experience of moderate stress ; 4) not taking medications affecting CNS function or physiologic measures (e.g. steroids or neuroleptics); 5) able to perceive aromas; 6) reporting no smell sensitivities or

allergies; 7) non-smoking for at least one year prior to enrollment; and 8) able to understand and follow study instructions and perform tests.

The rationale for recruiting moderately stressed adults over 50 for this study includes the fact that, with few exceptions, to date, studies evaluating aromatherapy typically utilized younger subjects and produced variable findings (Lee et al., 2012). A possible reason for such variability in findings might be that, while stress affects individuals across life span, younger healthy individuals have multiple compensatory mechanisms for overcoming detrimental effects of stress. However, the full impact of stress might become revealed better with increasing age when aging processes augment effects of stress leading to more significant clinical symptoms (Graham et al., 2006). Middle-aged and older adults might potentially be more sensitive to stress, express greater ranges of stress responses, and benefit the most from stress management, as increased stress reactivity with advancing age leads to accelerated aging and disease processes (Aguilera, 2011). Further, adults over 50 are significantly affected by expectancy bias (Oken et al., 2008). Next, variability in HPA axes measures important for this project might be reduced in older women (expected majority of the proposed RCT sample) due to menopause and absence of hormonal changes linked to menstrual cycle. These features provide significant advantages for using adults 50 or older in the proposed research and might help maximize detection of significant effects of aromatherapy if they exist. Additionally, for this RCT adults with PSS score ≥ 9 are sought because 9 is the mean stress level observed in healthy adults of similar age estimated from previous studies conducted in our

laboratory. Recruiting subjects with perceived stress level that is at or above estimated average stress level for the targeted population is hoped to ensure enrollment of subjects most sensitive to laboratory stressors and thus most likely to benefit from any potential stress-reducing aromatherapy effects.

Participants screened potentially eligible over the phone were invited for a laboratory visit. All study procedures were reviewed and approved by the Oregon Health & Science Institutional Review Board, and all participants signed the consent form prior to participating in any activities during the laboratory visit.

Telephone screening for eligibility

Interested volunteers called and completed a screening interview to determine preliminary eligibility. The interview consisted of describing the study goals and procedures and assessing the interested volunteer eligibility for the study. Those found eligible after the telephone screening were invited for a laboratory visit.

Study groups, randomization process, and blinding

Prior to the study visit each participant was randomized to a group based on aroma type (lavender, coconut, or water) and was also assigned to a subgroup based on a type of prime they received during the visit (prime or no prime) as described below.

Groups based on aromas. Each participant was exposed to one aroma during the testing portion of the study. There were 3 types of aroma used: putative stress-reducing (further referred as stress-reducing), detectable placebo aroma, and undetectable placebo aroma.

For the experimental stress-reducing aroma - a drop of organic lavender (*Lavandula angustifolia*) essential oil (Mountain Rose Herbs, Eugene, OR) was diluted in 15 ml of grapeseed carrier oil (Now Foods, Bloomingdale, IL).

For the detectable placebo aroma a teaspoon of organic virgin coconut (*Cocos nucifera*) base oil (The Ananda Apothecary, Boulder, CO) was diluted in 15 ml of grapeseed carrier oil (Now Foods, Bloomingdale, IL) and mixed until coconut oil was completely dissolved and solution appeared clear. The rationale for using coconut aroma as a detectable placebo came from its wide use as a base oil in many aromatherapy preparations, accompanied by the absence of stress-reducing effects attributable to lavender (Wildwood, 1996). Virgin coconut oil possesses a pleasant aroma that might be familiar to many people due to wide coconut use in culinary and cosmetic products.

For the undetectable placebo aroma, distilled water was used to provide the appearance of essential oil while emitting no odor and producing no effects typically attributed to aromatherapy.

To maximize participants' expectancy, participants received the information at the beginning of the study that several aromas were being tested for their stress-reducing properties and some aromas might be readily perceptible while others might be very diluted. Participants also were told that regardless of whether or not the participants were able to perceive their aroma, the aroma was expected to produce an effect. More information about the participants' instructions can be found in the study consent form (Appendix 1).

Aroma preparation and administration. All study aroma solutions were stored in cool dry place in identical unmarked15 ml amber vials and replaced every 2 months following general storage guidelines for solutions of essential and base oils (Wildwood, 1996).

During the visit, three drops of the aroma solution assigned to a participant were placed on a 5 x 5 mm cotton pad. The pad was then fixed to a small piece of transparent odor free tape that was attached to the participant's nose so that the pad infused with the aroma came to the midpoint between the participant's nose and upper lip. The cotton pad remained attached until the end of testing portion of the visit, but the aroma was not replenished during the testing.

Subgroups based on the presence of a prime. In addition to being assigned to a group based on aroma type, each participant was further randomized into a prime subgroup to assess verbally-mediated expectancy effects. Half of the participants in each group based on aroma were randomized to a prime subgroup, and the other half were randomized to a no-prime subgroup.

Participants in *prime* subgroup, just prior to aroma exposure, received a card that read: " You are about the experience a powerful relaxing and stress-reducing aroma. To experience it best, please close your eyes and inhale the aroma deeply. You may or may not perceive this aroma. This aroma is known to provide a profound relief from stress whether or not people are able to perceive it".

Participants in *no-prime* subgroup received a card that read:" You are about the experience an aroma that may or may not help reduce your stress level. Please close your eyes and inhale the aroma deeply. You may or may not

perceive this aroma. Aromatherapy can be effective whether or not people are able to perceive aromas."

Each participant was randomized into one of the groups based on aroma and into a subgroup based on a prime by a non-blinded research assistant using an adaptive randomization procedure (Pocock & Simon, 1975). The purpose of this procedure was to balance the distribution of age, gender, and stress score for aroma group allocation.

Blinding. To avoid any bias in evaluation of aroma and expectancy effects on cognitive function, all study visits were conducted by an assessor blinded to the participants' aroma group and prime subgroup. The blinded assessor was wearing a disposable active carbon nose filter (Breathe-Ezy Nasal Filters ®, Henderson, NV) for the duration of aroma exposure to avoid perceiving any odor. Additionally, participants were reminded throughout the study not to share any information about the aroma they experience or the absence of perception of the aroma to avoid un-blinding.

Randomization. Randomization was performed by a non-blinded research assistant who assigned participants to the study group and prime versus no prime subgroup. The non-blinded research assistant also prepared materials for the visit including the assigned aroma in an unidentifiable vial and a card that contained either a prime or no prime statement.

Diagram 1. RCT flow

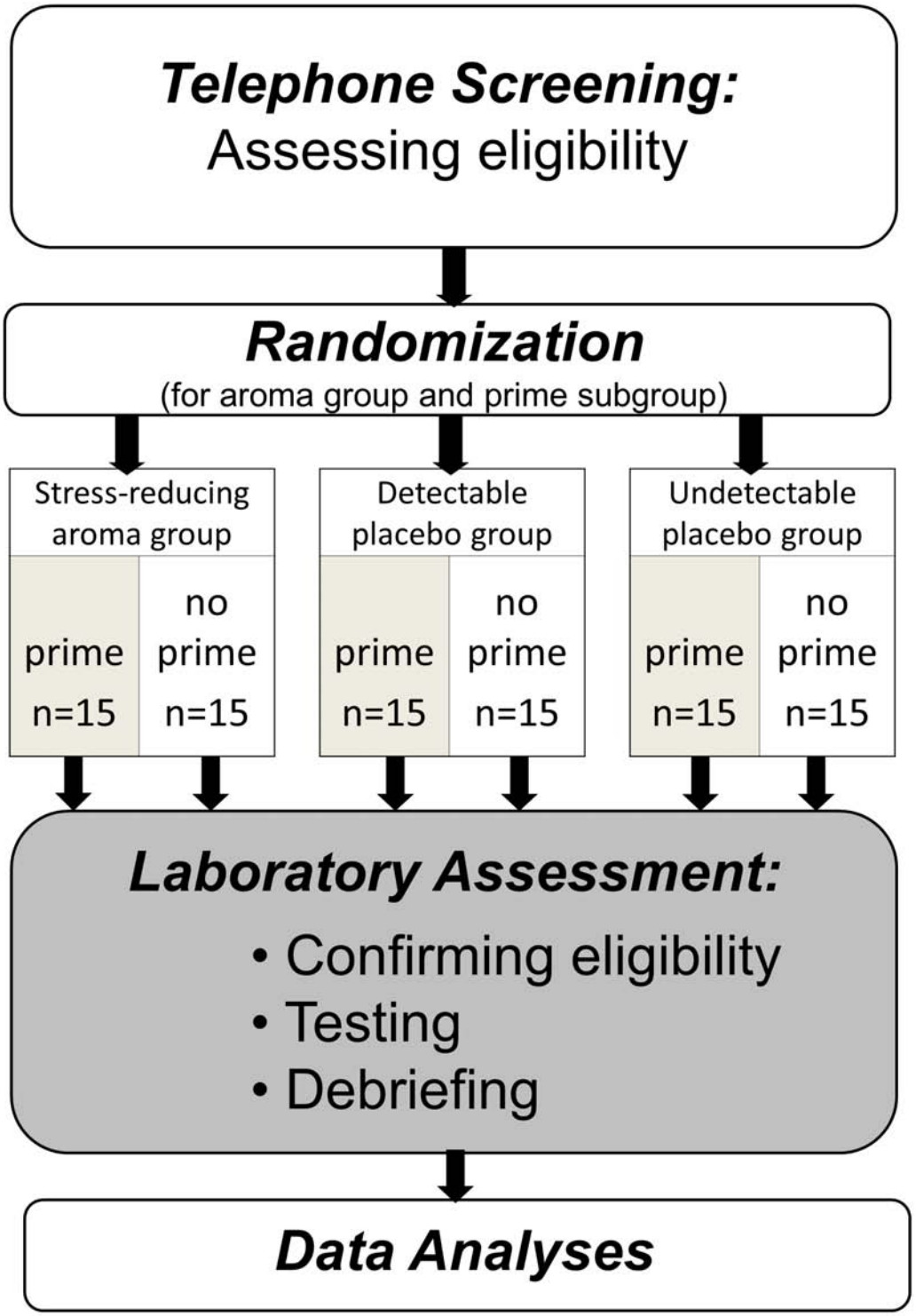
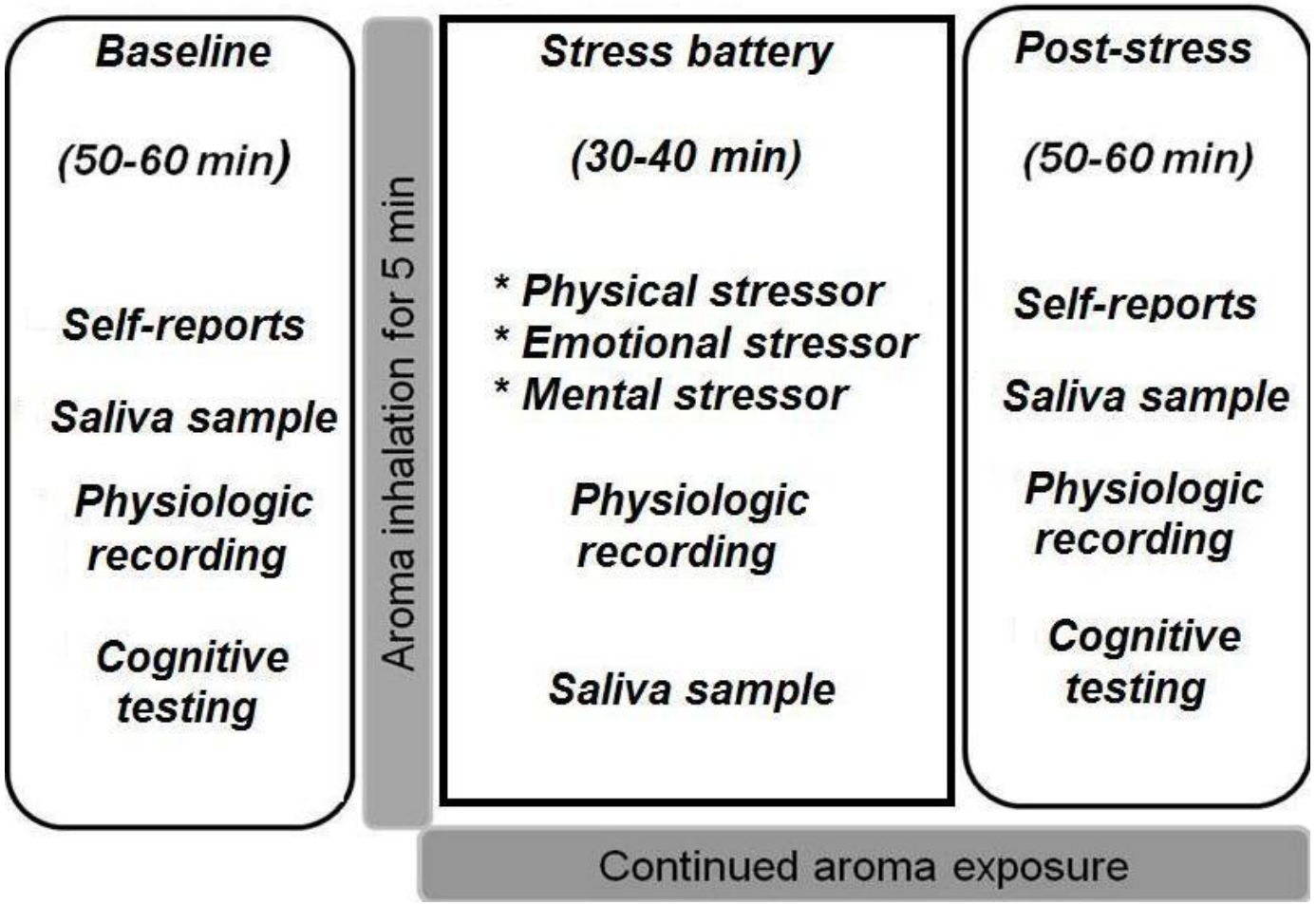


Diagram 2. Study visit Activities



Laboratory visit: procedures

All study visits started between 12 pm and 12:30 pm to minimize circadian effects. Participants were instructed to avoid any scented products or foods with strong odor on the day of the visit and were told to have a meal 30 min prior to their scheduled visit. Participants had access to drinking water during the study.

During the laboratory visit, participants signed the consent form, and their eligibility was confirmed by screening for general anosmia with a 3-item Quick Smell Identification Test (Sensonics, Inc., Haddon Heights, NJ) described previously (Jackman & Doty, 2005). Briefly, the 3-item Quick Smell Identification Test is a scratch and sniff test during which a participant sniffs 3 synthetic smells (in the following sequence: chocolate, banana, and smoke) and identifies each of the smells using a multiple choice presented next to the scented area. After the screening and confirming eligibility, the equipment for electrophysiological measures was set up, and participants proceeded with the visit that included a baseline assessment, a stress battery, and a post-stress assessment (Diagram 2). During the baseline participants completed self-report measures, provided the baseline measures of physiologic activity (EEG and respiration), and collected the first set of salivary measures. After the baseline each participant received their assigned aroma and a card with or without the prime and began inhaling their assigned aroma. Five minutes after initial exposure, participants underwent a stress battery followed by another set of physiologic recording and saliva measures. Finally, participants completed the post-stress assessment similar to the baseline assessment (with self-report measures, physiologic recordings, and

saliva collection). Subjective stress ratings were taken during baseline and post-stress assessments as well as after each stressor in the stress battery. The laboratory visit took about 4 hours. After completing the laboratory visit, the equipment for measuring electrophysiological responses was dismantled, and the non-blinded research assistant debriefed the participants about the purpose of the study (the debriefing scrip is available in Appendix 2). Finally, at the end of the visit participants received a modest reimbursement (at a rate $10.00 per hour) for their time and effort.

Stress battery

The stress battery consisted of emotional, physical, and mental stressor to elicit stress response in study participants. The chosen stressors are considered relatively mild but have been previously demonstrated to influence physiologic, endocrine, and cognitive functions.

Physical stressor. The Cold pressor task (Lovallo, 1975) was used as a physical stressor. During this task participants submerged their dominant hand into the container with ice-cold water and kept their hand submerged as long as they could tolerate it or until 3 min elapsed.

Emotional stressor. For the emotional stressor a 5-minute slideshow of unpleasant images from the International Affective Picture System (Lang, Bradley, & Cuthbert, 1999) was displayed on a computer screen. The images from categories “war”, “ violence”, “accident”, “suffering”, “ animals” with valence less than 5 and arousal level greater than 5 were chosen for the presentation. To

help ensure participants watched the slideshow, they were asked to push a button if the image of a snake appeared on the screen.

Mental Stressor. The Montreal Imaging Stressor Task was used as a mental stressor, and it is composed of computerized mental arithmetic items with a built-in failure algorithm and social evaluative threat component shown to elicit cortisol levels increase (Dedovic et al., 2005). The software for the MIST was generously provided by Dr. Jens Pruessner of McGill University.

Physiological measures

Electroencephalography. The EEG was recorded from 32-channel array (10/20 system) using the BioSemi Active Two EEG recording system (BioSemi BV, Amsterdam, Netherlands). Signa Gel (Parker Labs, Fairfield, NJ) was applied to create a stable electrical connection between each electrode and participant's scalp. The electro-oculogram (EOG) was recorded from the electrodes placed above the left external canthus and below the right external canthus. Electrode impedances cannot be measured from the BioSemi Active Two electrodes. However, the EEG recordings were monitored to adhere to the offset recording standards of the BioSemi Active Two system. The sampling rate was 1024 Hz. The specific EEG and ERP-related measures obtained from these recordings are described in detail in chapters 3 and 5, respectively.

Salivary measures

Saliva samples were collected using Sarstedt Salivettes (Sarstedt AG & Co, Numbrecht, Germany). Samples were stored in a refrigerator shortly after

collection and were centrifuged following the visit completion and stored at –80F prior to processing by the Oregon Clinical Translational Research Institute Core Laboratory. Saliva samples from each subject at baseline, after stress battery, and during the post stress assessments were run in the same assay batch. Information regarding specific salivary measures is presented in Chapter 3.

Self-report measures

The purpose of self-report instruments was to assess variables that might be affecting aromatherapy actions or influenced by aromatherapy actions.

Background variables. Participants answered questions about their age, race, ethnicity, education level, marital status, employment status, and income level. Previous aromatherapy use (frequency and purpose) was assessed using a custom questionnaire asking participants whether they used aromatherapy previously (ever) and in the previous 3 months, how often aromatherapy was used, and for what purpose (e.g. relaxation).

Perceived Stress Scale (PSS) (Cohen et al., 1983) was used to assess perceived stress level in the previous week to determine baseline stress level.

Neuroticism, a personality trait that has been reported to affect expectancy effects (Herz, 2009) was assessed with the Neuroticism-Extroversion-Openness (NEO) Five-Factor Inventory designed to give quick, reliable, and valid measures of the five domains of adult personality (Costa, Fagan, Piedmont, Ponticas, & Wise, 1992).

Expectancy of aromatherapy effect was assessed with a visual analog scale (VAS) ranging from 0 to 100mm. Participants put a mark on a line indicating the expected effect of aromatherapy on stress level (ranging from decreased stress to increased stress) and the overall effect they expected from aromatherapy (ranging from overall negative effect to overall positive effect).

Stress-related measures

Participants rated their subjective stress level at multiple time points using a VAS analog scale ranging from 0 (no stress) to 100 (extremely stressed).

The Positive and Negative Affect Schedule (PANAS) (Watson, Clark, & Tellegen, 1988) was used to assess positive (PANASpos) and negative (PANASneg) at the baseline and during the post-stress period.

State portion of *the State -Trait Anxiety Inventory (STAI)* (Spielberger & Vagg, 1984) was used to asses anxiety levels at the baseline and the post-stress period.

Measures of aroma hedonic qualities. At the end of aromatherapy exposure participants used VAS scales to rate the pleasantness (ranging from 0 = extremely unpleasant to 100 = extremely pleasant), and intensity (ranging from 0 = barely noticeable to 100 = extremely intense) of their assigned aroma.

Statistical analyses

IBM SPSS Statistics package version 19 was used for all data analyses.

Preliminary data screening included examining histograms and box plots to assess normality assumption and indicate potential outliers. The points identified in box plots as extreme outliers were excluded from further analyses. If serious violations of normality were detected, the transforms were applied (natural log, square root, Box Cox), and non-parametric tests were used if violations of normality assumption remained severe after attempting transforms.

Background variables. Group differences in baseline characteristics have been assessed using analyses of variance (ANOVAs) for continuous variables and chi-square analyses for categorical variables with group and subgroup assignment as between subject variables.

Manipulation check. Success of stress manipulation was verified by performing RM ANOVAs or Wilcoxon Rank-Sum tests conducted with the whole sample, with the time point (baseline vs. post-stress) as a within group variable for the physiologic and self-report stress-related measures.

Participant baseline characteristics

Baseline characteristics: well-matched groups and subgroups

The overall sample included ninety-two healthy adults (Mean age = 58.0, 79.3% females). The groups based on aroma and prime were matched on major background variables including age, gender, education, perceived stress, and personality traits (all p's > .05). Also, participants in different groups were similar in previous aromatherapy use (all p's > .05). Scores for stress-related measures of anxiety and affect were similar for the aroma and prime groups (all p's > .05).

However, participants in prime subgroup reported greater the subjective stress levels at baseline compared to those in no prime subgroup, $p = .01$.

The details of baseline characteristics based on aroma and prime groups are presented in Table 1.

Table 1. Baseline participant characteristics

Mean (SD)	Aroma groups			p	Prime groups		p
	Lavender n = 31	Coconut n = 31	Water n = 30		Prime n = 47	No prime n= 45	
Age	58.9 (6.6)	57.9 (6.0)	57.3 (5.9)	.72	59.1 (6.2)	56.9 (6.0)	.07
Female (%)	77.4	83.9	75.9	.72	85.1	72.7	.15
Education (years)	16.1 (.4)	16.2 (.4)	15.8 (.4)	.61	15.4 (.4)	16.6 (.4)	.06
PSS score	16.6 (5.3)	18.4 (5.7)	16.2 (5.9)	.33	17.9 (5.0)	16.2 (6.2)	.21
VAS stress	19.8(16.6)	18.9(17.4)	21.5(19.2)	.81	23.9(18.6)	16.0(15.7)	.01
STAI score	33.9 (6.7)	34.5 (7.6)	33.3 (9.4)	.92	33.6 (8.2)	34.3 (7.9)	.70
PANAS negative	13.6 (4.0)	12.8 (3.0)	13.2 (5.6)	.64	12.6 (4.2)	13.8 (4.4)	.19
PANAS positive	30.5 (9.1)	32.5 (5.7)	34.4 (6.4)	.10	32.5 (7.0)	32.3 (7.7)	.91
Aroma users (%)	51.6	38.7	46.4	.59	42.6	48.8	.55
Expected effect.	70.3 (2.3)	71.2(2.3)	71.7(2.4)	.96	71.6 (1.9)	70.6 (2.0)	.61
Expected stress	33.8 (3.3)	32.9(3.3)	34.5(3.4)	.87	33.1 (2.6)	34.4 (2.8)	.93

Abbreviations: SD = standard deviation, PSS = Perceived Stress Scale, VAS stress = visual analog scale stress rating, STAI = State and Trait Anxiety Inventory, PANAS = Positive And Negative Affect Schedule, Expected effect. = expected effectiveness of aromatherapy, Expected stress = expected stress level due to aromatherapy (from neutral = 50).

Stress battery effectiveness check using subjective measures

When the whole sample was assessed, participants reported feeling more stressed after the stress battery: compared to mean baseline stress (M= 20.0, SD = 17.6), average stress level reported during the stress battery was significantly greater (M = 63.2, SD = 18.4), $F(91) = 328.81$, $p < .001$. Furthermore, state anxiety also significantly increased from baseline (M = 34.0, SD = 8.0) to post-stress period (M = 36.6, 7.2), $F(91) = 10.01$, $p = .002$. Participants also reported a significant decrease in positive affect from baseline (M = 32.5, SD = 7.3) to post-stress period (M = 30.9, SD = 8.0), $F(91) = 6.49$, $p = .013$. No significant changes from baseline to post-stress period were observed on negative affect scores, $p > .10$. Changes in stress-related measures due to exposure to stress battery are presented in Figure 2 (subjective stress ratings) and Figure 3 (changes in subjective affect and anxiety).

Aroma hedonic qualities for different aromas

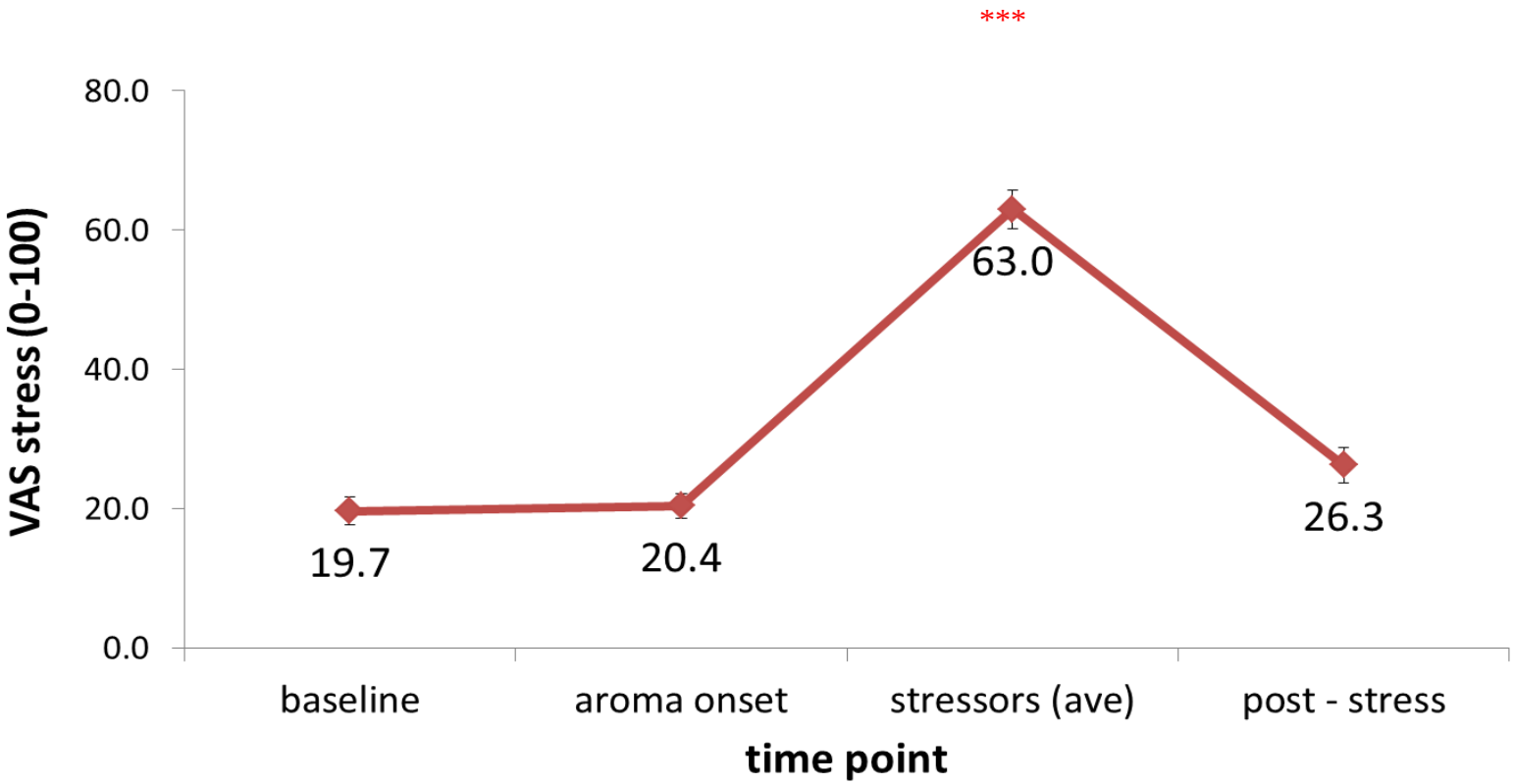
The results indicate that participants in different aroma groups had significantly different ratings for the intensity of their assigned aroma, $F(2, 76) = 14.8$, $p < .001$. Specifically, as indicated by Tukey HSD follow-up tests, the mean aroma intensity rating in the lavender group was 48.3 (SD = 26.4) significantly greater than the ratings in the groups exposed to coconut, M = 24.9 (SD = 26.2), $p < .001$ and water, M =14.2 (SD = 13.3), $p < .001$. No significant differences emerged between intensity ratings for water and coconut groups, $p = .35$.

Additionally, there was a significant difference in aroma pleasantness rating for the participants in different aroma groups, $F(2, 76) = 7.7$, $p = .001$.

Specifically, the water aroma received an average pleasantness rating of 57.2 (SD = 17.6), the coconut aroma received an average pleasantness rating of 65.3 (SD = 22.1), and the lavender aroma received an average pleasantness rating of 78.2 (SD = 19.6). Follow-up comparisons tests using Tukey HSD indicated that the pleasantness rating of the water was significantly lower than that of the lavender aroma, $p < .001$, with a non-significant trend for the difference between pleasantness ratings between the water and coconut aromas, $p = .09$. There were no significant differences in pleasantness ratings between the coconut and lavender aromas, $p = .18$. Figure 4 depicts group differences in aroma hedonic qualities.

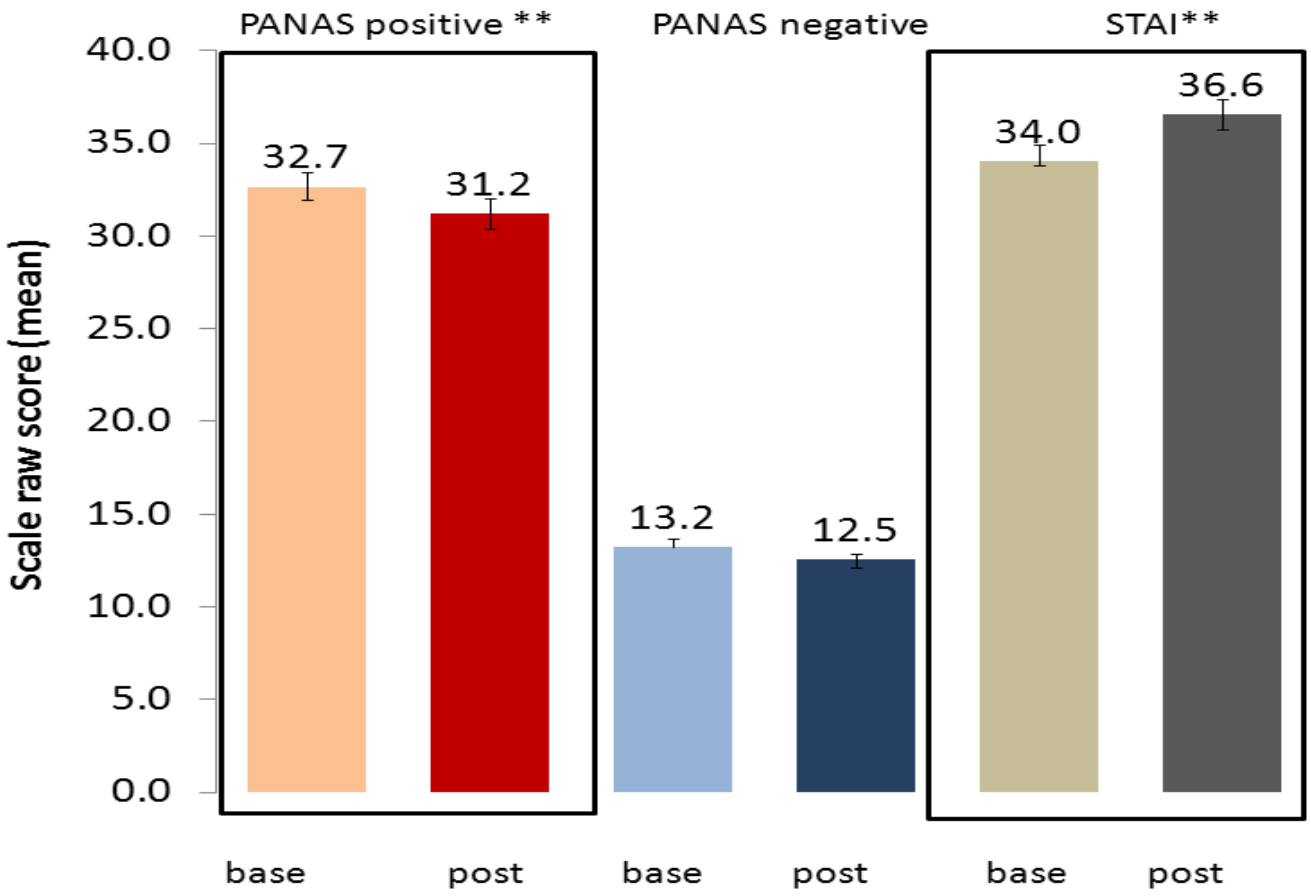
The results of analyses assessing aroma hedonics suggest that lavender aroma was the most intense aroma compared to both water and coconut aromas, which were more similar on the intensity ratings. Thus, the attempt to match lavender and coconut on intensity failed. However, the lavender and coconut groups had similar ratings for pleasantness of their aroma suggesting that matching on that variable was more successful.

Figure 2. Differences in subjective stress between baseline and stress time points



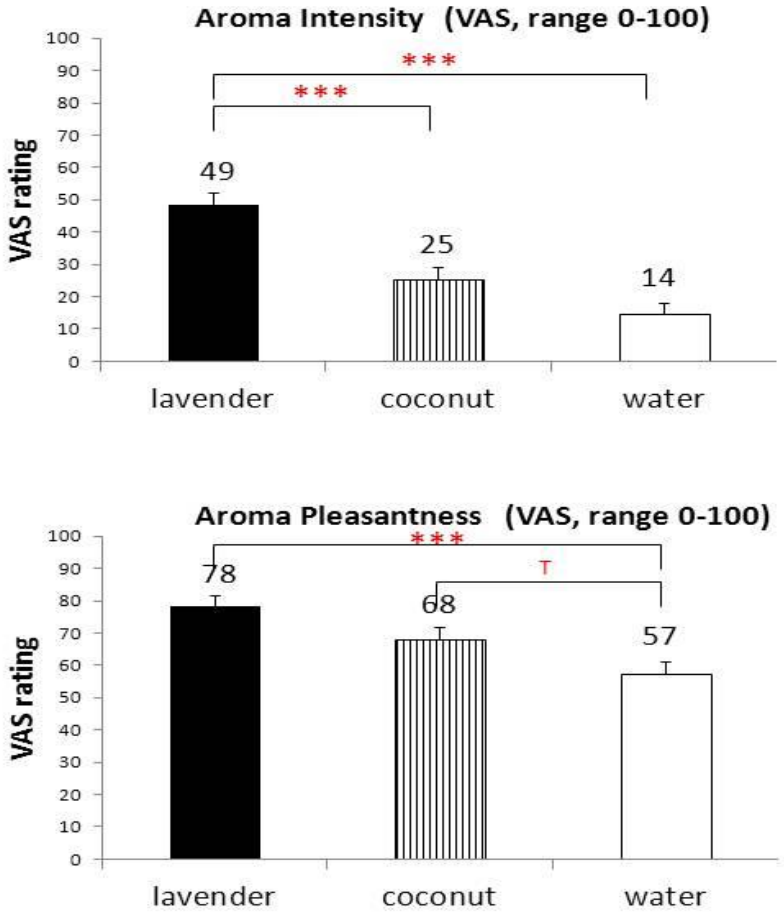
***p < .001 compared to the baseline value.
Values represent mean subjective stress ratings, Error bars represent standard Errors of the mean (SEm).

Figure 3. Changes in subjective measures from baseline to post-stress



**p ≤ .01.The numbers above the bars represent average scores. Error bars represent SEm.

Figure 4. Ratings of aroma hedonic qualities



T .10 > p > .05, ***p < .001

The numbers above the bars represent average group ratings. Error bars represent SEm.

The following chapters will address specific outcome measures: physiological markers sensitive to stress (addressed in Chapter 3), cognitive tests evaluating primarily high-order cognitive functions vulnerable to stress (addressed in Chapter 4 and Chapter 5), and brain responses to a cognitive task assessing attention, a higher-order cognitive function (addressed in Chapter 5). The Discussion section will summarize and integrate the results reviewed in different chapters and outlines limitation of the current work and suggestions for future research.

Chapter 3: Effects of aromatherapy on physiological stress markers following acute stress: the role of expectancy in aromatherapy actions

Introduction

Stress, commonly defined as a state of threatened homeostasis requiring an adaptive response (McEwen, 2002), induces a range of changes in autonomic and endocrine systems, psychological state, and behavior (Lupien et al., 2007; McEwen, 2008; Ulrich-Lai & Herman, 2009). While the stress response is adaptive and critical to survival, when called upon too often, it can become dysregulated leading to a broad range of health problems (McEwen, 2000). Chronic stress exposure leads to increased risk for depression, immune dysregulation, sleep disturbance, cardiovascular problems, diabetes, cancer, cognitive impairment and neurodegenerative diseases (Bremner, 1999; Chrousos, 2009; de Kloet, 2000; Graham et al., 2006; McEwen, 2008; McEwen et al., 1997). The negative stress consequences can be mitigated by stress management techniques (Esch & Stefano, 2010).

Complementary and alternative medicine (CAM) stress management approaches are becoming popular among the public and health care providers because of accessibility and cost effectiveness (Herman et al., 2005; Oken, 2004). Aromatherapy is one of the fastest growing CAM methods in the US due to its ease of application, fast action, and low cost (Butje et al., 2008; d' Angelo, 2002). Aromatherapy is an ancient non-invasive CAM therapy that uses volatile plant materials called essential oils for treatment and prevention of various human conditions (Edris, 2007). To date few controlled studies have been performed using aromatherapy as a stress-reducing intervention, and despite the

increasing use, efficacy of this CAM approach remains questionable (Herz, 2009; Kiecolt-Glaser et al., 2008; Lee et al., 2012).

The objective of the current study was to evaluate effects of a commonly used stress-reducing aromatherapy on several physiologic measures sensitive to stress. Previous studies focused on aromatherapy effect on particular physiological system, but the goal of this study was to assess the measures representing potentially different systems participating in stress response including sympathetic nervous system and endocrine system, thus allowing for broader conclusions about aromatherapy physiological effects in the face of stress. Objective measures used in this study have been selected because they were previously shown to respond to acute stress and, in some cases, to aromatherapy.

Electroencephalography (EEG) measures have been used in several previous studies assessing aromatherapy effects (Brauchli, Ruegg, Etzweiler, & Zeier, 1995; Diego et al., 1998; Hiruma, Yabe, Sato, Sutoh, & Kaneko, 2002). EEG frontal asymmetry, (EEG FA) has been used extensively to assess emotional state, and previous research has indicated that positive moods or reactions are linked to relatively lower alpha power in the left hemisphere (reflecting greater activation of the region), while negative moods or reactions are linked to relatively lower alpha power in the right hemisphere (reflecting greater activation of the region) (Davidson, 2004). EEG FA is believed to reflect the activity in the dorsolateral prefrontal cortex (Davidson, 2004) and can be affected by both stress and lavender aroma, according to previous studies (Kline,

Blackhart, Woodward, Williams, & Schwartz, 2000; Lewis, Weekes, & Wang, 2007; Sanders et al., 2002). However, the previous studies using EEG FA as an outcome variable in aromatherapy research lacked proper controls and did not evaluate placebo or expectancy effects that might play a role in aromatherapy actions.

Autonomic measures such as heart rate, blood pressure, and respiration rate are sensitive to stress (Ulrich-Lai & Herman, 2009) and commonly used in stress research (Hongratanaworakit; Pournemati, Azarbayjani, Rezaee, Ziaee, & Pournemati, 2009; Saeki, 2000). Previous studies evaluating autonomic measures have produced conflicting results regarding the effects of aromatherapy on measures such as heart rate and respiration rate. Some studies found a decrease in heart rate and respiration rate after aromatherapy (Buckle, 1993; Kuroda et al., 2005), but other studies showed no change due to aromatherapy (Dunn, Sleep, & Collett, 1995; Saeki, 2000).

Endocrine markers have also been successfully used in aromatherapy research. Salivary cortisol, a commonly used stress biomarker, is considered a reliable measure of hypothalamus-pituitary-adrenal (HPA) axis adaptation to stress (Hellhammer, Wust, & Kudielka, 2009). Some evidence suggests that cortisol levels might be affected by aromatherapy exposure (Atsumi & Tonosaki, 2007), but other studies report no specific aroma effects on cortisol levels (Kiecolt-Glaser et al., 2008; Toda, Den, Nagasawa, Kitamura, & Morimoto, 2005).

Another endocrine marker, salivary chromogranin A (CgA) is also receiving attention as a useful stress measure (Nakane, Asami, Yamada, & Ohira, 2002;

Toda et al., 2005). CgA is released along with catecholamines from the adrenal medulla and sympathetic nervous endings (Winkler & Fischer-Colbrie, 1992). CgA and cortisol were suggested to underlie different types of response to stress stimuli: CgA associated with sympathetic system activation is believed to be more suitable for assessing acute stress compared to cortisol that is secreted more slowly after HPA axis activation (Toda et al., 2005; Toda & Morimoto, 2008). Furthermore, CgA was shown to be affected by lavender aroma after a mental stressor (Toda & Morimoto, 2008). Overall, several physiologic measures sensitive to stress have been evaluated with aromatherapy with inconsistent results. Some of these inconsistent results are likely due to differences in study methods. The current study has been designed to provide a rigorous assessment of aromatherapy effects on physiological markers of stress using a single-blind RCT design.

In addition to assessing aromatherapy effects on a battery of physiological markers, this study aimed at evaluating some of the mechanisms involved in aromatherapy actions that currently remain unknown. The two leading hypotheses explaining reported aromatherapy benefits are pharmacological and psychological (Herz, 2009). The former states that aromas exert specific effects by olfactory stimulation acting like a drug affecting specific receptors, the view embraced by aromatherapy practitioners prescribing particular aromas to alleviate specific conditions (Hirsch, 2001). The latter attributes any aromatherapy actions to expectancy bias involving previously learned associations influencing psychological or emotional state (Howard & Hughes,

2008). This study focused on the role that expectancy might be playing in aromatherapy actions. Specifically, the role of expectancy bias was evaluated by manipulating aroma-mediated (presence of inert but perceptible aroma) and verbally-mediated (verbal suggestion of aroma effects) expectancies in study participants.

The hypothesis for this study was that the role of both pharmacological and expectancy effects will be supported, and different measures will be affected through different mechanisms.

Methods

Participants

Ninety-two healthy adults (Mean age = 58.0, 79.3% females) were recruited from the community using flyers and media announcements. Eligibility criteria and baseline characteristics of the participants were described in Chapter 2.

Study groups, randomization process, and blinding

Prior to the study visit each participant was randomized to a group based on aroma type (lavender, coconut, or water) and was also assigned to a subgroup based on a type of prime they received during the visit (prime or no prime) as described in Chapter 2.

Laboratory visit

Detailed visit information has been described in Chapter 2.

Briefly, participants signed the consent form after their eligibility was confirmed. After the screening and confirming eligibility, the equipment for

electrophysiological measures was set up, and participants proceeded with the visit that included a baseline assessment, a stress battery, and a post-stress assessment (Diagram 3). During the baseline participants completed self-report measures, provided the baseline measures of physiologic activity (EEG and respiration), and collected the first set of salivary measures. After the baseline each participant received their assigned aroma and a card with or without the prime and began inhaling their assigned aroma. Five minutes after initial exposure, participants underwent a stress battery followed by another set of physiologic recording and saliva measures. Finally, participants completed the post-stress assessment similar to the baseline assessment (with self-report measures, physiologic recordings, and saliva collection). Subjective stress ratings were taken during baseline and post-stress assessments as well as after each stressor in the stress battery.

Diagram 3. Time points for obtaining physiological measures

Baseline assessment	Aroma start	Stress battery	Stress end	Post-stress assessment
EEG, RR Saliva sample	EEG, RR		EEG, RR Saliva sample	EEG, RR Saliva sample

Abbreviations: EEG = electroencephalography, RR = respiration rate

Stress battery

The stress battery described in detail in Chapter 2 consisted of emotional, physical, and mental stressor to elicit stress response in study participants.

Electrophysiological measures

Electrophysiological measures were recorded during a 5-min restful awake state with an intermittent auditory vigilance task to prevent excessive drowsiness created and presented in EPrime 2.0 (Psychology Software Tools, Inc., Pittsburgh, PA). The task was performed several times during the visit (see Diagram 3) with eyes closed in a sitting position in a light and sound attenuated laboratory. During the task participants were required to push the right button on a two-button button box if they heard a high-pitch tone (2000 Hz) and left button if they heard a low-pitch tone (1000Hz). The auditory stimuli were introduced to participants prior task start and during the task they were presented for 1 second with a random inter-trial interval ranging between 4 and 14 sec).

Respiration rate. The respiration rate was obtained by using an elastic respiration belt (Ambu Sleepmate, Glen Burnie, MD) placed around participant's abdomen. The respiratory rate system was connected to the computer running the BioSemi Active Two EEG recording system (BioSemi BV, Amsterdam, Netherlands) and could be visualized and saved as the part of the Biosemi output file.

Respiration rate processing. Respiratory rate was processed using a custom macro run using Matlab R2007a version (MathWorks, Natlick, MA) that has shown strong correlations with the results obtained by counting (personal

communication with Dr. Wahbeh, OHSU). The respiration rate was obtained from the recordings that lasted 5 min each.

Electroencephalography. The EEG was recorded from 32-channel array (10/20 system) using the BioSemi Active Two EEG recording system (BioSemi BV, Amsterdam, Netherlands). Signa Gel (Parker Labs, Fairfield, NJ) was applied to create a stable electrical connection between each electrode and participant's scalp. The electro-oculogram (EOG) was recorded from the electrodes placed above the left external canthus and below the right external canthus. Electrode impedances cannot be measured from the BioSemi Active Two electrodes. However, the EEG recordings were monitored to adhere to the offset recording standards of the BioSemi Active Two system. The sampling rate was 1024 Hz.

EEG processing for frontal asymmetry (FA) measure. EEG FA was obtained from the data collected during 5-min intervals of resting awake state as described above. All processing was completed offline using Brain Vision Analyzer version 2.0 (Brain Products GmbH, Gilching, Germany) similar to previously described research (Anokhin, Heath, & Myers, 2006). Briefly, average local reference EEG (Nunez & Pilgreen, 1991) was filtered offline from 0.1 to 70 Hz (and 60 Hz notch filter was additionally applied). Data from one second following a tone onset were removed to exclude tone-related evoked potentials. Artifacts due to eye movements were removed using independent component analysis (Jung et al., 2000), and the data were visually inspected for gross artifacts that were removed. Remaining data were segmented into 2 seconds epochs, and the semi-automatic artifact rejection was applied to flag any epoch containing

gradient voltage step greater than 125 μV/20ms and amplitudes greater than ±75 μV that were further removed after manual check. The remaining artifact-free epochs were subjected to Fast Fourier Transform (FFT) with 40% Hanning window and 0.5 Hz resolution. The power spectra for individual epochs were averaged, and the measures of EEG spectral density were obtained for alpha band (8 –12.99 Hz). Square root values of power were used, and frontal hemispheric asymmetry was calculated as ((L-R)/(L+R))*100, where L and R are square root values at the homologous left and right hemisphere sites (using local average reference values at F3 and F4). With this calculation, FA negative values reflect lower alpha power (higher activation) in the left hemisphere linked to a more positive mood.

Salivary measures

Saliva samples were collected using Sarstedt Salivettes (Sarstedt AG & Co, Numbrecht, Germany). Samples were stored in a refrigerator shortly after collection and were centrifuged following the visit completion and stored at –80F prior to processing by the Oregon Clinical Translational Research Institute Core Laboratory. Saliva samples from each subject at baseline, after stress battery, and during the post stress assessments were run in the same assay batch.

Cortisol (Cort). Cortisol was measured using a commercially available ELISA kit (Salimetrics, State College, PA 16803). The method is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol. A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol

linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present Inter-assay %CV for two controls samples that were run in every assay were 9.8% and 4.7 % for 0.1 and 1.0 ug/dL respectively. Intra-assay %CV was 4.1% (1.0 ug/dL). Greater increase in cortisol level relative to baseline is indicative of greater HPA axis activation and suggests a greater response to stress (more stressed state). Another important aspect of cortisol response is the rate of change over time: faster return to baseline values indicate a more resilient system.

Chromogranin A (CgA). CgA was measured using a commercially available ELISA kit (Kamiya Biomedical Company, Seattle, WA 98168). The method is based on a competitive enzyme immunoassay using a highly specific antibody to human CgA (aa 344-374) coupled with a biotin-streptavidin affinity methodology. Inter-assay percent of CV was 14% for a 2.2 pmol/ml control sample. Average percentage difference between duplicate samples was 5.1%. Previous studies suggested that increased CgA levels shortly after a stressor are associated with greater mental stress (Toda & Morimoto, 2008).

Self-report measures

The purpose of self-report instruments was to assess variables that might be affecting aromatherapy actions or influenced by aromatherapy actions. Self-report measures included background variables, stress-related measures, and measures of aroma hedonic qualities. Chapter 2 contains more detailed information about the measures assessed in the study.

Statistical analyses

General analytical approach and procedures used for data screening and assessment of background variables as well as evaluating effectiveness of stress battery and aroma hedonic qualities for each aroma are described in Chapter 2.

Primary analyses

Family-wise adjustments for multiple comparisons were made for each set of the physiological measures (electrophysiological and salivary) using a False Discovery Rate approach (Benjamini & Hochberg, 1995). In this study, with 2 measures evaluated in each family, the smallest p value resulted from the analyses of a set is considered significant if less than .025, and a next in magnitude p value is considered significant if less than .05. The p values between .050 and .075 were considered trending on significance.

Analyses of aroma effects: Physiological measures

Participants in different groups and subgroups were similar on physiologic measures at baseline (refer to Table 1). However, there still was a considerable

variability in baseline levels of the physiological and endocrine markers used in this study. To account for some baseline variability in salivary and respiratory measures, stress and post-stress variables used in the analyses for Cort, CgA, and respiration were baseline corrected (representing proportion of the baseline value).

EEG frontal asymmetry (FA). Repeated Measures (RM) Analysis of covariance (ANCOVA) was used with time as a within subject factor (aroma start pre-stress, stress, post-stress), FA at baseline as a covariate, and group and subgroup assignments as between subjects variables.

Respiration rate (RR). RM ANOVA was used with time as a within subject factor (stress, post-stress), and group or subgroup assignments as between subjects variables to evaluate changes in RR. RR at each time point was presented as a proportion of baseline value.

Analyses of aroma effects: Salivary measures

RM ANOVAS were used with time as a within subject factor (stress, post-stress), and aroma group as a between subject variable to assess aroma effect on the salivary measures (cortisol and chromogranin A) after stress battery. Each salivary measure was presented as a proportion of baseline value.

Table 2. Baseline values of physiological variables for aroma and prime groups

Mean (SD)	Aroma groups			p	Prime groups		p
	Lavender n = 31	Coconut n = 31	Water n = 30		Prime n = 47	No prime n= 45	
EEG FA score	.015 (.15)	.006 (.12)	.042 (.14)	.66	.032 (.12)	.008 (.15)	.66
RR (resp/min)	15.9 (3.4)	15.8 (3.2)	14.6 (3.7)	.33	15.4 (3.6)	15.5 (3.4)	.93
Cortisol (ug/dL)	.13 (.08)	.14 (.07)	.11 (.08)	.14	.13 (.07)	.13 (.08)	.76
CgA (pmol/ml)	5.08 (3.4)	4.52 (2.8)	7.32 (8.8)	.44	4.98 (3.5)	6.29 (7.4)	.54

Abbreviations: SD = standard deviation, EEG FA = EEG frontal asymmetry, RR = respiration rate, CgA = Chromogranin A.

Secondary analyses

Secondary analyses were conducted to evaluate potential mechanisms involved in aromatherapy effects on physiological markers, to probe the role of different types of expectancy on physiological responses, and to assess the relationships among the physiological, stress-related, and aroma qualities variables. Due to exploratory nature of the analyses no adjustments for multiple comparisons were made for secondary analyses. The p value < .05 was considered significant for this set of analyses and trends were noted if p value was between 0.1 and .05.

Analyses of verbally-mediated prime effects and aroma x prime interactions

The analyses conducted to assess prime effects and aroma x prime interactions were similar to the analyses for aroma effect except both aroma group (lavender, coconut, water) and prime subgroup (prime, no prime) were used as between subject variables.

Analyses of aroma-mediated prime effects and aroma x prime interactions

The analyses conducted to assess aroma-mediated prime effects and aroma by prime interactions were similar to the previous set of analyses but only the two placebo aroma groups (coconut and water) were compared.

Correlations among cognitive and stress-and-aroma-related variables

Bivariate Pearson correlations were performed to evaluate relationships among cognitive performance variables and variables associated with aroma hedonic qualities, expectancy, and stress.

Results

Stress battery effectiveness check using objective stress measures

When the whole sample was assessed, several stress biomarkers showed significant change in response to stress battery. Participants' respiration rate was elevated during stress battery, ($M = 18.7$, $SD = 3.8$) compared to baseline ($M = 15.4$, $SD = 3.6$), $p < .001$. Cortisol levels were also elevated following stress battery ($M = .26$, $SD = .06$), compared to baseline ($M = .18$, $SD = .05$), $p < .001$. Additionally, levels of chromogranin A were significantly decreased after stress battery ($M = 4.5$, $SD = 0.4$), compared to baseline ($M = 5.6$, $SD = 0.6$), $p < .001$. There was no change in EEG frontal asymmetry patterns following stress, $p > .10$.

Group effects on stress-related self-reported measures

Subjectively, participants also reported feeling more stressed after the stress battery as was described in Chapter 2. However, when different group responses were compared, RM ANOVAs indicated no significant effects of aroma group or a prime subgroup for STAI, PANASpos, and PANASneg, all p's > .05.

There was a trend for a time x aroma interaction for the measure of subjective stress, $F(1, 75) = 3.07$, $p = .05$, partial $\eta^2 = .08$. Follow-up analyses suggested people in coconut group tended to report higher stress during post-stress assessment compared to those in lavender or water group, $p = .07$.

Overall, while there were changes in subjective stress-related measures in response to stress battery for the whole sample, there were no significant

differences in participants' stress, anxiety, and affect ratings due to aroma groups or prime subgroups at post-stress assessment.

Because there were significant differences in aroma hedonics ratings (described in Chapter 2), the analyses for the stress biomarkers were performed with and without aroma intensity rating or aroma pleasantness rating as a covariate to probe whether variance related to aroma hedonic qualities were contributing to significance of the group differences.

Primary analyses: aromatherapy effects on physiologic function

Figure 5 presents information about changes from baseline in objective measures at stress and post-stress assessments.

EEG frontal asymmetry

RM ANCOVA indicated no significant effects or interactions, all p's > .10. However, the data suggested similarity between lavender and coconut group. When the analyses were run with perceptible aroma group (perceptible aroma vs. non-perceptible aroma) as a between group factor and time (aroma start pre-stress, stress, post-stress) as a within group factor the main effect of scent was present, $F(1,80) = 4.70$, $p = .03$, partial $\eta^2 = .06$. Specifically, the participants in groups exposed to perceptible aromas (lavender and coconut) displayed significantly lower relative EEG FA (indicative of a more positive mood and affect) during initial aroma exposure, immediately after stress battery, and at post-stress assessment compared to participants in water group who displayed progressively greater relative EEG FA as the study continued.

Covarying for aroma intensity and aroma pleasantness rating: Intensity. The results remained unchanged when aroma intensity rating was added as a covariate in the main model with aroma groups. When the analyses were run with perceptible vs. non-perceptible aroma groups, there was a main effect of time, $F (2, 71) = 3.58$, $p = .03$, partial $\eta^2 = .09$ not noted previously and a trend for main effect of aroma presence $F (1, 72) = 4.12$, $p = .046$, partial $\eta^2 = .05$, similar to the analyses conducted without covarying for intensity rating.

Covarying for aroma intensity and aroma pleasantness rating: Pleasantness. The results remained unchanged when aroma pleasantness rating was added as a covariate in the main model with aroma groups. When the analyses were run with perceptible vs. non-perceptible aroma groups, the previously found main effect of aroma presence became non-significant, $F (1, 68) = 2.46$, $p = .121$, partial $\eta^2 = .04$. Though there was a smaller n for this analysis due to lost data for pleasantness rating, the result of this additional analysis suggest that any effect on EEG FA might be mediated by aroma pleasantness.

Respiration rate

Aroma effects: RM ANOVA indicated a significant time x aroma interaction, $F (1, 84) = 4.15$, $p = .019$, partial $\eta^2 = .09$. Post-hoc analyses revealed that, while RR in water group remained stable over time, there were changes in RR during stress battery in lavender group ($p = .036$) and at post-stress in coconut groups ($p = .047$). The differences between groups at any time point did not reach statistical significance, p's $> .05$.

Covarying for aroma intensity and aroma pleasantness rating: Intensity. The results remained relatively unchanged when aroma intensity rating was added as a covariate in the main model with aroma groups. Even with reduced sample size due to lost intensity rating data, there was a significant time x aroma interaction, $F(2,76) = 3.65$, $p = .031$, partial $\eta^2 = .09$.

Covarying for aroma intensity and aroma pleasantness rating: Pleasantness. Similarly, the results remained unchanged when aroma pleasantness rating was added as a covariate in the main model with aroma groups. Even with reduced sample size due to lost reliability rating data, there was a significant time x aroma interaction, $F(2,72) = 4.09$, $p = .021$, partial $\eta^2 = .102$.

Therefore, the results indicated that aromatherapy effect on RR was not likely moderated or mediated by the hedonic qualities of the aroma; however, the rigorous tests of mediation/moderation were not performed.

Primary analyses: aromatherapy effects on salivary measures

Cortisol

Despite a significant change in cortisol levels due to stress battery exposure, RM ANOVA indicated no significant aroma effect or aroma x time interaction, all p's > .10. All aroma groups displayed similar patterns of change. Though the mean increase in stress cortisol level for lavender group was the least steep, the group levels did not differ significantly at either stress or post-stress assessment due to high variability (Figure 5).

Covarying for aroma intensity and aroma pleasantness rating: Intensity. The results remained unchanged when aroma intensity rating was added as a covariate in the main model with aroma groups.

Covarying for aroma intensity and aroma pleasantness rating: Pleasantness. The results remained unchanged when aroma intensity rating was added as a covariate in the main model with aroma groups.

Chromogranin A (CgA)

RM ANOVA indicated aroma effect, $F(2, 87) = 4.03$, $p = .021$, partial $\eta^2 = .09$. Post hoc tests using Tukey HSD indicated that people in water group showed significantly lower CgA levels on average at two assessment points compared to the average CgA levels at the same time points in lavender ($p = .037$) and coconut ($p = .045$) groups.

Covarying for aroma intensity and aroma pleasantness rating: Intensity. When the analyses were run with intensity rating as a covariate, the previously found main effect of aroma became non-significant, $F(2, 78) = 1.67$, $p = .194$, partial $\eta^2 = .04$. The results suggest that aromatherapy effect on CgA might be potentially mediated by the hedonic qualities of the aromas.

Covarying for aroma intensity and aroma pleasantness rating: Pleasantness. Similarly, when the analyses were run with pleasantness rating as a covariate, the previously found main effect of aroma became non-significant, $F(2, 74) = 2.66$, $p = .111$, partial $\eta^2 = .06$.

Figure 5.Changes in objective stress-related measures relative to baseline for different aroma groups

Figure 5 Legend

a. For the EEG asymmetry, a represents the main effect of scent, p = .03, with participants in groups exposed to perceptible aromas (lavender and coconut) displaying lower EEG FA relative to their baseline during the study compared to participants in water group who displayed progressively greater relative EEG FA as the study continued.

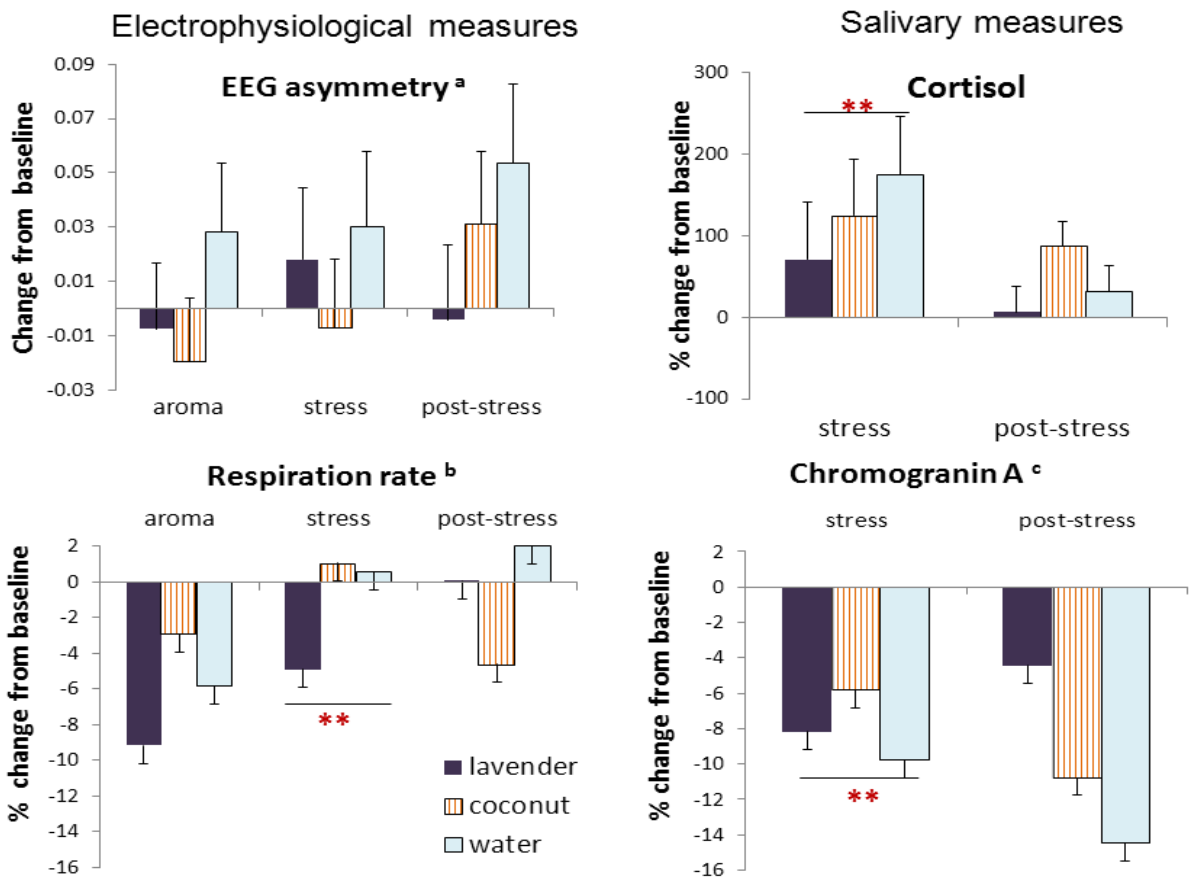
b. For the RR, b represents a time x aroma interaction, p = .019, with water group displaying stable RR over time, while lavender group showing decrease following stress, p = .036, and coconut groups showing change at post-stress assessment, p = .047.

c. For CgA, c represents the main effect of aroma effect, p = .021, with people in water group showing lower CgA levels on average at two assessment points compared to the average CgA levels at the same time points in lavender (p = .037) and coconut (p = .045) groups.

The data presented in an untransformed form to allow for easier interpretation

Aroma data point represents 5 min period after initial aroma exposure before stress battery exposure

Figure 5. Changes in objective stress-related measures relative to baseline for different aroma groups



**p < .01(change for the whole sample compared to baseline).

Secondary analyses

RM ANOVAs did not indicate any effect of prime or interactions involving a prime for RR, EEG FA, or CgA, all p's > .10.

Cortisol. RM ANOVA indicated a trend for prime x time interaction, $F (1, 84) = 3.94$, $p = .051$, partial $\eta^2 = .05$ suggesting differences in slopes of change for the two subgroups, with no prime subgroup showing a steeper slope for decrease in cortisol levels from stress to post-stress compared to prime subgroup. However, there were no significant differences between prime and no prime subgroups at either stress or post-stress time points.

The role of expectancy for EEG FA and CgA, as well as for RR in placebo groups

To assess whether any changes in physiologic measures could arise solely due to the presence of perceptible scent (evaluating aroma-mediated expectancy), only two placebo groups (coconut and water) were compared.

RM ANOVAs did not indicate any effect of aroma or prime or interactions involving aroma or prime for cortisol, $p > .10$.

EEG frontal asymmetry. RM ANCOVA suggested a trend for a 3 way time x aroma x prime interaction, $F (1, 50) = 2.78$, $p = .073$, partial $\eta^2 = .05$ and a trend for the main aroma effect, $F (1, 50) = 3.06$, $p = .087$, partial $\eta^2 = .06$. Specifically, people in coconut group displayed lower levels of frontal asymmetry indicating less distress compared to people in water group at all time points during the study. This difference was driven mainly by the people in no prime subgroup, as

patterns frontal asymmetry were more similar for the participants in prime subgroups during stress and post-stress assessments.

Respiration Rate. RM ANOVA indicated a trend for prime effect for RR, $F(1, 54) = 3.53$, $p = .066$, partial $\eta^2 = .06$. Specifically, participants in prime subgroup tended to have lower RR during stress battery and at post-stress assessment compared to participants in no prime subgroup. Interestingly, priming did not affect RR in lavender group.

Chromogranin A. RM ANOVA indicated the main effect of aroma, $F(1, 56) = 5.30$, $p = .025$, partial $\eta^2 = .09$. Specifically, people inhaling coconut aroma demonstrated smaller change in CgA levels during stress and post-stress assessments compared to people inhaling water.

Relationships among stress biomarkers and self-reports

Pearson correlation analyses did not indicate any significant correlations between the stress biomarkers or between stress biomarkers and subjective variables, all p's > .10.

Non-significant trends were observed for relationship between RR following stress battery and VAS stress, $r = .18$, $p = .090$, and between RR following stress battery and expected change in stress rating, $r = -.18$, $p = .099$.

Because the groups based on aroma showed diverging patterns on several stress biomarkers, correlational analyses were repeated for each of the aroma groups.

Only in lavender group EEG FA following stress was related to cortisol levels at the same time point, $r = .356$, $p = .05$. Additionally in lavender group, EEG FA

following stress was related to aroma pleasantness rating, r = .426, p =. 02. Further CgA level after stress was related to NEO Neuroticism subscale score, r = .457, p = .01.

No relationships between stress markers appeared for coconut group except for a trend indicating association between CgA level following stress and aroma intensity rating, r = .325, p = .098.

For participants in water group, RR following stress was related to cortisol level, r = -.325, p = .098, VAS stress, r = .388, p = .04, and expected change in stress rating, r = - .425, p = .02 at the same time point. Furthermore, in water group, EEG FA following stress was trending on being associated with aroma pleasantness rating, r = -.361, p = .08 and expected change in stress rating, r = .388, p = .04 at the same time point.

Overall, lack of significant relationships among biomarkers and stress-related measures is likely due to divergent changes in different measures in different groups.

Discussion

The present study investigated the effects of lavender aromatherapy on several physiological markers following stress battery. Additionally, the study also assessed the roles of aroma-mediated and verbally-mediated expectancy in aromatherapy actions.

The results of the study indicated that stress-induced changes in several physiologic measures: EEG FA, RR, and CgA were influenced by aroma

inhalation. Some but not all of the aroma effects were linked to aroma hedonic qualities, and verbally-mediated expectancy might have also influenced some of the observed changes.

Aromatherapy effects on stress biomarkers

We evaluated utility of lavender aromatherapy for stress-reduction, and because of that, evoking stress response was a critical part of the study. The stress battery used in the study was successful. There were significant changes from baseline values following the stress battery in most of the stress biomarkers including salivary cortisol, respiration rate, and chromogranin A. Additionally, there were significant post-stress changes from baseline in several self-report measures including subjective stress, anxiety, and affect measures.

Interestingly, while respiration rate, cortisol, and chromogranin A were affected by stress battery in the whole sample, only RR and CgA showed differences due to aroma group assignment. Also, EEG FA did not show significant change due to stress exposure; however, influences of aroma were observed for different aroma groups after the baseline. Below the results observed in the study are discussed for each physiologic measure separately.

EEG frontal asymmetry. Previous research suggested that exposure to lavender might be associated with greater relative left frontal EEG activation (reflected in lower EEG FA that is associated with increased approach-oriented response and alleviation of depressed mood) (Sanders et al., 2002). However, in our study EEG FA patterns following stress in lavender group (while showing only small changes in EEG FA due to stress) did not differ significantly from the

patterns observed in the placebo groups. Some reasons for such differences in findings between our and previous studies might be due to the fact that in Sanders et al. study (2002) participants did not undergo stress battery prior to aromatherapy, and also the comparison group in the previous study was a different active aroma (rosemary) rather than a control condition.

Interestingly, when the participants were grouped by aroma type (perceptible vs. not perceptible), the main effect of aroma type emerged with people in water group showing a progressive increase in EEG FA (associated with increase in distress and depressed mood) while the groups inhaling perceptible aromas showed lower levels of EEG FA across all time points. Furthermore, analyses using aroma hedonic qualities as covariates suggested that aroma effects on EEG FA might be mediated by aroma intensity and aroma pleasantness.

Such results are in agreement with a previous study that found divergent EEG FA patterns due to exposure to pleasant aroma (vanilla) compared to exposure to neutral (water) or unpleasant (valerian) aromas (Kline et al., 2000). That study also showed EEG FA patterns associated with pleasant aroma similar to the patterns observed in our study due to presence of perceptible (both rated as pleasant) aromas. Contrary to our findings showing increased EEG FA in water group at all time points, Kline et al. (2000) study demonstrated no EEG FA changes after exposure to neutral (water) or unpleasant (valerian) aromas. This difference in findings was likely due to the fact that participants in the study by Kline and colleagues (2000) did not undergo stressful procedures.

Furthermore, according to our results, to produce EEG FA patterns reflective of less distressed state the aroma needs to be perceptible and pleasant but likely does not need to possess any active stress-reducing qualities in order to be effective: using the perceptible placebo aroma in our study possessing a pleasant smell (coconut) resulted in lower EEG FA following stress exposure compared to using non-perceptible placebo (water). Therefore, EEG FA differences between water and perceptible aromas observed after stress exposure are likely arising due to aroma-mediated expectancy.

Respiration rate. Previous studies reported conflicting findings regarding effects of aromatherapy on RR (Buckle, 1993; Saeki, 2000). In our study the results indicated time by aroma interaction for RR with people in water group showing little change in RR compared to people in lavender and coconut groups who showed significant changes in RR following stress battery: in lavender group RR decreased from baseline levels after stress battery and returned to baseline levels at post-stress assessment, and in coconut group RR was slightly increased from baseline levels immediately after stress battery and decreased from baseline levels at post-stress assessment. Buckle and colleagues (Buckle, 1993) reported slower and deeper respiration following massage with essential oils in post-operative patients. Such results are similar to the results in our study observed for participants in lavender group who showed slowed average breathing rate after aroma exposure. Furthermore, aroma effects on RR were not affected by aroma hedonic qualities and aroma-mediated expectancy influence seems unlikely. Interestingly, RR stress-related changes after exposure to

placebo aromas might have been influenced by verbally-mediated expectancy (with those in prime subgroup showing decreased RR after stress exposure compared to increased RR in no prime subgroup participants), but this result was only observed at the trend level and must be interpreted with caution. Interestingly, RR stress-related changes were not influenced by prime in the lavender group. A decrease in RR after exposure to lavender aroma appears to be independent of expectancy effects and is consistent with reported sedative and relaxation properties of lavender essential oil likely attributable to the effects the major component of the lavender essential oil linalool (Cavanagh & Wilkinson, 2002; Holmes et al., 2002; Levenhagen, 2008; Toda & Morimoto, 2008; Wildwood, 1996).

Cortisol. No stress-related changes in salivary cortisol were observed due to aromatherapy even though it has been significantly affected in the whole sample. Previous research found lower cortisol levels in response to lavender compared to rosemary aroma (Atsumi & Tonosaki, 2007). In our study, lavender group had also the smallest increases in salivary cortisol levels after stress battery compared to other groups. However, similar to some studies evaluating cortisol changes following stressor (cold pressor task or mental stress) (Kiecolt-Glaser et al., 2008; Toda & Morimoto, 2008), the changes in cortisol levels between lavender and control aromas in the current study were not significant.

Chromogranin A. The results of this study indicated significant main effect of aroma on CgA levels following stress with people in water group displaying different patterns (greater progressive decrease in CgA levels) from those

observed in people from lavender and coconut group. Furthermore, aroma hedonic qualities might be mediating the observed differences suggesting critical role of aroma-mediated expectancy in the observed patterns. CgA is receiving attention as a potential marker of stress, particularly mental stress. Some previous studies suggest that CgA levels are usually increased immediately following acute stress, and CgA is more suited to assess acute stress than cortisol levels indicating that CgA and cortisol changes represent different systems playing the role during stress response (Nakane et al., 2002; Toda & Morimoto, 2008). In our study we observed a significant decrease in CgA levels; however CgA levels were measured 10 minutes after onset of mental stressor, which occurred after physical and emotional stressors in the stress battery. Therefore, our results indicate later changes in CgA levels that only few previous studies assessed. When CgA levels were measured several minutes after stressors, the initially increased CgA levels due to stress showed a significant decrease in a group inhaling lavender aroma but not in a control (no aroma) group (Toda & Morimoto, 2008). Our findings are similar, but the conclusions about possible mechanisms associated with CgA change are likely different. While Toda (2008) suggests that CgA stress-related changes in lavender group (compared to no aroma group) are likely due to lavender ability to suppress the activity of sympathetic nervous system that CgA change reflects, our results suggest that CgA levels are affected by the presence of aroma in general (at least by presence of lavender and coconut aromas used in our study).

Furthermore, hedonic qualities of aroma such as intensity and pleasantness appear to mediate the aroma effects on CgA levels.

To the best of our knowledge, this study is unique in introducing a control group with inert aroma, which allowed us to assess the role of perceptible smell in aromatherapy actions. Most previous studies utilized no odor control group or a control group with a different essential oil or aroma that possesses different types of properties (e.g. lemon, rosemary, etc.). Our results with comparisons made to both non-perceptible and perceptible placebo aromas point to a significant role of aroma-mediated expectancy and aroma hedonics because more similarities were observed between the patterns for perceptible aromas than between the patterns for placebo aromas.

Interestingly, while group effects were observed for physiological measures, participants in different aroma groups did not differ in patterns of change from baseline to post-stress on measures of subjective stress level, anxiety, and positive and negative affect. The dissociation between the results of objectively and subjectively measured outcomes has been reported before in aromatherapy research (Heuberger, Redhammer, & Buchbauer, 2004). However, it is more common to observe significant subjective changes due to aroma in the absence of objective changes (Diego et al., 1998; Knasko, 1992). Some believe that physiological changes due to aromatherapy might be meaningful only if they correlate with subjective evaluations (Herz, 2009). It is certainly advantageous for aromatherapy recipients to feel subjective relief after aromatherapy, however some research demonstrates that important benefits might still be observed

without subjective awareness of significant relief from aromatherapy. For example, research suggests changes in agitation behaviors, sleep, and cognitive function could be evident without aromatherapy recipients knowledge of aromatherapy or without significant subjective changes in measures of stress, anxiety, or affect (Goel et al., 2005; Holmes et al., 2002).

Overall our findings are consistent with expectancy effects playing a major role in aromatherapy actions. Presence of a pleasant aroma was associated with different patterns of stress-related changes in two physiologic measures compared to the absence of aroma. According to our results lavender is likely to have specific effects beyond those related to expectancy on some measures (e.g. sedative effects observed on slowing of respiration rate); however the measures potentially sensitive to specific lavender effects like respiration rate can be influenced by verbally-mediated expectancy in placebo aromas. Aromatherapy as an intervention has been cautiously received by conventional medicine providers due to lack of rigorous evidence for its effects and little understanding of its mechanism (Lee et al., 2012). Research including our study points to the significant observable physiologic effects associated with aroma presentation. Some of these effects, according to our study, potentially arise from aroma pharmacological effects, but others are likely to be influenced and enhanced by expectancy effects. The evidence is accumulating that the mechanisms underlying aromatherapy action are likely to combine both pharmacological properties of specific aromas and psychological expectancy effects. Maximizing rather than combating participants or patients' expectancy

might be a useful strategy for obtaining optimal health benefits from CAM approaches like aromatherapy because some of the aromatherapy effects arise from non-specific expectancy effects.

Chapter 4: Potential mechanisms of aromatherapy effects on cognitive function following acute stress: contribution of aroma hedonic qualities and expectancy effects

Introduction

Acute stress can affect different cognitive functions (de Kloet, 2000; McEwen & Sapolsky, 1995), with higher-order cognitive functions such as attention, executive function, and working memory being particularly susceptible to the detrimental effects of stress (Arnsten, 2009; McEwen & Sapolsky, 1995; Wolkowitz, Reus, Canick, Levin, & Lupien, 1997). Stress reduction might be a promising strategy for preventing both short- and long-term cognitive impairment (Kremen, Lachman, Pruessner, Sliwinski, & Wilson), and some most popular stress-reducing interventions have roots in the complementary and alternative medicine (CAM) approaches that might provide a safe and cost-effective alternative to conventional medicine approaches (Harris, Cooper, Relton, & Thomas, 2012; Herman et al., 2012). One of the popular CAM approaches, aromatherapy, or therapeutic use of essential oils from plants, has been suggested as a method for stress reduction and cognitive facilitation (Moss et al., 2003; Moss, Hewitt, Moss, & Wesnes, 2008; Motomura et al., 2001; Muzzarelli et al., 2006). Earlier studies have indicated that aromatherapy can affect both stress level and cognitive function (Kline et al., 2000; Komiya et al., 2006; Krebs, 2006; Kuroda et al., 2005; Lis-Balchin, 1997; McCaffrey, 2008; Moss et al., 2003; Moss et al., 2008; Motomura et al., 2001), but more rigorous recent research has not found any reliable objective aromatherapy effects (Howard & Hughes, 2008; Kiecolt-Glaser et al., 2008). To date, the evidence for aromatherapy effectiveness as a method for stress reduction and cognitive facilitation is

equivocal, and understanding of the mechanisms underlying aromatherapy actions is lacking (Herz, 2009; Hirsch, 2001; Hobbs, 1997).

Previous research on the effect of odors of different origins on cognitive function has identified four potential mechanisms that can be used to explain how aromatherapy might work (Jellinek, 1997; Johnson, 2011). The first mechanism is pharmacological and odor-specific: it involves direct impact of volatile compounds on neural activity after activating olfactory receptors. The second mechanism is hedonically-driven, with aromas producing mood changes based on the odor hedonic qualities (such as pleasantness and intensity) following odor exposure with secondary effects on cognition. The third mechanism is purely psychological, with any benefits arising from odor resulting from expectancies or beliefs related to odor qualities. The forth mechanism is contextual/associative, with odors producing specific effect resulting from previous associations of the odor with particular stimulus, mood, or behavior. To the best of our knowledge, only few studies have evaluated potential mechanisms involved in aromatherapy actions (Howard & Hughes, 2008; Kiecolt-Glaser et al., 2008).

This study was designed to assess the relevance of the first 3 mechanisms listed above for the effects of stress-reducing aromatherapy on cognitive function. To evaluate any potential pharmacological effects from aroma inhalation, we used lavender as an experimental stress-reducing aroma. Lavender is one of the most researched essential oils shown effective for stress reduction and cognitive enhancement in the face of stress (Field et al., 2008; Kim et al., 2007; Kline et al., 2000; Sanders et al., 2002; Toda & Morimoto, 2008).

Lavender actions were compared against the actions of two placebo aromas. One of the placebo aromas was an odorless and inert substance (water) intended to serve as a general control. The other placebo aroma (coconut) was intended to produce a pleasant smell without any known direct effects on stress or cognition (Wildwood, 1996). The coconut aroma was used to investigate how aroma hedonic qualities might contribute to aromatherapy effects on cognitive function. Finally to assess the role of expectancies and beliefs about the aroma in facilitating cognitive performance we manipulated participants' expectations about the aromas they inhaled by giving half of the people in each aroma group a prime suggesting that the aroma to which they are exposed is a powerful stress-reducing agent. The second half of the people in each group received a neutral statement about the assigned aroma (no prime).

The main hypothesis was that lavender aromatherapy would augment cognitive function after stressful experience to a greater extent than placebos but expectancy would play an important role in the effects of aromatherapy.

Methods

Participants

Ninety-two healthy adults (Mean age = 58.0, 79.3% females) were recruited from the community as described in Chapter 2.

Study groups, randomization process, and blinding

Prior to the study visit each participant was randomized to a group based on aroma type (lavender, coconut, or water) and was also assigned to a subgroup

based on a type of prime they received during the visit (prime or no prime) as described in Chapter 2.

Laboratory visit

Detailed visit information has been described in Chapter 2.

Briefly, participants completed the visit that included a baseline assessment, a stress battery, and a post-stress assessment. During the baseline participants completed a self-report measures packet and a cognitive battery (Diagram 2). After the baseline each participant received their assigned aroma and a card with or without the prime. After completing the baseline assessment participants began inhaling their assigned aroma. Five minutes after initial exposure, participants started a stress battery followed by the post-stress assessment similar to the baseline assessment. Subjective stress ratings were taken during baseline and post-stress assessments as well as after each stressor in the stress battery. The laboratory visit took about 3-4 hours. After completing the laboratory visit, the non-blinded research assistant debriefed the participants about the purpose of the study, and participants received a modest reimbursement for their time and effort.

Stress battery

The stress battery consisted of emotional, physical, and mental stressor to elicit stress response in study participants and was described in greater detail previously in Chapter 2.

Self-report measures. The purpose of self-report instruments was to assess variables that might be affecting aromatherapy actions or influenced by

aromatherapy actions. The detailed information about the self-report measures have been provided in Chapter 2. Briefly, participants provided background information about their age, race, ethnicity, and education level. They also reported previous aromatherapy use using a custom questionnaire, rated their baseline stress in the previous week using Perceived Stress Scale (PSS) (Cohen et al., 1983), and provided information about their personality traits using Neuroticism-Extroversion-Openness (NEO) Five-Factor Inventory (Costa et al., 1992) designed to give quick, reliable, and valid measures of the five domains of adult personality.

Expectancy of aromatherapy effect was assessed with a visual analog scale (VAS) ranging from 0 to 100mm. Participants put a mark on a line indicating the expected effect of aromatherapy on stress level (ranging from decreased stress to increased stress) and the overall effect they expected from aromatherapy (ranging from overall negative effect to overall positive effect).

Stress-related measures. These measures have been described in Chapter 2 and included subjective stress as well as measures of anxiety and positive and negative affect.

Measures of aroma hedonic qualities. At the end of aromatherapy exposure participants used VAS scales to rate the pleasantness (ranging from 0 = extremely unpleasant to 100 = extremely pleasant), and intensity (ranging from 0 = barely noticeable to 100 = extremely intense) of their assigned aroma.

Participants were also asked whether they could perceive the aroma and recognize it.

Cognitive measures

The cognitive tests have chosen based on their relatively short duration, and because of previous findings suggesting their sensitivity to stress or expectancy effects (Oken et al., 2008; Wechsler, 1997). Alternate versions of the tests were used when appropriate during different assessment periods.

The Telephone Interview for Cognitive Status (TICS) (Welsh et al., 1993) was used to assess potential participants' cognitive function and screen for any cognitive impairment prior to study enrollment.

The Stroop Color-Word Test Golden version, or Golden Stroop (GS), (Golden, 1978), a measure of selective attention and cognitive flexibility was used to assess executive functions. Only word reading and color naming conditions were used. The interference score calculated as a difference between word reading and color naming conditions was used as the outcome variable for subsequent data analyses.

The Digit Span Backward (DSB) from the WAIS-III (Wechsler, 1997) was used to test working memory. Maximum length of correctly recalled series was used as the outcome measure for this test.

The Letter-Number Sequencing (LNS) from the WAIS-III (Wechsler, 1997) (Herz, 2009) was used to assess attention and working memory. The summary score for this subtest will be used as an outcome variable for the statistical analyses.

Simple reaction time (SRT) test was used to evaluate processing speed as previously described (Oken et al., 2008). The test was presented on the computer with participants pushing the button as quickly as possible when a circle appeared on the screen. Twenty-four stimuli were presented, and the median reaction time (RT) for correct responses was used as a variable for the statistical analyses.

Statistical analyses

General statistical procedures for evaluating background differences between study groups, check of stress battery effectiveness and assessment of hedonic qualities of the aromas were presented in Chapter 2.

Primary analyses

Cognitive performance. A variable representing a change in percentage from baseline to post-stress assessment was calculated and as a dependent variable in the data analyses for each cognitive test. A 3 (aroma groups: lavender, coconut, water) by 2 (prime groups: prime, no prime) Analysis of Covariance (ANCOVA), with years of education as a covariate, was performed for each cognitive variable to assess whether changes in cognitive performance differed between groups based on aroma and groups based on prime. When a significant group difference was observed for aroma type, planned comparisons using simple contrasts evaluating difference between the experimental (lavender) group and each placebo group were performed.

To control for increased probability of Type I error due to multiple comparisons, a False Discovery Rate approach (Benjamini & Hochberg, 1995)

was used to determine statistical significance of the results. According to this approach, with four outcome measures used for primary analyses, the smallest p value obtained in the primary analyses set must be lower than .0125 to be considered significant, the next in magnitude p value must be lower than .025 to be considered significant, the third in magnitude p value must be lower than .0375 to be considered significant, and finally the largest p value to be considered significant must be lower than .05.

Secondary analyses

Secondary exploratory analyses were conducted to assess the impact of aroma hedonic characteristics and different types of expectancy on cognitive performance.

Aroma hedonic qualities and group differences in subjective measures. ANOVAs were used to evaluate group ratings of aroma hedonic qualities as well as changes in self-reported measures of stress, affect, and anxiety. For the self-reported measures of stress, affect, and anxiety, a score obtained during the post-stress assessment was converted to the percent of baseline score on the same instrument, and the percent of baseline variable was used in the analyses.

Correlations among cognitive and stress-and-aroma-related variables. Bivariate Pearson correlations were performed to evaluate relationships among cognitive performance variables and variables associated with aroma hedonic qualities, expectancy, and stress.

Simple mediation analyses. If the ANCOVA for cognitive test was significant for group, a simple mediation analysis was done for that test. Potential mediators

tested consisted of the variables that were related to cognitive performance and the measures that were affected by group. The potential mediators were evaluated in a regression by entering group along with education covariate, and the single mediator considered. The variable was considered a potential mediator if its inclusion into the regression equation cause the group effect to become non-significant.

Results

Baseline Characteristics. The details of most of baseline characteristics based on aroma and prime groups have been previously presented elsewhere (Table 1 of Chapter 2). Briefly, the groups based on aroma and prime were well-matched on major background variables including age, gender, perceived stress, and personality traits (all p's > .05). However, the participants allocated to the no prime group showed a trend for more years of education compared to the prime group (p = .06). Though the mean group difference was only about one year, and participants from different groups did not differ on their performance on all cognitive tests at baseline (all p's > .05), the length of education was added as a covariate to the analyses of cognitive performance. The baseline performance on cognitive tasks was similar for participants in all groups and subgroups (Table 3).

Table 3. Baseline cognitive performance

Mean (SD)	Aroma groups			p	Prime groups		p
	Lavender n = 31	Coconut n = 31	Water n = 30		Prime n = 47	No prime n = 45	
TICS score	39.0 (.7)	38.5 (.7)	37.2 (.7)	.55	38.1 (.5)	38.3 (.6)	.82
SRT (Median RT, ms)	275.3 (12.2)	242.8 (12.4)	282.2 (12.6)	.06	267.7 (10.0)	265.8 (10.2)	.89
DSB score	8.5 (.4)	8.4 (.4)	8.3 (.4)	.95	8.3 (.3)	8.4 (.3)	.82
LNS score	20.2 (.5)	20.2 (.5)	19.5 (.5)	.58	20.1 (.4)	19.8 (.4)	.56
GS interference	59.4 (2.0)	54.5 (2.0)	54.6 (2.0)	.14	55.4 (1.6)	57.0 (1.6)	.50

Abbreviations: TICS = Telephone Interview for Cognitive Status, SRT = Simple reaction time task, DSB = Digit Span Backward Task, LNS = Letter Number Sequencing task, GS = Golden Stroop task.

Primary analyses: aromatherapy and expectancy effects on cognitive performance

Preliminary data screening of the cognitive performance measures was done to assess whether the assumptions for ANCOVA were seriously violated. Exploratory analyses indicated the presence of a significant outlier for the SRT. The outlier was likely caused by the program error and was excluded from any further analyses. Additionally, data from 1 subject for the SRT task have been lost due to a computer problem during the assessment. Due to these issues, the sample size for the SRT task was reduced to 90. Further examination of the data showed that data for SRT and LNS had slightly skewed distributions; however no transformation was applied to either measure. Type III sum of squares were used to correct for the slightly unequal cell numbers. The Levene tests indicated no significant violations of homogeneity of variance assumption for any cognitive test measures.

Figures 6a and 6b contain group patterns in percent change from baseline for cognitive performance on different cognitive tasks. Table 4 shows the results of the statistical analyses for the main effects and interactions for each of the cognitive measure.

Table 4. The results of the Analyses of covariance for cognitive measures with years of education used as covariate

Source	aroma df = 2, 85			prime df = 1, 85			aroma x prime df = 2, 85		
	F	$P\eta^2$	p	F	$P\eta^2$	p	F	$P\eta^2$	p
SRT*	1.56	.04	.22	**8.32****	**.09**	**.01**	1.98	.05	.15
DSB	**4.48****	**.10**	**.01**	.33	.00	.57	.14	.00	.87
LNS	0.37	.01	.69	.003	.00	.95	.48	.01	.52
GS	1.18	.03	.31	2.98	.03	.09	**3.51**^{T}	**.08**	**.03**

Abbreviations: SRT = Simple reaction time task, DSB = Digit Span Backward Task, LNS = Letter Number Sequencing task, GS = Golden Stroop task, $P\eta^2$ = Partial η^2.

*Degrees of freedom for the SRT: for aroma and aroma x prime, df = 2, 83 and for prime, df = 1, 83

^{T} P< .10< .05, ** p ≤ .01.

Figure 6 Legend

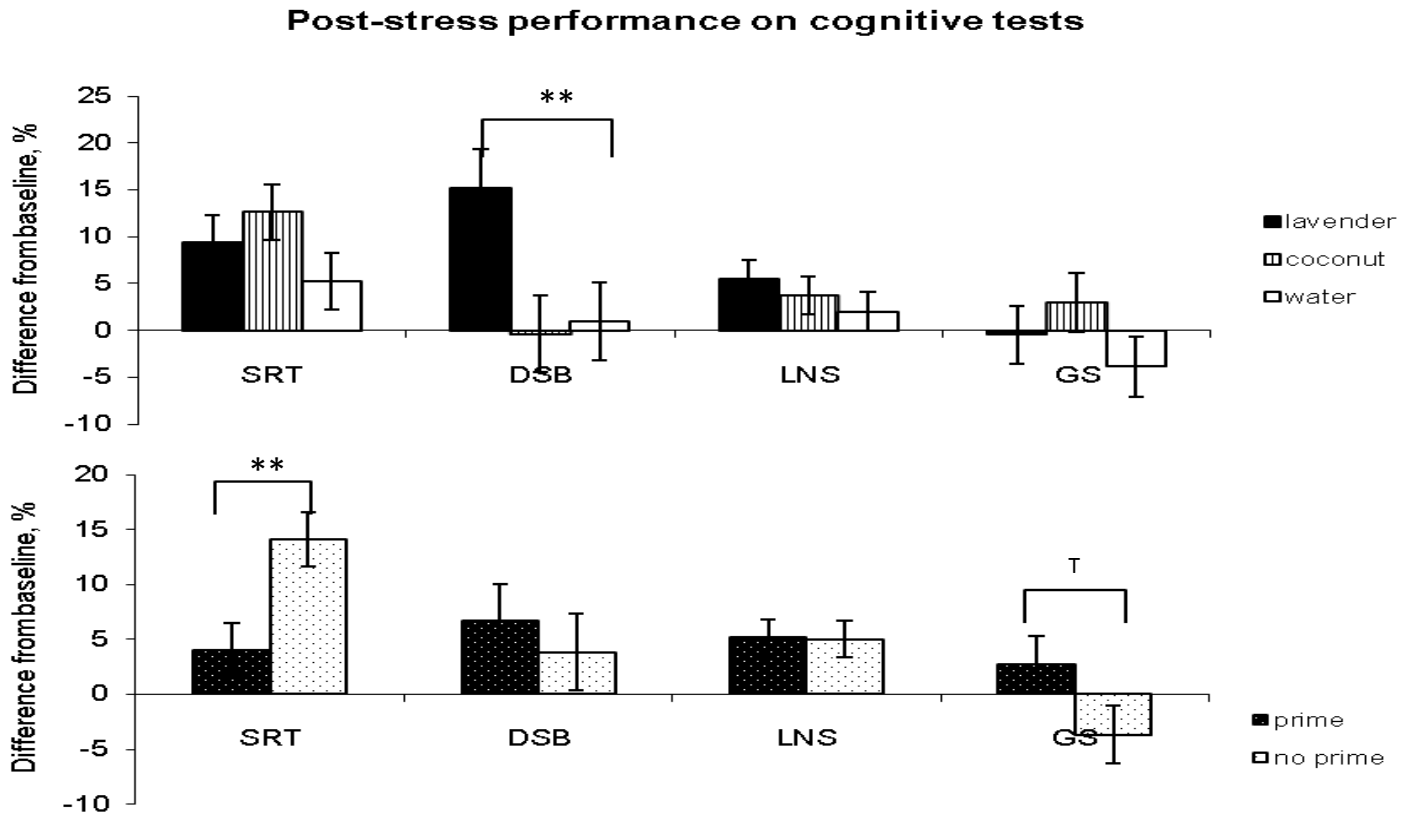
Figure 6. Post-stress performance on cognitive tasks for the aroma groups (Panel A) and prime subgroups (Panel B). The data is presented as mean percent difference from baseline performance with the untransformed mean values presented for easier interpretation (Box-Cox transforms were applied during study analyses). Error bars represent standard errors of the mean.

A. The only significant difference in cognitive performance between aroma groups was a post-stress increase in DSB working memory task score in lavender group compared to the placebo groups.

B. For the effects of prime subgroup, there was a smaller increase in median reaction time on the SRT processing speed task in the primed subgroups regardless of aroma assignment compared to a change in non-prime subgroups. Additionally, there was a trend for non-primed subgroups to display less color-word interference compared to primed subgroups.

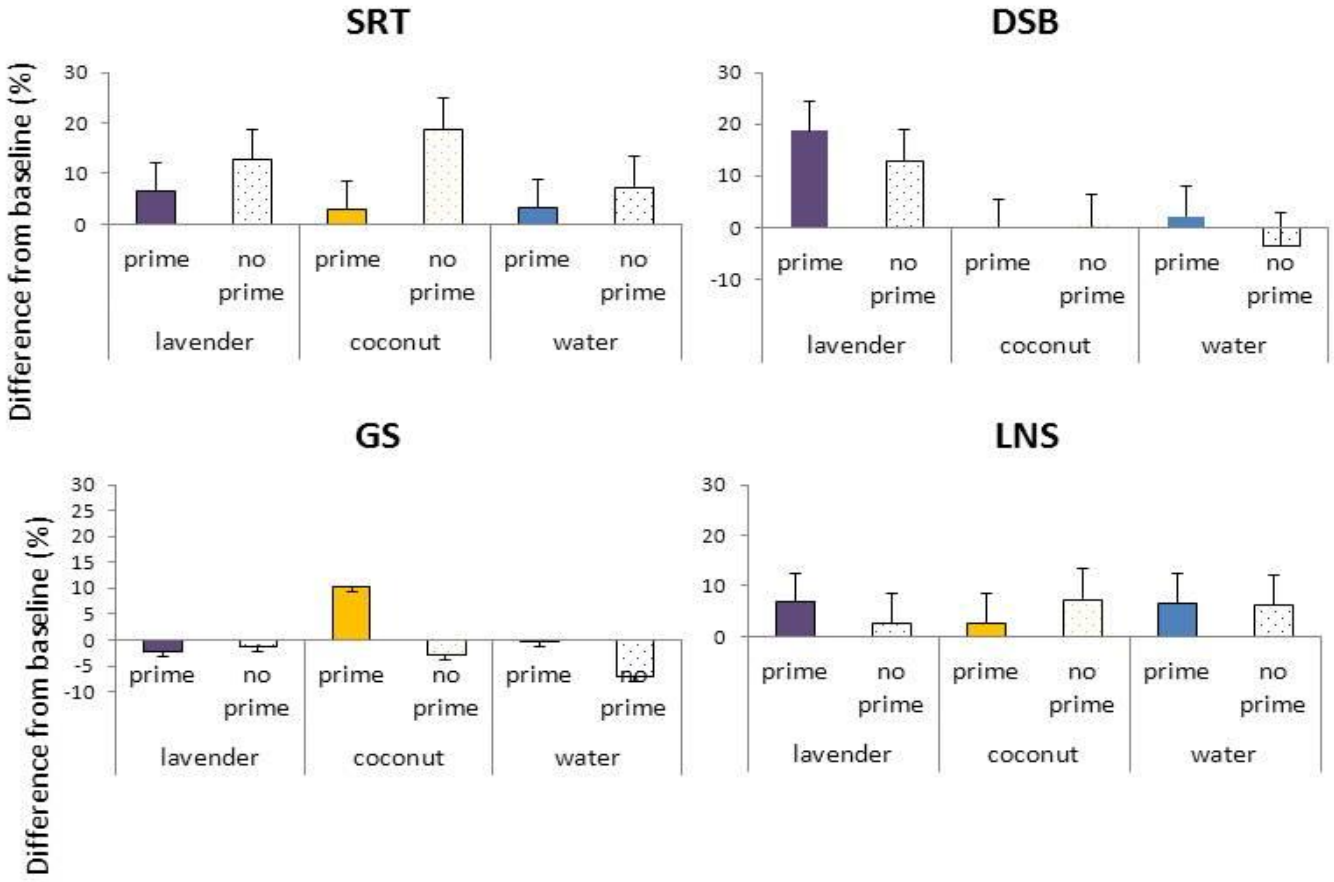
Figure 6b. Post-stress performance on cognitive tasks for participants exposed to different aroma groups and prime subgroups. The data are presented as mean percent difference from baseline performance with the untransformed mean values presented for easier interpretation

Figure 6a. Post-stress performance on cognitive tasks for aroma groups (Panel A) and prime subgroups (Panel B).



Abbreviations: SRT = Simple Reaction Time Task, DSB = Digit Span Backward Task, GS = Golden Stroop, LNS = Letter Number Sequencing Task.
** $p \leq .01$, T $.10 > p > .05$

Figure 6b. Post-stress performance on cognitive tasks for participants exposed to different aroma groups and prime subgroups.



Abbreviations: SRT = Simple reaction time task, DSB = Digit Span Backward Task, GS = Golden Stroop task, LNS = Letter Number Sequencing task.

Aroma effects. The percent change from baseline in cognitive performance on the SRT task, LNS task, and GS task did not significantly differ between the groups exposed to different aromas, all p's > .10. However, the groups exposed to different aromas showed a difference in the percent change from baseline on the DSB task, $F(2, 85) = 4.48$, $p = .014$, partial $\eta^2 = .095$. Simple contrasts performed to compare specific group differences revealed that participants in the lavender group demonstrated 15% increase in the DSB score from baseline performance at the post-stress assessment which translates into an average of one point gain in the score obtained at the post-stress assessment. The improvement in the DSB performance observed in lavender group was in contrast to the lack of change in the DSB performance of both coconut group participants with an average of 0.4% decrease in the DSB score from baseline, $p = .008$ and water group participants with an average of 0.9% increase in DSB score from baseline, $p = .016$.

Expectancy (prime) effects. The percent change from baseline in cognitive performance on the DSB and LNS tasks was similar for the participants regardless of the type of prime they received, all p's > .10. A non-significant trend for the prime effect appeared for the percent change in the GS interference from baseline, $F(1, 85) = 2.98$, $p = .088$, partial $\eta^2 = .034$ with participants receiving no prime displaying a decrease in interference during post-stress assessment compared to their baseline performance, and participants receiving a prime displaying an increase in interference during post-stress assessment compared to their baseline performance. The only significant difference due to prime effect

appeared for performance on the SRT task, F (1, 83) = 8.32, p = .005, partial η^2 = .091. Specifically, during the task those who received a prime that they were inhaling a powerful stress-reducing aroma showed a significantly smaller (on average about 4 % or 8 ms) increase in reaction times compared to their baseline performance than those who did not receive a prime with an average 14% (or about 35 ms) increase in reaction time from their baseline performance. Thus, while all participants showed some slowing on SRT task at the post-stress assessment compared to their baseline performance, those receiving a prime displayed less slowing than those not receiving a prime.

Aroma by prime interactions. No significant interactions were observed for the percent change from baseline in cognitive performance on the SRT, DSB, and LNS tasks, all p's > .10. For the GS interference, the ANCOVA indicated an aroma by prime interaction that was at a trend level after adjusting for multiple comparisons, F (2, 85) = 3.15, p = .034, partial η^2 = .076. Upon closer examination, participants in groups receiving placebo aromas and no prime had decreased interference on Stroop task at post-stress assessment compared to their baseline performance while for the lavender group this was reversed: those in lavender group receiving a prime had a decrease in their interference score during post-stress assessment compared to baseline performance and those receiving no prime displayed increase in interference during the post-stress assessment.

Secondary analyses: exploring the role of other factors

The role of aroma hedonic qualities. Previous research (Herz, 2009) identified a number of characteristics that might influence aroma effects. Perception of the aroma was evaluated in this study while the group differences in aroma intensity, and aroma pleasantness were reported previously (Chapter 2). The results for the aroma perception (summarized in Figure 7) indicated that the patterns of aroma perception differed between the groups, Pearson X^2 = 35.02, $p < .001$. In the lavender group, 29 (94%) participants indicated that they perceived the aroma, with one person (3%) unsure about whether or not she could perceive the aroma and one (3%) unable to perceive the aroma. The participants guessed the aroma being lavender in 52% cases, and other guesses included “herbal”, “peppermint”, “roman chamomile”, “roses”, “rosemary”, “musk”, and “something in nature”. In the coconut group, 13 (43%) participants could perceive the aroma, 11 (37%) were unsure about their ability to perceive the aroma, and 6 (20%) were unable to perceive the aroma. For those who perceived the aroma, the guesses varied between “nut”, “cinnamon”, “cookie”, cotton candy”, gingerbread”, “vanilla”, “grass”, “baby wipes”, and “tobacco”. In water group 9 (30%) participants indicated that they could perceive the aroma, 6 (20%) were unsure about their ability to perceive the aroma, and 15 (50%) were unable to perceive the aroma. For those who perceived the aroma, the guesses about what the aroma was varied between “”flower/floral”, fresh laundry”, “linen”.

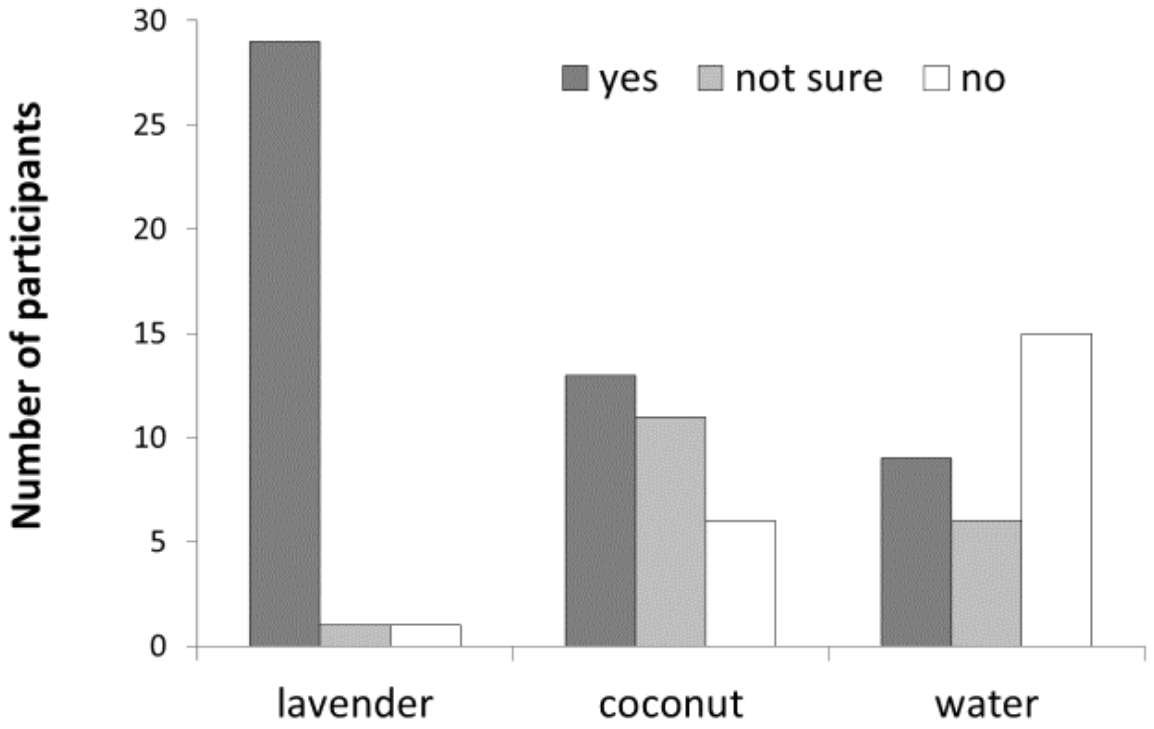
Furthermore, as previously reported in Chapter 2 participants in different aroma groups had significantly different ratings for the intensity and pleasantness of their assigned aroma.

The results of analyses assessing aroma hedonics suggest that lavender aroma was the most recognizable and intense aroma compared to both water and coconut aromas that were both less recognizable and more similar on the intensity ratings. Thus, the attempt to match lavender and coconut on intensity failed. However, the lavender and coconut groups had similar ratings for pleasantness of their aroma suggesting that matching on that variable was more successful. There were no significant prime by aroma interactions for these variables.

Figure 7. Perception of the assigned aroma between different aroma groups.

Figure 7 Legend. The bars represent number of participants in different categories of aroma perception (yes = perceived the aroma, not sure = unsure whether the aroma was perceived, no = did not perceive the aroma). There was a significant group difference for the patterns of perception, Pearson $X^2 = 35.02$, $p < .001$.

Figure 7. Perception of the assigned aroma between different aroma groups.



Group differences in stress-related variables. Previous research indicated that some of the effects on cognitive function attributed to aroma might be due to changes in alertness, stress level, and affect (Johnson, 2011). To test whether changes in affect and stress level might be important for effects attributed to aroma, the aroma groups were compared on these variables.

There were no significant effects of aroma on percent changes from baseline on subjective measures of positive and negative affect or anxiety, all p's > .10.

Alertness was not assessed with a specific scale but approximated using an "alert" item from the PANAS. There was a significant effect of an aroma, X^2 = 6.73, p = .034. Specifically, those in the lavender group reported decreased alertness during post-stress assessment compared to those in the water group, p = .029, with no other group differences noted for this measure, all other p's > .10.

The aroma groups were also compared on subjective stress rating following the stress battery. There was a non-significant trend, X^2 = 4.81, p = .09 for the effect of the aroma on stress level percent change from baseline, with participants in the lavender group reporting lower subjective stress.

The role of expectancy. The groups provided similar ratings of expected aromatherapy effectiveness to reduce stress and expected change in stress level after the aromatherapy (all p's > .05).

To test whether there was a specific effect of expectancy in the absence of any effect potentially associated with stress-reducing properties of aroma, the analyses were conducted using only two placebo groups, water and coconut.

To assess the role of expectancy due to the presence of a pleasant aroma, the placebo groups were compared on cognitive changes after stress battery using MANCOVA with four cognitive measures, years of education as a covariate, and aroma (water vs. coconut) as a between-group factor. To make sure the difference was due to aroma perception, the analyses were repeated with all group participants and then with participants who could not perceive the assigned aroma in the coconut group excluded. Both analyses provided similar results and indicated no significant aroma effect when placebo groups were compared, Wilks' $\Lambda = .93$, $F(1, 49) = .90$, $p = .47$, partial $\eta^2 = .073$ suggesting that the presence of perceptible placebo coconut aroma was not sufficient to produce a significant change in cognitive performance compared to water.

To assess the role of verbally-mediated expectancy enhanced by using a prime, the placebo groups were compared on cognitive changes after stress battery using MANCOVA with four cognitive measures, years of education as a covariate, and prime (prime vs. no prime) as a between-group factor. The analyses indicated a significant overall effect of prime when only placebo groups were compared, Wilks' $\Lambda = .82$, $F(1, 56) = 3.00$, $p = .026$, partial $\eta^2 = .19$. Specifically, compared to those receiving no prime, participants receiving a prime showed less slowing on the SRT task, $F(1, 56) = 7.01$, $p = .01$, partial $\eta^2 = .11$ but an increased interference on the Stroop task, $F(1, 56) = 4.64$, $p = .035$,

partial η^2 = .08. The change in Stroop interference was driven by an increased reading speed in prime group, p = .038, but decreased speed for naming colors, p = .035 compared to the no prime group.

Relationships among cognitive measures and stress-related and hedonic variables. Bivariate Pearson correlations were performed for the variables that could potentially affect the aroma and prime effects on cognitive performance. Significant correlations were observed between percent change from baseline performance on the DSB and aroma intensity rating, r = .27, p = .01 and between percent change from baseline performance on the DSB and subjective stress rating at post-stress assessment, r = -.33, p = .002. There were a non-significant trend for percent change from baseline performance on the DSB to be positively related to aroma pleasantness rating, r = .20, p = .08 and for percent change from baseline performance on the SRT to be positively related to expected change in stress rating, r = .18, p = .09 Table 5 has more information about the correlation coefficients.

Table 5. Zero-order bivariate correlations between cognitive and expectancy and stress-related variables

Variable	1	2	3	4	5	6	7	8	9	10
1. SRT (% change)	1	-.147	-.014	-.063	.183^{T}	-.053	-.063	-.025	-.089	-.004
2. DNB score (% change)		1	-.075	.010	-.162	-.080	.267*	.196^{T}	.012	-.331**
3. LNS score (% change)			1	.233*	-.051	.042	.130	-.051	.167	-.096
4. GS interference (% change)				1	-.021	.050	.028	.026	.033	-.107
5. Expectancy of stress change					1	-.402**	-.009	-.083	-.079	.152
6. Expectancy of aroma effect						1	-.086	.085	.264*	.127
7. Aroma intensity rating							1	.543**	-.113	-.052
8. Aroma pleasantness rating								1	-.049	-.156
9. PANAS alertness rating									1	.053
10. Stress level at post-stress										1

Abbreviations: SRT = Simple reaction time task, DSB = Digit Span Backward Task, LNS = Letter Number Sequencing task, GS = Golden Stroop task, PANAS = Positive And Negative Affect Schedule.

^{T} .10 > p > .05, * p < .05, ** p < .01.

To further evaluate the role of the factors that potentially contributed to the change in post-stress performance on DSB and SRT tasks the simple mediation analyses were conducted. Each variable that related to cognitive performance on the DSB and SRT tasks or was different between the study groups was entered individually as a factor into the regression equation containing cognitive variable as a dependent variable and a group variable as a predictor. As noted in Table 6, aroma intensity rating was the only variable which inclusion caused the p value for the effect of aroma group to become non-significant suggesting that aroma intensity rating might be mediating relationships between change in the DSB task baseline performance and aroma group. None of the other potential mediators tested appeared to significantly affect the relationship between the performance on a cognitive test and the group.

Table 6. Potential mediation of the aroma effect on DSB and the prime effect on the SRT

Possible mediator	Aroma effect on the DSB performance [a]	Prime effect on the SRT performance [b]
Post-stress alertness level	-.289 (.009)	N/A
Post-stress stress level	-.231 (.025)	N/A
Aroma intensity rating	**.-222 (.071)**	N/A
Aroma pleasantness rating	.-253 (.033)	N/A
Expected stress change	N/A	.310 (.004)
Expected aroma effectiveness	N/A	.329 (.003)

Abbreviations: DSB = Digit Span Backward Task, SRT = Simple reaction time task

a. effect on cognitive function without mediator : standardized beta = -. 255, p = .014.

b. effect on cognitive function without mediator: standardized beta = .304, p = .005.

Aroma intensity rating was the only variable that significantly affected the p value of the aroma effect on the DSB performance (bold font to emphasize significant change).

Discussion

Our study indicated that cognitive performance following acute stress was differentially affected by specific aromas and enhanced by suggestion of aroma effectiveness for stress reduction. Here we discuss how our results can be interpreted in the light of the four potential mechanisms involved in cognitive facilitation by aromas proposed by Jellinek (1997).

Evidence for pharmacological mechanism involved in lavender effect on cognitive performance

The lavender aromatherapy was beneficial for performance on one of the working memory tests used in our study, Digit Span Backward, with participants in the lavender group showing an average of one point improvement on their DSB score at post-stress assessment from their baseline score on this test. The participants in the placebo groups showed virtually no change in performance on this test post-stress. These findings are in accord with previous accounts of aromatherapy being capable of inducing changes in cognition (Diego et al., 1998). Studies assessing lavender aromatherapy specifically also reported significant cognitive changes such as more accurate performance on math computations following lavender aromatherapy exposure compared to a group exposed to rosemary aromatherapy (Moss et al., 2003). Lavender essential oil has been advertised for its anxiolytic and relaxation effects and ability to reduce stress (Bradley, Starkey, Brown, & Lea, 2007; Field et al., 2008; Levenhagen, 2008). The link between stress and cognitive performance has been supported in several studies, and is especially relevant for the higher-order cognitive functions

due to detrimental effects of acute stress on prefrontal cortex (Arnsten, 2009; Bremner, 1999; Lupien et al., 2007). Therefore, the facilitative effect of lavender on working memory, the function most likely affected in a stressful situation (Arnsten, 2009; Schoofs, Wolf, & Smeets, 2009), is also consistent with lavender‘s putative tress-reducing properties. Previous studies demonstrated lavender’s effects on both subjective and objective measures of stress and anxiety (Holmes et al., 2002; Lehrner et al., 2005; Toda & Morimoto, 2008; Woelk & Schlafke). Our results, however, did not indicate a significant reduction in subjective stress in the lavender group compared to placebo groups. People in the lavender group did rate their stress level after the stress battery lower than people in other groups, but the difference was at a trend level and did not reach the conventional significance level. Also, though subjective stress level after stress exposure was significantly related to the DSB task performance, stress was not mediating the relationship between aroma group and the DSB task performance in our sample.

Evidence for hedonically-driven mechanism

Contrary to the findings observed in this experiment, some previous studies indicated that exposure to lavender during cognitive tasks does not influence cognitive function or results impaired performance on tasks assessing memory and attention compared to controls (Moss et al., 2003). In Moss et al. study aromas were intended to be below detection threshold, and participants were not aware about using aromas in the study. Research suggests that perception of the aroma and hedonic characteristics of aroma play an important role in the effects

that the aroma produces (Herz, 2009; Johnson, 2011). In the current experiment not only most of the participants in the lavender group could perceive the aroma, but over a half of them could also correctly identify it.

Furthermore, in our study hedonic qualities of lavender aroma were distinct from those of the placebo aromas. For example, lavender aroma had a greater pleasantness rating compared to water aroma. Additionally, lavender aroma was rated differently from both water and coconut aromas on aroma intensity. Moreover, the aroma intensity rating in our study appeared as a potential mediator of the observed relationship between performance on the DSB task and aroma group (Table 6). These findings are indicative of the critical role of hedonically-driven odor effects for the aroma effects on cognition. However, the explanation for the hedonically-driven mechanism proposed by Jellinek (1997) suggests that the effects on cognition are secondary to an increase in mood-related measures. In our study no differences between groups exposed to different aromas were found for state anxiety as well as positive and negative affect measures.

Furthermore, the comparison between the two placebo aromas used in this study, one with the undetectable and the other with detectable aroma (that also had a pleasantness rating similar to lavender) yielded no group differences in cognitive performance. The significant effects of lavender along with the lack of differences between the placebo aromas with different hedonic qualities might have different interpretations. One possible interpretation is that lavender possesses some pharmacological properties that influence cognitive

performance on the DSB task; the evidence consistent with this (pharmacological) mechanism has been reviewed above. The second interpretation might be that similar pleasantness rating might be insufficient to produce similar effects on cognition when the intensity of the aromas and the ability to perceive them differ. Intensity or salience of the aroma can influence arousal or alertness level and impact cognitive performance. Alertness is a basic aspect of attention affecting most cognitive processes and can be viewed as related to and influencing the speed of information processing (Ilmberger et al., 2001). Contrary to the expected influence of aroma intensity on alertness, participants in the lavender group rated their alertness at post-stress assessment similar to that in the coconut group but lower than in the water group, which is consistent with sedative and relaxing properties of lavender lending more weight to the pharmacological explanation of lavender aromatherapy actions. Further, the groups inhaling different aromas were similar on their performance on SRT task post-stress, a task assessing speed of information processing. Curiously, although performance on the SRT task was not affected by aroma type, it was significantly influenced by prime type.

Evidence for psychological mechanism: role of expectancy

Expectancy effects have been previously shown to play an important role in facilitating cognitive performance and aromatherapy actions (Campenni et al., 2004; Howard & Hughes, 2008; Oken, 2008; Oken et al., 2008). In our study a verbal suggestion of stress reduction due to aroma was beneficial for the performance on the SRT processing speed task regardless of the aroma type

that participants inhaled: those receiving a prime demonstrated less slowing on the SRT task during post-stress assessment compare to the peers receiving no prime. Though short of reaching conventional significance level, there was also a positive relationship between performance improvement on the SRT task and the baseline rating of the expected change in stress level due to aromatherapy suggesting that both general expectations relating to aromatherapy as a therapeutic approach and specific expectation of improvement enhanced by a verbal suggestion of aroma efficacy for stress reduction might play a role in facilitating cognitive changes. Furthermore, our results indicated prime effect for the Golden Stroop interference score when only placebo aromas were compared: those who received a prime had an increased interference on GS task compared to those who received no prime. The change in GS interference was driven by an increased reading speed in the prime group, $p = .038$ compared to the no prime group. Therefore, it appears that the verbal suggestion of the stress-reducing effect had similar influence on both tasks where prime effect was significant: the speed of responding was increased for both reaction times on the SRT and reading on the word reading portion of the GS.

Evidence for contextual/associative mechanism of action

Though this mechanism was not designed to be evaluated in our study some of the results indicate that it might also contribute to the aromatherapy effects. Lavender aroma, the most recognized aroma in the study with over 50% of participants correctly identifying it, was associated with improved performance on the DSB task, but the placebo aromas that were not as recognizable were not

associated with change in performance on that or any other cognitive tasks. Lavender-scented product are widely used in massage oils, spa treatments, and other products intended to make environment peasant and peaceful. It is tempting to speculate that due to its popularity in environments intended for enjoyment and relaxation, for many people lavender aroma might have been associated with memories of pleasant experiences or relaxation.

Overall, according to our data, no single mechanism could fully account for the results observed in this study. Interestingly, aroma and prime manipulations affected separate aspects of cognitive performance in our study suggesting that different mechanisms might account for the observed aromatherapy actions. Our results lend some support to all assessed mechanisms likely involved in odor facilitation of cognition – pharmacological, hedonically-driven, contextual-associative, and purely psychological arising from expectancy alone. Our data suggests that changes in working memory assessed by the DSB were likely arising from either the pharmacological effects attributable to lavender or lavender's hedonic qualities, or the combination of those. The contextual/associative explanation of the lavender action also cannot be ignored due to popularity and recognizability of this aroma. Changes in reaction time and GS performance were clearly associated with the verbally-mediated expectancy, which robustly affected the speed of processing independent of aroma qualities, either hedonic or pharmacological. Choosing the right outcome measures might be critical to pinpoint specific effects associated with aroma: if in our study we did not assess working memory we could not observe any significant effects of

aroma that appeared to be specific to that measure. Including a more extended battery of outcome measures might be beneficial for evaluating different effects of aromatherapy on different aspects of cognition in future studies.

Conclusions

Our study indicated that cognitive performance following acute stress was facilitated by aromatherapy exposure: exposure to lavender aroma but not placebo aromas was associated with improved performance on a working memory task while a verbal suggestion of aroma effectiveness for stress reduction regardless of aroma type was associated with facilitation on a speed of processing task. Mechanisms underlying observed effects of aromatherapy on cognition were different for the different tasks. Thus, overall consequences of aromatherapy exposure are produced by different mechanisms, each of which could be manipulated to produce the optimal effect on functioning.

Chapter 5: Effects of stress-reducing aromatherapy on go-nogo task following acute stress: an evoked related potentials (ERP) study

Introduction

Aromatherapy, the use of essential plant-based oils for therapeutic purposes, is an ancient practice (Lis-Balchin, 1997) that has been growing in popularity in the Western cultures in the last decades (Lee et al., 2012). A growing number of research studies assessing aromatherapy effects suggested that aromatherapy is effective for a variety of conditions depending on the essential oil used (Halcon, 2002; Halm, 2008; Herz, 2009; Holmes et al., 2002; Hongratanaworakit & Buchbauer, 2006) with the strongest evidence indicating aromatherapy effects for relaxation and stress reduction (Rimmer, 1998; Sayette & Parrott, 1999; Seo, 2009; Setzer, 2009; Shiina et al., 2008; Wilkinson et al., 2007). However, a recent review of systematic reviews evaluating available evidence for aromatherapy efficacy in healthcare concluded that the currently existing evidence is not convincing for aromatherapy effectiveness for any condition (Lee et al., 2012). The main reason for the lack of conclusive evidence included poor quality of primary research studies assessing aromatherapy effects: small sample size, lack of adequate control groups, and paucity of objective measures in some cases were all cited (Herz, 2009; Lee et al., 2012). The dearth of convincing evidence for aromatherapy actions is also linked to the lack of understanding for the mechanism of aromatherapy actions. Suggestions for future research evaluating aromatherapy effects included designing more rigorous studies (Lee et al., 2012).

This study was designed to address the issues raised in previous systematic reviews and evaluate the effects of commonly used stress-reducing

aromatherapy. Furthermore, a specific emphasis of the study was on understanding the role expectancy effects might play in producing aromatherapy actions.

One of the main uses of aromatherapy is for relaxation and improvement in personal efficiency and functioning (Ilmberger et al., 2001), and the essential oil most widely used for these goals is lavender (*Lavandula angustifolia*) (Bowles, 2003; Buckle, 2003; Field et al., 2008; Fujii et al., 2008; Herz, 2009). There is research evidence for positive lavender effects on agitated behaviors (Holmes et al., 2002; Lin, Chan, Ng, & Lam, 2007), on biomarkers of stress (Atsumi & Tonosaki, 2007; Sanders et al., 2002; Takatsuji et al., 2008; Toda & Morimoto, 2008), anxiety levels (Field et al., 2008; Fujii et al., 2008; Kritsidima et al., 2010; Motomura et al., 2001), and cognitive functioning (Diego et al., 1998). However, some studies indicate no effects of lavender for the same conditions (Howard & Hughes, 2008; Kiecolt-Glaser et al., 2008). Such disagreements between the findings might be due to multiple factors, but one of the most critical factors influencing the results of the study is choosing the appropriate outcome measures. Many early aromatherapy studies relied heavily on subjective assessments that could potentially bias the results. Furthermore, many previous studies did not employ blinding of assessors to the study condition, which also can lead to biasing the results (Lee et al., 2012). Additionally, the choice of the control group for the research study can also critically influence the findings.

This study was an attempt to address all the above issues by designing a study using objective outcome measures, including two different control groups,

and blinding assessors to the assigned condition as well as possible. In this study we evaluated the stress-reducing properties of lavender by assessing its effects on higher order executive function. Executive functions are involved in planning, error correction, adaptation to novel situations, and response inhibition (Posner & Dehaene, 1994; Sehlmeyer et al., 2010), and they are extremely susceptible to the effects of acute stress (Arnsten, 2009; McEwen & Sapolsky, 1995; Qin, Hermans, van Marle, & Fernandez). In this study we specifically assessed indices of response inhibition through evaluating event related potentials (ERPs) in a visual go-nogo (GNG) task, in a paradigm where subjects respond to one target stimulus (go condition) and withhold responses to another target stimulus (nogo condition). Evaluating ERP in a go-nogo paradigm allows for objective assessment of cognitive function (attention and response inhibition) and yields both behavioral and functional brain data. In some studies the ERP evaluation approach has been shown more sensitive to the manipulations or different conditions than the data available from behavioral task (Zhang, Zhao, & Xu, 2007) indicating that evaluating brain patterns might in some cases to be more sensitive to subtle effects of interventions. Previous research with odors using a go-nogo task indicated significant effects of odors on event related potentials and suggested odors may enhance cognitive activity and inhibitory processing of motor response (Iijima, Osawa, Nishitani, & Iwata, 2009).

Inhibitory control assessed by go-nogo task is a central component of executive function critical for normal mental processes (Zhang et al., 2007), and

has been studied particularly by evaluating nogo N200 and nogo P300 components.

While the usual N200/P300 components are related to attentionally mediated processing of salient stimuli, these components during the go-nogo task are also considered to represent different sub-processes of response inhibition (Sehlmeyer et al., 2010). It has been shown to be associated with activity in anterior cingulate cortex (ACC) and other frontal areas of the brain (Rubia et al., 2001; Zhang et al., 2007). ERP indices for response inhibition have been demonstrated to be related to anxiety (Sehlmeyer et al., 2010), depressive symptoms (Zhang et al., 2007), and stress-induced alcohol–related processing (Ceballos, Giuliano, Wicha, & Graham). Furthermore, the ERP indices are influenced by acute stress (Ceballos et al.).

Briefly, N200 occurs approximately 200 ms after stimulus onset (with longer latency usually observed in older adults compared to younger adults (Schroeder, Lipton, Ritter, Giesser, & Vaughan, 1995). Previous studies suggested that N200 tends to be of larger amplitude and shorter latency in good vs. bad inhibitors (Falkenstein, Hoormann, & Hohnsbein, 1999). Further, stress has been shown to enhance the N200 amplitude in previous research (Ceballos et al., 2012). P300 occurs approximately between 250 and 650 ms after stimulus onset. Differences in P300 latency are related to function speed and overall cognitive performance with shorter latencies associated with superior cognitive performance (Polich, 2007). In addition to latendy and amplitude of the component peak, other ERP measures of interest include peak area.

Our main hypothesis was that if lavender aromatherapy is beneficial for stress reduction as previously suggested (Holmes et al., 2002; Kline et al., 2000), exposure to lavender aromatherapy prior to stress induction will minimize detrimental stress effects on performance on go/nogo task and ERP indices related to inhibitory control.

Methods

Participants

Eighty-one healthy adults (Mean age = 58.2, 79 % females) were recruited from the community by posting flyers in public places and through media announcements. The participants described below represented a subset of participants from a larger clinical trial of the aromatherapy effects on cognitive and stress markers (detailed in Chapter 2). The reasons for reduced sample for ERP measures include data loss due to problems with computerized go-nogo task as well as data loss due severe artifact contamination of some data that precluded further data analyses.

Study groups, randomization process, and blinding

Prior to the visit participants were randomized to a group based on aroma type (lavender, coconut, or water) and to a subgroup based on a type of prime they received during the visit (prime or no prime). Some methodology details are presented below but more details are presented in Chapter 2.

EEG/ERP measurement

EEG was recorded from 32-channel array (10/20 system) using the BioSemi Active Two EEG recording system (BioSemi BV, Amsterdam, Netherlands). Signa Gel (Parker Labs, Fairfield, NJ) and Ten20 conductive paste (Weaver and Company, Aurora, CO) were applied to create a stable electrical connection between each electrode and participant's scalp. The electro-oculogram (EOG) was recorded from the electrodes placed above the left external canthus and below the right external canthus. Additionally the single-ended signals were converted to differential signals offline with electrodes from right and left hemisphere referenced to the average of both mastoid electrodes. Electrode impedances cannot be measured with the standard electrodes when using Biosemi Active Two system. However, the EEG recordings were monitored to adhere to the offset recording standards of the Biosemi Active Two system. The sampling rate was 1024 Hz.

Laboratory visit

Detailed visit procedures are described in Chapter 2. Briefly, all eligible participants were fitted with the EEG system for ERP recording as described above. After that, participants proceeded with the visit that included a baseline assessment, a stress battery, and a post-stress assessment. During the baseline participants completed self-report measures and computer tests. After the baseline, each participant received their assigned aroma and a card with or without the prime. After completing the baseline assessment participants began inhaling their assigned aroma. Five minutes after initial aroma exposure, the stress battery was initiated for each participant and was followed by the post-

stress assessment similar to the baseline assessment. Subjective stress ratings were obtained during baseline and post-stress assessments as well as after each stressor in the stress battery. The laboratory visit took about 4 hours. After completing the laboratory visit, the EEG system was disassembled, and the non-blinded research assistant completed the study debriefing with each participant about the purpose of the study. All participants received a modest reimbursement for their time and effort following the study completion.

Stress battery

The stress battery consisted of emotional, physical, and mental stressor to elicit stress response in study participants. The chosen stressors are considered relatively mild but have been previously demonstrated to influence physiologic, endocrine, and cognitive functions as previously described in Chapter 2.

Self-report measures

Self-report instruments included to assess variables potentially affecting responses to aromatherapy are described in Chapter 2.

Cognitive measures

The Telephone interview for cognitive status (TICS) (Welsh et al., 1993) was used to assess potential participants' cognitive function and screen for any cognitive impairment prior to study enrollment.

Simple reaction time (SRT) test was used to allow participants to practice prior to starting go-nogo task and to evaluate processing speed as previously described (Oken et al., 2008). The test was presented on the computer with

participants pushing a mouse button as quickly as possible when a letter “O” appeared on the screen. Twenty-four stimuli were presented.

Go-Nogo (GNG) task was performed after the SRT task. It included presentation of 200 uncued stimuli on a computer monitor (80% letter “O” go stimuli, and 20% letter “Q” nogo stimuli) that were presented for 100 ms on the screen at pseudo-random order with an inter-trial interval that varied randomly between 1000 and 1300 ms.

Both computer tasks were presented by EPrime software version 2 (Psychology Software Tools, Inc, Sharpsburg, PA) in a dimly lit room. The black stimuli appeared on a white computer monitor located at a viewing distance of 80 cm. The subjects pushed the response button with their preferred hand. The main outcome measures were percent of errors, and median reaction time (RT), along with the RT standard deviation (SD) calculated from correct responses. RT SD was used as a marker of response variability. Variability of response times has been suggested as a marker of diverted attention (Bartolomeo, Sieroff, Chokron, & Decaix, 2001).

ERP Data Processing. All processing was completed offline using Brain Vision Analyzer version 2.0 (Brain Products GmbH, Gilching, Germany). Average mastoid reference EEG was filtered offline from 0.1 to 70 Hz (and 60 Hz notch filter was additionally applied). Artifacts due to eye movements were removed using independent component analysis (Jung et al., 2000), and epochs containing other artifacts (e.g. high frequency movement artifact) were removed. The data were processed into segments based on stimulus type. A segment

included 100 ms of activity prior to stimulus onset and 1000 ms of activity following the stimulus onset. Further, the data for each stimulus type were averaged using correct trials, and ERP components were determined. Components of interest included N200 and P300. The N200 was defined as the most negative peak occurring between 170 and 350 ms after stimulus onset and was measured relative to baseline. The P300 was defined as the most positive peak occurring between 250 and 600 ms after stimulus onset and was measured relative to baseline. Peaks and amplitudes were assessed with a semi-automatic detection function available in Brain Vision Analyzer and checked manually. The N200 and P300 areas were calculated automatically for the specified time range. The parameters for area estimations were based on the ERP waveforms averaged from all subjects. The N200 area was calculated for the time range between 150 and 250 ms, and P300 area was calculated for the time range between 250 and 600 ms after stimulus onset.

Statistical analyses

General statistical procedures related to data screening and evaluation of background variables and stress battery effectiveness were described in Chapter 2.

Some of the ERP data were lost due to severe contamination with artifacts. Slight differences in sample sizes for different measures reflect these data loss issues.

Primary analyses

Behavioral data. Repeated Measures Analyses of Variance (ANOVA) with time (baseline vs. post-stress assessment) used as a within-subject factor, aroma group (lavender, coconut, water) and prime subgroup (prime, no prime) used as between subject factors were used to assess main effects and interactions in behavioral performance. If a significant difference between groups or subgroups was indicated for one of the baseline variables affecting cognitive performance (e.g. education, age), that variable was used as a covariate in the subsequent analyses. To minimize Type I error due to multiple comparisons, for this set of analyses a Bonferroni adjustment was used to determine significant values. The p value of less than .017 (.05/3) was considered statistically significant for this group of tests, and values between .017 and .05 were considered trending on significance.

Neurophysiological data: ERPs. Repeated Measures Analyses of Variance (ANOVA) with time (baseline vs. post-stress assessment) and stimulus type (go vs. nogo) used as within subject factors, and aroma group (lavender, coconut, water) and prime subgroup (prime, no prime) used as between subject factors, were performed to assess different two-way and three-way interaction patterns in each of the neurophysiological variables: peak amplitude, peak latency, and overall area associated with the peak. Because N200/P300 components are typically fronto-centrally maximal for nogo stimuli and centro-parietally maximal for go stimuli (Johnstone, Pleffer, Barry, Clarke, & Smith, 2005) and visual inspection of ERP waveforms indicated most robust effects at Cz, the analyses were carried out using the Cz data. Additionally, because patterns for nogo trials

were of primary interest, nogo trials were the main focus of the follow-up analyses when an interaction was suggested. To control for an increased probability of Type I error due to multiple comparisons for each of the ERP components of interest, a Bonferroni adjustment applied separately to aroma and prime effects has been used to determine statistical significance of the results. The p value of less than .017 (.05/3) was considered statistically significant for this group of tests, and values between .017 and .05 were considered trending on significance.

Secondary analyses

Correlations among cognitive and stress-and-aroma-related variables. Bivariate Pearson correlations were performed to evaluate relationships among post-stress cognitive performance variables and ERP variables, as well as variables associated with aroma hedonic qualities, expectancy, and stress. No adjustments for multiple comparisons were utilized for secondary exploratory analyses, $p < .05$ was considered significant, and the results were considered trending on significance when p level was between .05 and .10.

Results

Baseline Characteristics. To check whether random assignment of the participants to the groups and subgroups resulted in well-matched groups, baseline characteristics were compared among groups. Similarly to the whole sample, for the subsample of participants described in this chapter, the groups based on aroma and prime were well-matched on major background variables

including age, gender, perceived stress, and personality traits (all p's > .05). However, the participants allocated to no prime group had more years of education compared to prime group (p = .04). Though the mean group difference was about a year, and participants from different groups did not differ on their baseline performance, the length of education was added as a covariate to the analyses of behavioral data.

Previous aromatherapy use. Similarly to the whole sample described in Chapter 2, participants in different groups were similar in previous aromatherapy use and aromatherapy use during the previous 3 months (all p's > .05). The details of baseline characteristics based on aroma and prime groups are presented in Table 7.

Stress battery effectiveness check. Stress battery check to confirm that participants experienced stress indicated that, similar to the data described in Chapter 2, significant increases in subjective stress level and state anxiety, as well as a decrease in positive affect were found in this subsample. This finding suggested that the induction of subjective feeling of stress by a stress battery was successful.

Table 7. Baseline participant characteristics

Mean (SD) unless otherwise noted	Aroma groups			P value	Prime groups		P value
	Lavender (n = 27)	Coconut (n = 27)	Water (n = 27)		Prime (n = 40)	No prime (n = 41)	
Age	59.1 (7.1)	57.5 (6.2)	56.5 (5.1)	.35	59.0 (6.2)	56.2 (5.9)	.06
Female (%)	77.8	85.2	74.1	.59	82.5	75.6	.45
Education (years)	16.0 (2.1)	15.8 (1.9)	16.0 (3.2)	.93	15.3 (2.3)	16.6 (2.6)	.04
TICS score	38.5 (3.9)	39.2 (3.3)	37.0 (3.9)	.11	38.4 (3.6)	37.9 (4.0)	.60
PSS score	15.7 (5.1)	18.2 (5.0)	16.2 (6.1)	.28	17.0 (4.9)	16.4 (6.0)	.64
STAI score	33.2 (7.2)	34.0 (7.4)	33.1 (9.8)	.90	32.6 (8.4)	34.3 (7.9)	.35
PANAS negative	13.5 (4.2)	12.9 (3.0)	13.4 (6.0)	.27	12.7 (4.7)	13.9 (4.4)	.45
PANAS positive	32.0 (9.4)	32.8 (5.0)	33.9 (6.7)	.64	33.3 (6.6)	32.5 (7.8)	.60
Previous aroma use (%)	55.6	40.7	42.3	.49	40.0	52.5	.26
Expected effectiveness	70.4 (13.5)	71.4 (13.0)	71.5 (12.1)	.95	71.4 (11.7)	70.8 (13.8)	.84
Expected stress change	33.6 (19.9)	33.2 (17.4)	34.4 (18.3)	.99	33.6 (17.5)	33.8 (19.4)	.99
SRT, Median RT	279.15 (59.8)	245.7 (38.0)	282.6 (97.5)	.08	272.1 (86.8)	266.3 (51.5)	.97

Abbreviations: SD = standard deviation, TICS = Telephone Interview for Cognitive Status, PSS = Perceived Stress Scale, STAI = State and Trait Anxiety Inventory, PANAS = Positive And Negative Affect Schedule, Expected effectiveness = expected aromatherapy effectiveness for stress reduction, Expected stress change = expected change in stress level from neutral VAS score of 50 (less than 50 – decreased stress, greater than 50 – increased stress, SRT = Simple reaction time task, RT = reaction time

Primary analyses: Behavioral results

Participants in different groups demonstrated similar baseline performance on all of the GNG task outcome measures, all p's > .10 (Table 8a and 8b). Due to the fact that participants in all groups demonstrated similar performance on go trials displaying almost no errors of omission (response rate > 99%) during both baseline and post-stress assessment, this variable was not used in further analyses of group differences. The variables of interest included median RT, response variability (RT SD), and percent of errors of commission. The changes in these variables were compared among different groups to assess whether behavioral performance of participants exposed to different aromas or primes were affected differently.

GNG median reaction time for go trials. Repeated Measures ANCOVA indicated that there was a trend for the effect of time on median RT values, $F (1, 75) = 4.82$, $p = .03$, partial $\eta^2 = .06$ with participants on average displaying shorter median RT to go stimuli at post-stress assessment. This general post-stress RT decrease is a common phenomenon that is believed to be due to learning. There was also a time by prime interaction, $F (1, 75) = 4.52$, $p = .037$, partial $\eta^2 = .06$ at a trend level after adjusting for multiple comparisons. The results of follow-up analyses indicated that participants in the prime subgroup regardless of the aroma they experienced had a significant decrease in their median RT, $F (1, 39) = 19.13$, $p < .001$ unlike the participants in the no prime subgroup, $p > .05$.

GNG response variability (using SD). Repeated Measures ANCOVA indicated a trend for time by prime interactions in GNG response variability, $F(1, 75) = 4.94$, $p = .029$. The results of post-hoc analyses indicated that participants in the prime subgroup regardless of the aroma they experienced had a significant decrease in variability of responses at post-stress assessment, $F(1, 39) = 18.61$, $p < .001$ compared to the participants in the no prime subgroup who did not show a significant change in variability of responses, $p > .05$.

GNG errors of commission. Repeated Measures ANCOVA did not detect any differences in percent GNG errors of commission, all p's > .10 indicating similar error rate for all participants regardless of the time point and group or subgroup assignment.

Overall, no effect of aroma or group on behavioral performance on GNG task was demonstrated at a conventional significance level. A trend for prime by time interaction was suggested for both median RTs and response variability. For both of these variables participants receiving a prime regardless of aroma experience demonstrated results potentially indicative of improved attention during the post-stress assessment (manifested in greater reduction in median RT and decrease in response variability beyond general learning effects) compared to participants receiving no prime.

Table 8a. Behavioral performance in the go-nogo task by aroma group

Mean (SD)	Lavender		Coconut		Water	
	base	post	base	post	base	post
Go (% correct)	99.5 (.9)	99.6 (.8)	99.4 (.8)	99.3 (1.3)	99.3 (1.4)	99.5 (1.2)
Nogo (% correct)	84.5 (12.9)	87.5 (11.6)	78.4 (15.5)	81.5 (16.0)	83.7 (13.5)	85.9 (14.4)
Median RT	366.3 (60.9)	346.9 (44.6)	354.3 (62.2)	334.7 (49.0)	355.2 (67.5)	342.1 (56.7)
RT SD	87.1 (27.9)	76.8 (22.7)	83.1 (23.3)	78.9 (21.1)	84.3 (33.3)	76.1 (29.7)

Abbreviations: RT = reaction time, SD = standard deviation

Dependent Variables: Go = Performance on go trials indicates errors of omission rate, Nogo= performance on nogo trials indicates errors of commission rate, Median RT = median reaction time is a measure of processing speed, RT SD = reaction time standard deviation is a measure of response variability used as a marker of diverted attention.

Table 8b. Behavioral performance in the go-nogo task by prime group

Mean (SD)	Prime		No prime	
	base	post	base	post
Go (% correct)	99.2 (1.3)	99.3 (1.3)	99.6 (.6)	99.6 (.9)
Nogo (% correct)	81.9 (13.5)	84.3 (14.4)	82.6 (14.8)	85.8 (14.0)
Median RT[a, b]	356.6 (59.6)	333.2 (48.2)	360.5 (66.9)	349.48 (51.0)
RT SD[a, b]	86.7 (26.4)	74.6 (23.4)	83.0 (30.1)	79.3 (25.7)

Abbreviations: RT = reaction time, SD = standard deviation

[a] = prime x time interaction, trend level at $0.05 < p < 0.017$, [b] = significant change in prime subgroup, $p < 0.05$

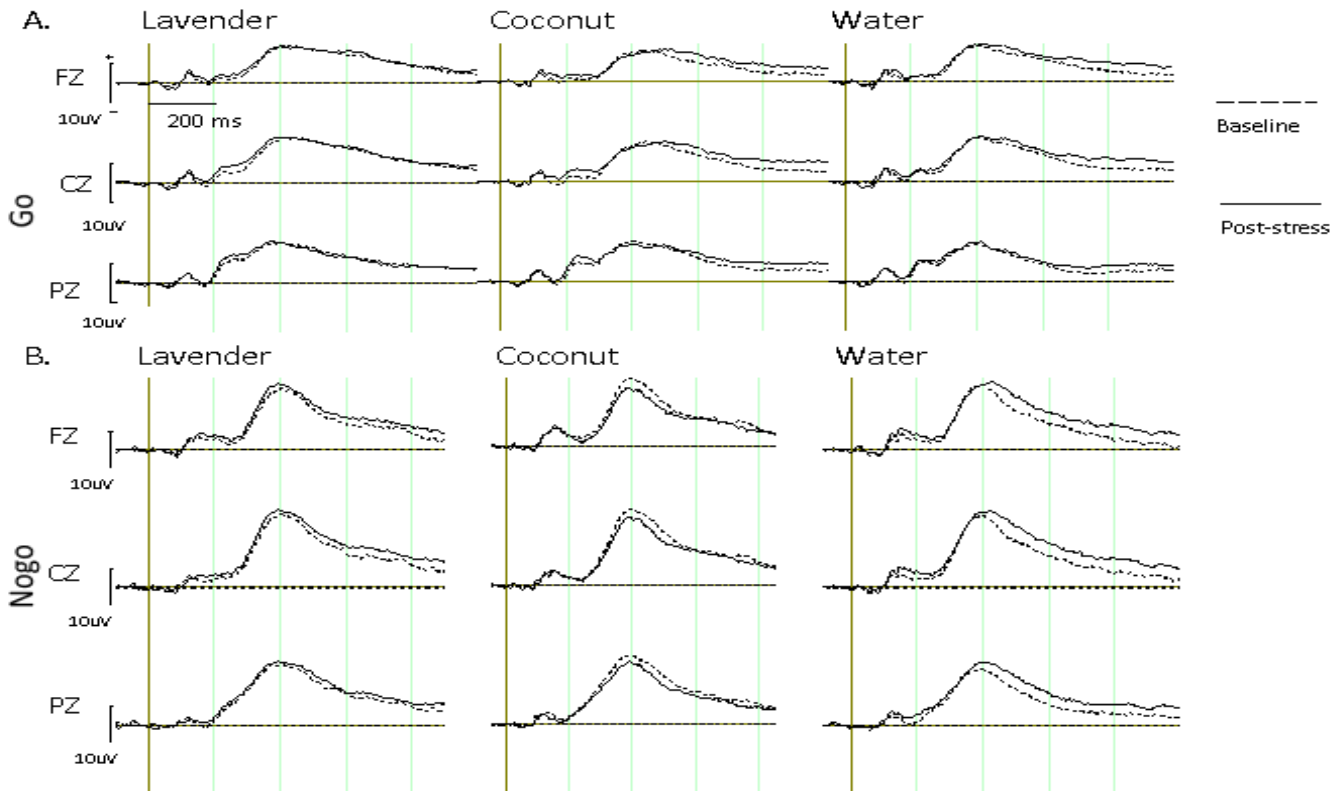
Dependent Variables: Go = Performance on go trials indicates errors of omission rate, Nogo= performance on nogo trials indicates errors of commission rate, Median RT = median reaction time is a measure of processing speed, RT SD = reaction time standard deviation is a measure of response variability used as a marker of diverted attention.

Primary analyses: ERP results

Group and subgroup comparisons of ERP measures at baseline indicated no differences for participants in different prime subgroups, all p's > .05 and no differences for participants in different aroma groups for most of the variables except for the variable denoting area associated with N200 in go trials, p = .01. All other variables were similar for participants in different groups at baseline, all p's > .05. Please refer to Figures 8 and 9 for ERP waveforms for different aroma groups and prime subgroups, respectively. As expected, all participants displayed a distinct go-nogo effect in both N200 and P300 components with nogo trials stimuli eliciting larger N200 and P300 compared to go trials stimuli.

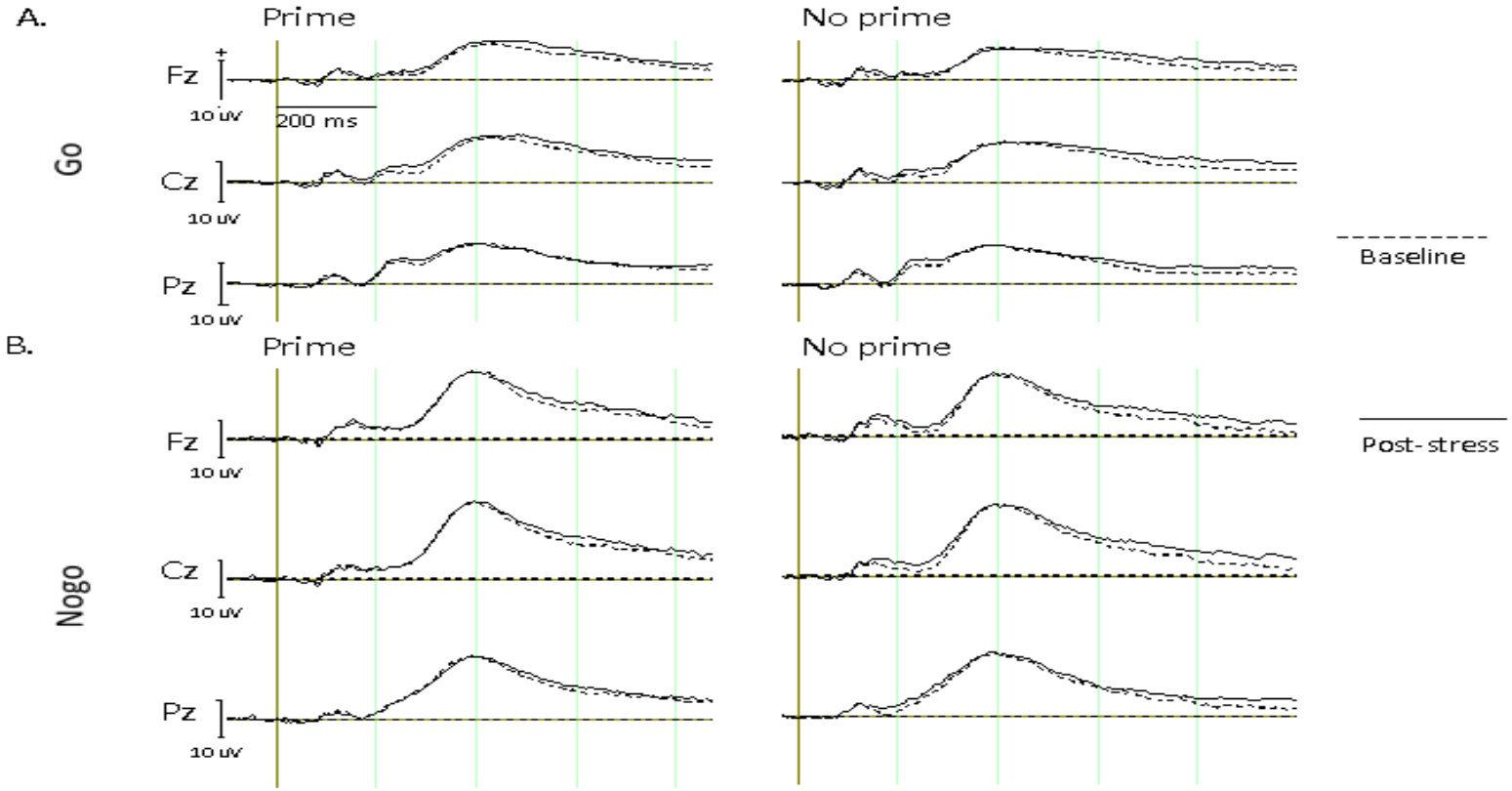
The following analyses assessed differences in patterns of ERP components between aroma groups and prime subgroups with the main focus on nogo stimuli (latency, amplitude and area measures). As a general rule decreased latencies and increased amplitudes (and areas) are indicative of more preserved cognitive function.

Figure 8. ERP waveforms for groups based on aroma



Waveforms are presented separately for go (Panel A) and nogo (Panel B) stimuli for the three electrode locations and three aroma groups. The dashed line represents baseline waveform, solid line represent post-stress waveform. Positive values are plotted upward. Note that ERP waveforms in lavender group are very similar between baseline and post-stress time points.

Figure 9. ERP waveforms for subgroups base on prime.



Waveforms are presented separately for go (Panel A) and nogo (Panel B) stimuli for the three electrode locations and three aroma groups. The dashed line represents baseline waveform, solid line represent post-stress waveform. Positive values are plotted upward.

N200

N200 Peak amplitude. RM ANOVA revealed a trend for the three-way time x stimulus type x aroma interaction, $F (2, 66) = 3.49$, $p = .036$, partial $\eta^2 = .10$ and time x stimulus type x prime interaction, $F (1, 66) = 7.59$, $p = .008$, partial $\eta^2 = .10$.

Analyses following up on these 3-way interactions indicated that there was a significant time effect for go trials: a lower N200 amplitude was displayed during post-stress assessment compared to baseline, $F (1, 68) = 5.18$, $p = .026$, partial $\eta^2 = .07$. For the nogo trials an aroma x time interaction was not statistically significant, $F (1, 71) = 2.44$, $p = .095$, partial $\eta^2 = .06$, and there was no main time effect, $p > .05$. No other group or prime effects were noted.

Further, analyses revealed a trend for a time x prime interaction, $F (1, 68) = 5.11$, $p = .027$, partial $\eta^2 = .07$ for nogo trials. Specifically, during nogo trials participants in no prime subgroup displayed a lower N200 peak amplitude during post-stress assessment than their baseline values, $F (1, 33) = 7.68$, $p = .009$, compared to participants in prime subgroup who showed little change in N200 peak amplitude between the two assessments.

N200 Peak latency. RM ANOVA revealed the main effect of stimulus type, $F (1, 66) = 7.08$, $p = .010$, partial $\eta^2 = .10$ (nogo peak latency < go peak latency) as well as suggested a trend for a three-way interactions for time x stimulus type x prime, $F (2, 66) = 4.77$, $p = .033$, partial $\eta^2 = .07$.

Additional analyses indicated that the groups did not differ on this measure during go trials. During nogo trials participants receiving a prime had a post-stress decrease in peak N200 latency compared to participants in no prime condition whose peak latency was slightly increased post-stress, $F(1,68) = 3.03$, $p = .086$, but this result did not reach even a trend level after adjusting for multiple comparisons.

Area associated with N200. RM ANOVA revealed the trend for the main effect of time, $F(1, 67) = 5.29$, $p = .025$, partial $\eta^2 = .07$ (baseline area < post-stress area) and a significant effect of stimulus type $F(1, 67) = 34.16$, $p < .001$, partial $\eta^2 = .34$ (nogo area > go area). Furthermore, the analysis indicated a three-way time x stimulus type x aroma interaction, $F(2, 67) = 9.22$, $p < .001$, partial $\eta^2 = .22$.

As noted above, analyses of go trials indicated a significant group difference in N200 areas at baseline, so no further analyses were attempted due to pre-existing group differences on that measure. Additional analyses comparing changes in N200 area for nogo trials from baseline to post-stress assessment between lavender and placebo group participants indicated a trend for time x experimental aroma interaction, $F(1, 70) = 4.56$, $p = .036$, partial $\eta^2 = .06$. Specifically, participants in lavender group displayed a different pattern (little change in N200 area during two study assessments) compared to participants exposed to placebo (who demonstrated an increase in N200 area at post-stress assessment from baseline values).

P300

P300 Peak amplitude. RM ANOVA revealed the main effect of stimulus type, F (1, 66) = 172.72, p < .001, partial η^2 = .73 (nogo peak amplitude > go peak amplitude), and a trend for the time x stimulus type interaction, F (1, 66) = 4.70, p = .034, partial η^2 = .07 suggesting that the difference between go and nogo peak amplitudes were reduced during post-stress assessment compared to baseline difference. No other significant effects or interactions were detected.

P300 Peak Latency. RM ANOVA revealed the main effect of stimulus type, F (1, 66) = 860.73, p < .001, partial η^2 = .93 (nogo peak latency < go peak latency). A trend for time x prime interaction was also detected, F (1, 66) = 4.13, p = .046, partial η^2 = .06. Follow-up analyses revealed no prime subgroup differences for go and nogo trials, even though participants in different subgroups displayed divergent patterns over time: those receiving prime showed a decrease in P300 latency and those receiving no prime showed an increase in P300 latency at post-stress assessments compared to baseline values. The results did not reach a significance level, F (1, 72) = 3.17, p = .079.

Area associated with P300. RM ANOVA revealed the main effect of stimulus type, F (1, 66) = 156.76, p < .001, partial η^2 = .70 (nogo P300 area > go P300 area). A trend for a three-way stimulus type x time x aroma interaction was revealed, F (1, 66) = 3.91, p = .025, partial η^2 = .11.

Follow-up analyses indicated that, while no significant time differences were observed for lavender and coconut groups on nogo P300 area variable, a significant difference over time (increased nogo P300 area at post-stress

assessment) was evident for water group, F (1, 23) = 7.23, p = .01, partial η^2 = .23.

Relationships among GNG behavior, ERP measures, and aroma-related measures

The relationships among behavioral and functional brain data were evaluated to attempt to link specific ERP components to behavioral performance on cognitive tests and subjective feelings of alertness and aroma hedonic qualities. Table 9 includes information about relationships among GNG behavioral and ERP results as well as some aroma-related variables.

Links between behavioral and ERP results. There were several significant correlations linking behavioral data GNG task and ERP data collected at post-stress assessment.

First, as is common, median reaction time on go trials on GNG task was positively related to response variability (measured by SD), r = .69. p < .001. Furthermore, a non-significant trend was noted for relationships between median reaction time on go trials and nogo P300 area, r = -.37, p = .07.

Next, response variability on go trials was related to mean nogo N200 area, r = .55, p = .004, and nogo P300 area, r = -.45, p = .02. The response variability measure was also influenced by alertness level, r = -.47, p = .01. No correlations emerged for the percent of errors of commission at post-stress assessment.

Links among different ERP indices. Several relationships were noted for nogo N200 variables: N200 amplitude was positively related to N200 area, r = .56, p = .004, P300 amplitude, r = .55, p = .005, and showed a trend for correlation with nogo P300 area, r = .46, p = .07. Additionally, nogo N200 and P300 latencies were related, r = .995, p < .001. Next, nogo P300 amplitude was strongly related to nogo P300 area, r = .94, p < .001.

Aroma-related variables and GNG measures. Expected effectiveness of aromatherapy was related negatively to P300 nogo amplitude, r = -.44, p = .03 and positively to alertness level r = .44, p = .02. Alertness level at post-stress assessment was negatively related to variability of response on GNG task, r = -.47, p = .01 and showed a trend to correlate to median reaction time on GNG go trials, r = -.35, p = .08. No correlations were noted between aroma intensity and pleasantness ratings and any of the GNG task measures or ERP variables.

Table 9. Relationships among aroma-related, and behavioral, and ERP measures at post-stress assessment

Variable	2	3	4	5	6	7	8	9	10	11	12	13
1 GNG Med. RT	**.692****	.176	-.054	.051	.120	-.221	.040	*-.373*^{T}	-.082	-.065	-.246	*-.346*^{T}
2. GNG SD	1	-.146	.232	.274	**.550****	-.262	.253	**-.449***	-.233	.113	-.209	**-.470***
3. GNG nogo % errors		1	-.166	-.309	-.099	-.132	-.278	-.147	.304	-.166	.211	.151
4. N200 nogo amplitude			1	.138	**.559****	**.547****	.124	*.376*^{T}	-.171	.121	-.025	-.165
5. N200 nogo latency				1	.048	-.192	**.995****	-.308	-.288	.112	-.153	-.110
6. N200 nogo area					1	.260	.063	.189	-.316	.157	-.055	-.252
7. P300 nogo amplitude						1	-.189	**.942****	**-.441***	.094	-.051	-.147
8. P300 nogo latency							1	-.297	-.291	.105	-.135	-.106
9. P300 nogo area								1	-.112	-.091	-.058	-.074
10. Expected aroma effect									1	-.252	*.364*^{T}	**.443***
11 Aroma Intensity										1	*.352*^{T}	-.112
12 Aroma Pleasantness											1	.287
13. Alertness level												1

Abbreviations: GNG = Go-nogo task, Med. RT = Median reaction time, SD = standard deviation, ERP = event related potential

^{T} = .10 < p < .05, *p < .05, **p < .01

Discussion

The purpose of this study was to evaluate effects of stress-reducing aromatherapy on go-nogo task performance and to assess the role of different types of expectancy in the aromatherapy actions.

Behavioral results

The version of go-nogo task used in this study proved to be easy for participants as indicated by low error rates for both errors of commission and errors of omission. Often easy behavioral tasks like the go-nogo paradigm fail to differentiate the performance in different groups or over time when healthy adults are tested (Zhang et al., 2007). Similar to previous studies, the behavioral results from our GNG task did not reach the conventional level of significance after adjusting for multiple comparisons. However, the trends observed in our study suggested that compared to participants in no prime condition, participants in prime condition regardless of actual aroma they experienced had a decrease in their median RT along with a decrease in variability of responses. These results suggest that verbally-mediated expectancy might be beneficial for performance on the GNG task and possibly influence attention processes in general.

ERP results

Typically N200/P300 components are studies in relation to attentionally mediated processing of salient stimuli. However more research indicates that these components during the go-nogo task are also considered to represent different sub-processes of response inhibition (Sehlmeyer et al., 2010).We

focused primarily on nogo N200 and nogo P300 components representing sub-processes of response inhibition, a component of the executive cognitive function (Eimer, 1993; Sehlmeyer et al., 2010) that was recently shown sensitive to stress (Ceballos et al., 2012). These components might be sub-served by prefrontal areas (Kawashima et al., 1996; Rubia et al., 2001) and anterior cingulate cortex (Beste, Willemssen, Saft, & Falkenstein, 2009; Bokura, Yamaguchi, & Kobayashi, 2001; Falkenstein, 2006) usually considered vulnerable to acute stressors (de Kloet, 2000). Our results indicate that the stress battery indeed elicited a stress response in our sample as evidenced from a post-stress increase in subjective stress and anxiety levels as well as from a decrease in positive affect.

N200. N200 occurs approximately 200 ms after stimulus onset (with longer latency usually observed in older adults compared to younger adults (Schroeder et al., 1995). Stress has been shown to enhance the N200 amplitude in previous research (Ceballos et al., 2012), but our study did not demonstrate an overall significant increase in N200 amplitude after stress battery. On the contrary, the trend for time effect indicated that N200 amplitudes were on average lower at post-stress assessment compared to baseline values. Some studies suggested that N200 tends to be of larger amplitude and shorter latency in good vs. bad inhibitors (Falkenstein et al., 1999) so our results could be interpreted in a way that at post-stress assessments participants on average displayed less inhibitory control than at baseline .

With regard to nogo N200 amplitude, our results indicated that N200 amplitude might be influenced by verbally-mediated expectancy of stress reduction. Specifically, when no prime was presented participants displayed a lower nogo N200 amplitude at post-stress assessment compared to baseline; however for those receiving a prime regardless of the assigned aroma little change in nogo N200 amplitude between the two time points was observed. If a decrease in nogo N200 amplitude coupled with an increase in N200 latency is indeed associated with poorer inhibition as previously proposed (Falkenstein et al., 1999) then receiving a prime suggesting stress reducing properties of the aroma might have prevented the participants from experiencing decrements in inhibitory control following stress battery. This notion is further supported by observing a decrease in nogo N200 peak latency in those receiving a prime in contrast to an increase in nogo N200 peak latency in those not receiving a prime.

Our results indicated that verbally-mediated expectancy plays an important a role in nogo N200 indices. In contrast, no significant effects on N200 amplitude or latency due to a specific aroma were revealed. However a trend involving aroma groups was suggested for the N200 area. Specifically, nogo N200 area was increased at post-stress assessment compared to baseline in both groups exposed to placebo aromas, but little change in nogo N200 area was evident for participants in lavender group. This might indicate that inhibitory processing at post-stress assessment was less affected in participants from the lavender group compared to that in participants from placebo groups. Overall, patterns of nogo

N200 indices between different prime and aroma groups are consistent with the notion that exposure to lavender aroma and enhancing verbally-mediated expectancy to aromatherapy in general might help preserving the pre-stress ERP patterns in N200 component even after stress exposure. Changes in nogo N200 indices at post-stress assessment observed in other groups are consistent with mild deterioration in earlier stages of response inhibition processing.

P300. Consistent with previous studies (Elmer 1993, Sehlmeyer 2010) greater P300 amplitudes and areas as well as shorter P300 latencies were observed for nogo trials compared to go trials. A trend for time by prime interaction was suggested for nogo P300 latency component. The patterns observed for nogo P300 latency were paralleling those observed for nogo N200 latency: an increase in nogo P300 latency at post-stress assessment for participants not receiving a prime and a decrease in nogo P300 latency for participants receiving a prime. Differences in P300 latency are related to function speed and overall cognitive performance with shorter latencies associated with superior cognitive performance (Polich, 2007). Therefore, our results suggest that giving a prime about stress-reducing aroma effects to study participants might have benefited their cognitive function.

Similarly to nogo N200 area, nogo P300 area was associated with differences in groups based on aroma: nogo P300 area for lavender group participants did not show significant difference between the two study assessments and was not significantly affected by stress battery. A trend was indicated for coconut group where the nogo P300 area was slightly decreased at

post-stress assessment compared to baseline. Furthermore, a significant increase in nogo P300 area at post-stress assessment was observed for water group. P300 area is usually significantly correlated with P300 amplitude, and research suggests several explanations for P300 amplitude changes. One of the explanations is that P300 amplitude indexes attentional resources; therefore when attentional resources are decreased (as is likely after stress exposure), the P300 amplitude tends to be smaller and P300 latency tends to be longer (Polich, 2007). Such a pattern is consistent with the pattern observed in a coconut group displaying slight decrease in nogo P300 area. Another possible explanation for the changes observed in nogo P300 area stems from the evidence pointing to the relationship between an increase in anxiety and increased P300 amplitudes (Karch et al., 2008; Sehlmeyer et al., 2010). This pattern is consistent with that displayed by the water group. Indeed, the participants in the study demonstrated a significant increase in state anxiety following the stress battery so exposure to odorless inert water might have left participants in water group more vulnerable to the consequences of increased anxiety. It is of interest that the two placebo groups had divergent ERP changes on nogo P300 area following stress, suggesting that aroma-mediated expectancy (presence of aroma vs. absence of aroma) might be playing a role in aromatherapy effect. Coconut base oil is considered an inactive substance for producing aromatherapy effects (Wildwood, 1996) so the observed ERP changes are unlikely to occur as a result of pharmacological effect of coconut oil. While interpretations of aroma effects on P300 area in placebo groups are ambiguous, it is notable that people in lavender

group did not demonstrate significant changes in nogo P300 area following stress similarly to the pattern observed for the nogo N200 area. The N200/P300 waveform was largely unchanged after stress exposure in lavender group; however, the waveform showed post-stress changes in the groups receiving placebo aromas. This is consistent with the explanation that stress-reducing and anxiolytic properties of lavender aroma might buffer detrimental effects of acute stress helping to protect cognitive function after stress exposure.

As expected behavioral performance was related to some of the ERP results: response variability linked to attention was related to both nogo N200 and nogo P300 areas with smaller response variability correlating to more pronounced N200 and P300 components. Median reaction times were associated with nogo P300 area at a trend level. Both behavioral measures were also related to alertness level that was in turn influenced by expectation of an aromatherapy effect. Neither aroma intensity nor aroma pleasantness were associated with GNG task measures or ERP data. This is in contrast to the data suggesting intensity of the aroma might play an important role in affecting cognitive function and physiologic stress markers after exposure to stress (described in Chapter 4).

Many of the reported results in our study did not reach conventional significance level after adjustment for multiple comparisons likely because of subtlety of the behavioral and ERP modifications resulting from exposure to stress and aromatherapy in healthy participants. However, we believe the conclusions are supported because of a strong agreement between behavioral data and ERP results.

Conclusions

Overall, our ERP results suggest that lavender aroma might possess pharmacological properties that help buffer effects of acute stress on cognitive function. In addition, evidence from this study indicates that verbally-mediated expectancies might underlie some changes in behavior and brain function after exposure to aromatherapy.

Chapter 6: General Discussion

Overview of the results

The goal of this work was to subject lavender aromatherapy commonly used for stress reduction to rigorous testing to assess its effects on physiological and cognitive functioning. In addition to evaluating lavender effects on multiple measures sensitive to stress, this work also tested the role of aroma-mediated and verbally-mediated expectancies to make some contribution to understanding of the mechanisms underlying aromatherapy actions.

One of the exciting findings from this study was the beneficial effects of lavender on working memory performance on the Digit Span Backward task with participants in lavender group showing an average 1-unit increase in their working memory capacity (described in Chapter 4) that was not previously reported. The Digit Span Backward task evaluates working memory, a higher-order cognitive function that is expected to be negatively affected by acute stress, and the findings of lavender effects on this function provide support for lavender stress-reducing effects but also don't rule out that lavender might have direct effects on some cognitive processes. The results reported in Chapter 5 that indicate that those who inhaled lavender during stress exposure showed negligible changes in ERP waveform observed during a go-nogo task assessing another higher-order function potentially vulnerable to stress, attention. These findings suggest protective effects of lavender aromatherapy on cognitive function after acute stress. The mechanism of these protective effects needs to be evaluated in the future.

Another interesting set of findings related to the potentially beneficial effect of verbally-mediated expectancy on behavioral performance on several cognitive tasks (Described in Chapters 4 and 5). According to the data reviewed in these chapters, priming aromatherapy recipients on expected aromatherapy effects might produce beneficial effects congruent with priming statement on cognitive function evident in ERP changes and behavioral performance on cognitive tests of processing speed and executive functioning.

Aroma-mediated expectancy associated with the presence of a perceptible aroma regardless of its putative pharmacological effects was also found important in this study. Aroma-mediated expectancy was underlying aromatherapy effects on physiologic measures of EEG frontal asymmetry and chromogranin A after stress induction (described in Chapter 3). Hedonic qualities of aroma including aroma intensity and aroma pleasantness influenced aroma-mediated expectancy and were critical for producing such effects.

Further, the results of this work provide support for calming and stress-reducing effects of lavender aromatherapy inhaled during stress exposure for physiological measures (Chapter 3). Specifically, in Chapter 3, exposure to lavender aromatherapy was associated with some reduction in respiration rate following stress indicative of a more relaxed state, as well as with changes in two additional stress markers (EEG frontal asymmetry and CgA) suggesting the utility of lavender for buffering detrimental stress effects.

Overall, the results show that observable aromatherapy effects are produced by a combination of mechanisms that involve aroma-specific pharmacological properties and general aroma properties as well as participants' expectations.

Is there evidence for lavender-specific stress-reducing effects?

The conventional way of assessing treatment or intervention efficacy is to show that the experimental intervention produces superior and clinically significant effect compared to the effects observed due to control conditions. The current work shows several effects observed exclusively in the group exposed to lavender aromatherapy during acute stressor and not in the groups exposed to placebo aromas. The beneficial effects of lavender aromatherapy indicative of reduced stress were evident from changes on objective measures sensitive to stress exposure including patterns of ERP N200/P300 components observed during go-nogo task, as well as influences on respiration rate, and performance on a working memory task. However, though lavender was assessed as a stress-reducing aroma, exposure to this aroma was not associated with improvements on subjective ratings of stress, anxiety, or mood.

It is curious that lavender aromatherapy widely used for stress reduction and relaxation did not produce a significant subjective relief of stress, as indicated by the lack of group differences on subjective measures of stress and anxiety. Some researchers argue that changes in objective measures due to aromatherapy are only meaningful when correlated with subjective evaluations, and in the absence of subjective responses observing physiological changes have questionable validity (Herz, 2009). Previous aromatherapy research

suggests that it is not uncommon to observe aromatherapy-related changes in objective measures that are not accompanied by similar changes in subjective measures and vice versa (Goel et al., 2005; Motomura et al., 2001; Toda & Morimoto, 2008). It is unclear why people in aromatherapy group did not report greater stress relief on subjective measures in the current study. Several potential explanations of lack of subjective differences between aroma groups in the presence of significant objective changes are possible.

The first explanation includes using subjective measures that might not be sensitive to the subtle effects of aromatherapy even though the same measures were sensitive to the within-subject changes due to stress exposure. Another potential explanation for the lack of group differences in subjective measures is the nature of the current protocol: post-stress assessment of subjective measures occurred after a long visit, including a stress battery and a set of challenging cognitive tasks, at the time when participants might be greatly affected by fatigue and hunger, the two powerful stimuli that might have overshadowed any potentially more subtle effects of stress-reducing aromatherapy. Finally the lack of group differences on subjective measures could be due to the presence of stress-reducing effects of similar magnitude in placebo conditions, the effects arising from aroma-or verbally-mediated expectancy that were observed on several objective measures and could have influenced subjective experiences as well.

Despite the absence of subjective effect of lavender on stress and anxiety measures, lavender aromatherapy was related to observed changes in behavior:

participants randomized to the lavender group demonstrated a significantly more improved performance on a working memory task performance compared to the performance on the same task by people randomized to the placebo groups. The observed post-stress average score increase on the working memory task by one unit correlates with increasing one's digit span roughly by one digit, which is a functionally meaningful improvement (increased capacity to manipulate more units of information in working memory). It is curious that out of the two tasks assessing working memory used in the study, the beneficial effects of lavender were only observed for one, Digit Span Backward, task, but not for the more challenging Letter-Number Sequencing task. Previous studies produced conflicting results regarding lavender effects on cognitive function after stress: some observed decrements in working memory and impaired reaction times on memory and attention based tasks (Moss et al., 2003) while others reported improved speed and accuracy on math calculations that require both memory and attention functions (Diego et al., 1998). The difficulty level of the assessed tasks might be critical for affecting the study results and producing different effects. It is possible that lavender exposure might be beneficial for the performance on tasks of moderate rather than high difficulty.

Specific mechanism for the observed lavender effects cannot be clearly determined from the currently available data, but several possible explanations are reviewed below. One explanation might involve pharmacological actions of lavender aromatherapy (potentially effects of lavender constituent linalool that showed a sedating effect in animal studies) resulting in reducing sympathetic

activation and buffering stress-related effects on prefrontal cortex that sub-serves executive functions including working memory. The mechanism by which lavender aromatherapy might buffer stress effect on cognitive performance is not known. The evidence from our study suggests that it is unlikely that lavender aromatherapy interferes with HPA axis activation and release of cortisol thus influencing PFC function, because no specific lavender effects on post-stress cortisol levels were found in the current work and several previous studies (Kiecolt-Glaser et al., 2008; Toda & Morimoto, 2008). However, lavender effect on cortisol levels was reported in earlier research (Atsumi & Tonosaki, 2007).

Aroma effects on cognitive function can also arise from improved mood due to exposure to a pleasant aroma, which is also unlikely in the current study due to the lack of specific effects on mood in the lavender group. Another possibility is a change in cognitive performance due to arousing effect of a high intensity olfactory stimulus. Indeed, there was a significant between-group difference for the aroma intensity ratings, and lavender aroma was rated the most intense of all the aromas used in the study (Chapter 2). Moreover, aroma intensity rating was suggested as having potentially mediating effect on producing improvements in the Digit Span Backward working memory task (Chapter 4). However, if the presence of intense aroma was indeed affecting the results of the working memory task, it is doubtful that this effect was due to increased arousal or alertness because people in the lavender group reported a decreased post-stress alertness level compared to the water group. In general, it is problematic that the groups with perceptible aromas in the current study were not matched on

aroma intensity, thus making it impossible to dissociate effects potentially influenced by aroma intensity from the specific pharmacological aroma effects. Matching experimental and placebo aromas on aroma intensity would be important for assessing the role of aroma intensity in aromatherapy actions.

Furthermore, lavender aromatherapy might have produced its effects due to prior conditioning and associating the aroma with previously experienced relaxed and stress-free states. This possibility was not directly tested in the current work. However, in the current study lavender aroma was more readily perceived and recognized compared to other study aromas, and as a popular scent used in care products and during services intended for relaxation, stress-reduction, and wellness it is likely to have been encountered by study participants previously (Cavanagh & Wilkinson, 2002; Denner, 2009). Therefore the explanation of aromatherapy effects by prior conditioning cannot be completely dismissed. The effects of lavender aromatherapy due to prior conditioning might be potentially linked to changes in autonomic function such as lowering respiration rate consistent with relaxation and stress reduction. Improvements in working memory performance after inhaling lavender might be a little harder to explain by prior conditioning with the stress-reducing (and not cognitively enhancing) aroma.

Overall, the evidence provided by this work is most consistent with lavender essential oil or its components having a direct pharmacological effect (linalool is currently considered most likely responsible for lavender effects). For example, animal studies suggested linalool inhibits $GABA_A$ binding reception acting in manner similar to that of anxiolytics benzodiazepines that enhance GABA effects

(Cavanagh & Wilkinson, 2002). This is just one potential explanation for lavender calming and sedating effects observed in reduction of respiration rate. Cognitive facilitation on working memory task is likely secondary to calming and stress-reducing influences of lavender on the autonomic and central nervous system. The precise neurobiological mechanism of lavender action in humans remains unclear as to my knowledge no attempts to elucidate it have been taken. Potential mechanisms of lavender action suggested by rodent research might not be relevant to humans so further research addressing this issue is warranted.

Important concern in assessing utility of any intervention is its risk to benefit ratio. Aromatherapy in general and lavender aromatherapy in particular are known for low incidence of side effects (Butje et al., 2008; Denner, 2009; Wildwood, 1996). In the current study only two participants out of 92 reported adverse experiences (headache) during the study that were potentially linked to aroma presence. These participants were in different groups (one inhaled lavender and one inhaled coconut aroma) and had their headache subside at the end of the study. A recent systematic review of adverse events associated with aromatherapy found 71 cases of adverse events due to aromatherapy (lavender was among the essential oils used in the studies) ranging from mild to severe including one fatality (Posadzki, Alotaibi, & Ernst, 2012). However, other reports show that if aromatherapy used as directed with diluted essential oils and inhalation as the main method of administration, the incidence of side effects is low, and the side effects are usually mild, such as a change in alertness or agitation level or presence of headache (specific essential oils have specific side

effect profiles) (Fung, Tsang, & Chung, 2012). Still, assessing side effects associated with aromatherapy and evaluating potential interactions essential oils might have with other drugs are the topics that need to be addressed in subsequent studies. Current general agreement in literature is that risks associated with inhaling diluted lavender essential oil are low (Denner, 2009; Levenhagen, 2008; Perry et al., 2012) especially when compared to the side effects of currently available pharmacological treatments for anxiety and stress reduction such as benzodiazepines (Perry et al., 2012).

The role of expectancy in aromatherapy actions

In addition to observing effects of lavender aromatherapy that were not evident in placebo groups, several measures following stress exposure were affected by lavender and coconut aromas (i.e. perceptible aromas) in a similar way, emphasizing the role of aroma-mediated expectancy in aromatherapy actions. Additionally, several objective measures were influenced by the prime, a verbal suggestion of assigned aromatherapy effectiveness in reducing stress, highlighting the importance of verbally-mediated expectancy for aromatherapy actions.

Evidence for aroma-mediated expectancy effects. Aroma-mediated expectancy was likely playing a role in producing effects on two physiologic measures: EEG frontal asymmetry and chromogranin A levels (Chapter 2). For both of these measures patterns of change due to exposure aromas had more similarities between the detectable aromas than the patterns observed due to exposure to water. Furthermore, the results suggested that the effects of

aromatherapy on these two measures were affected by aroma intensity and pleasantness ratings. These results are in agreement with previous research that indicated that presence of a pleasant odor might affect EEG activity patterns differently than the presence of an unpleasant odor (Kline et al., 2000). Relative left frontal activation, observed in groups inhaling perceptible aroma, is linked to positive emotions and approach behaviors that might be arising due to the presence of a pleasant stimulus (aroma). Furthermore, previous research on aromatherapy indicated that changes in some measures (e.g. anxiety) were similar between a group experiencing aromatherapy and a group experiencing a pleasant smelling inert stimulus (hair conditioner) (Wiebe, 2000); comparable effects were observed in the current work with experimental and placebo aromas producing analogous effects.

Further, effects of the presence of a perceptible aroma on post-stress CgA levels observed in the current study are equivalent to those observed in Toda & Marimoto's research (Toda & Morimoto, 2008). However, that study only used two comparison groups (lavender and no odor group), and the conclusion suggested a specific stress-reducing effect of lavender manifested in changes in stress biomarker CgA. Our results expanded this conclusion and indicated that changes in CgA, indicative of stress reduction, arise from the presence of not just lavender but also in the presence of another pleasant aroma such as coconut. While to the best of the author's knowledge, coconut base oil is considered inert (Wildwood, 1996), and no known studies indicated specific pharmacological effects of coconut on stress markers, the possibility of coconut exerting specific

effects on some objective stress measures cannot be completely excluded. Therefore, to generalize the finding of aroma-mediated expectancy effects on EEG patterns and CgA levels due to the presence of any pleasant aroma, future studies should test and compare the effects of several pleasant aromas on objective measures including EEG FA and CgA. Additionally, effects attributable to aroma-mediated expectancy should be evaluated for other measures that were not included in the current study. Finally, the current work confirmed the importance of a perceptible placebo group to help distinguish non-specific effects of aroma presence. Perceptible placebo aroma condition is an important control that should be included when aromatherapy efficacy is assessed.

Evidence for verbally-mediated expectancy effects. Verbally-mediated expectancy was also found important in aromatherapy effects in current study and was related to several changes in objective outcome measures. Specifically, suggestion of the powerful stress reducing effects of assigned aroma (referred to as a prime) regardless of the actual aroma inhaled by a participant, was associated with reduced post-stress reaction times on the cognitive tasks assessing speed of processing and attention, as well as with decreased variability of response on the attention based task (Chapters 4 and 5). Additionally, those receiving a prime tended to show greater post-stress reductions in respiration rate compared to those receiving no prime (Chapter 3); however the effects of the prime on respiration rate were only evident for placebo aromas, and were not observed for lavender aroma. Power of suggestion has been previously assessed in aromatherapy research. Campenni and colleagues

(Campenni et al., 2004) showed that suggestions related to the effects of an odor (relaxing, stimulating, or none) played a significant role in influencing physiological measures (heart rate and skin conductance) in the direction predicted by the suggestion after exposure to ambient odors or lavender, neroli, and placebo. Similarly, expectancies enhanced by suggesting aroma effects rather than specific aroma effects accounted for changes in subjective and physiologic measures in more recent studies (Howard & Hughes, 2008; Kiecolt-Glaser et al., 2008). In one study participants reported subjective changes in mood, number of physical symptoms, and changes on task performance due to exposure to pleasant, unpleasant, or neutral ambient odor congruent with the suggested aroma effects when no actual aromas were present (Knasko, Gilbert, & Sabini, 1990). This study indicated that presence of aroma is not necessary for verbally-mediating expectancy to produce its effects. In agreement with this, prime effects on objective outcome measures in our study were observed regardless of the presence or absence of perceptible aroma. Furthermore, an aroma hedonic qualities were not linked to any of the effects associated with verbally-mediated expectancy enhancement. The variables that were related to the measures affected by verbal suggestion included expectancy of the aromatherapy stress-reducing effect and alertness level.

Interestingly, in the current study the effects of the prime suggesting stress-reducing properties of the assigned aroma affected performance on cognitive tests, particularly speed and variability of responses. These effects on cognitive performance due to verbally-mediated expectancy are distinct from the effects on

cognitive function observed due to lavender aroma exposure, indicating potentially different neural mechanisms underlying the responses to lavender and responses to the presence of verbal suggestion. Some studies suggest that expectancy effects might have an additive influence on the measures affected by the specific aroma (Johnson, 2011), but our data demonstrated that specific effects observed due to lavender exposure were not further enhanced by expectancies, and the effects due to aroma-or verbally-mediated expectancy were observed on different sets of measures. Therefore our results suggest that the mechanisms underlying aroma specific effects and underlying expectancy effects are independent.

In addition to the beneficial prime effect on speed of processing and attention based tasks, trends in ERP results were shown indicating that verbal suggestion of aromatherapy effect might affect brain function (Chapter 5). Specifically, N200/P300 waveforms appeared more preserved in those who received a prime.

The evidence linking verbally-mediated expectancy effects, ERP effects, improved performance on processing speed and attention based cognitive tasks, and alertness is consistent with verbally-mediated expectancy producing some effect on brain networks associated with attention. Exact mechanism of such effects needs to be elucidated in future studies.

Expectancy, placebo effects, and search for mechanisms

The results of the current work indicate that lavender aromatherapy might possess pharmacological properties affecting autonomic function measures and

cognitive processes. However, the data also supports the important role of expectancy effects (both aroma and verbally mediated) in aromatherapy actions. Please refer to Figure 10 for the summary.

Expectation of benefits due to verbal suggestion or context (e.g. presence of aroma) are important components of placebo effects, the effects observed after administration of an inert treatment or intervention (Benedetti, 2008). Historically placebo effects have been viewed as nuisance that needs to be controlled for to reveal the true effect of the intervention or treatment of interest. However, research confirms therapeutic benefits in some treatments such as antidepressants that can be largely attributed to placebo effects (Kirsch, 2009).

From previous studies (Campenni et al., 2004; Howard & Hughes, 2008; Knasko, 1995; Knasko et al., 1990) and current work it appears that many beneficial effects of aromatherapy may occur due to placebo effects. In recent years there has been a shift in attitudes towards placebo effects: instead of viewing placebo effects as an annoyance some researchers and clinicians are investigating ways to harness placebo effects due to their potential to produce real health benefits by simulating therapeutic intervention with minimal side effects (Price, Finniss, & Benedetti, 2008). Research to date has identified multiple placebo responses relevant to multiple clinical conditions; each of them is likely driven by a specific psychological and neurobiological mechanism that depends on the context for introducing the placebo (Price et al., 2008). The mechanisms of placebo responses relevant for reducing stress and anxiety are not well-researched, but previous studies suggested that neurochemical and

psychophysical responses resulting from placebo effects might be an example of reward processing with ventral basal ganglia dopamine neurotransmission serving as an underlying mechanism across various forms of placebo-associated expectations (Scott et al., 2007). Furthermore, previous research demonstrated that when a robust placebo response associated with anxiety reduction was observed, fMRI showed changes in regional blood flow in the anterior cingulate cortex and lateral orbotofrontal cortex (Petrovic et al., 2005). It is becoming clear that placebo responses associated with aromatherapy have actual biological effects on the brain and body and are more than response bias. Evaluation of exact mechanisms associated with placebo responses in aromatherapy is an important goal for future studies.

Limitations of the current work

As any research study, the current work has several limitations, and major limitations related to the choice of the study sample and study aromas are noted below.

First, participants in the current study were 50 years or older, primarily women, generally healthy but reporting at least moderate amount of baseline stress level. Our sample also included mostly people who were well-educated, interested in aromatherapy (with about half of the sample having previous aromatherapy experiences), and expecting positive effects from aromatherapy (as evidenced from Table 1, Chapter 2). Therefore, some or all of the findings regarding aromatherapy effects found in this study might not generalize to other

groups including people younger than 50, clinical populations, those naive to aromatherapy, and people with low expectations about this CAM approach.

Second, the essential oil and its concentration were study-specific. Many previous studies provided little or no details about essential oils used for aromatherapy and their concentrations; however specific information regarding essential oils and their composition is critical and might be explaining some of the differences observed in previous studies. For example, different lavender cultivars (e.g. *Lavandula angustifolia, Lavandula latifolia, Lavandula stoechas, or Lavandula x intermedia)* can have considerable differences in levels of the major essential oil constituents and, therefore, can have slight differences in therapeutic actions (Cavanagh & Wilkinson, 2002). Furthermore, even for the same cultivar, levels of major essential oil constituents might vary among different batches (Cavanagh & Wilkinson, 2002). Additionally, different concentrations of essential oils might have very specific effects on participants (Atsumi & Tonosaki, 2007). The information about the major constituents of the essential oil used in the current work is available in Appendix 1. The results of the current study might be most relevant to the doses and preparations of the aroma stimuli used in the current work and might differ from the results of other studies where different concentration or preparation of the lavender aromatherapy were utilized. To date, little effort has been made to standardize aromatherapy essential oils and base oils used in research and health care settings, and this is an important issue to address in the future.

Another limitation of this work has to do with the choice of perceptible placebo aroma. Virgin coconut base oil has been chosen due to its reputation for being an inert substance that is suitable to be used as the base oil for different aromatherapy preparations. Also, no known pharmacological stress-reducing effects are associated with coconut (Wildwood, 1996). Additionally, virgin coconut oil possesses a perceptible aroma that is typically considered pleasant. In this study coconut aroma was expected to serve as a control for the presence of a perceptible odor and was supposed to have similar hedonic properties to the experimental lavender aroma. However in reality, coconut aroma was rated similarly to lavender aroma on pleasantness but not intensity (Figure 2, Chapter 2) and was perceived and recognized by fewer participants compared to the lavender aroma (Chapter 4). Therefore, in our data, dissociating effects of aroma and effects of aroma intensity might be problematic. Furthermore, though coconut is not used as an active aromatherapy substance and is not known for stress-reducing effects (Wildwood, 1996), the possibility of some specific pharmacological effects due to coconut exposure cannot be completely excluded. Therefore, more studies using various pleasant smelling but inert substances must corroborate the findings related to aroma-mediated expectancy described in this work.

Overall, despite the limitations, this work contributes important evidence of aromatherapy physiologic and expectancy effects for stress reduction in a population that has increased vulnerability to stress (Graham et al., 2006).

Future directions

The results of the current study suggest a number of possibilities for future research on aromatherapy for stress reduction and in general.

The data from the current study indicate that different outcome measures might be sensitive to different aspects of aromatherapy (e.g. there might be aroma-specific effects due to constituents of essential oil, but expectancy effects associated with aromatherapy might affect processing speed, aroma hedonic qualities might influence the degree of physiological changes). To elucidate the mechanisms and systems involved into complex aromatherapy actions a wider variety of outcome measures (subjective, physiological, endocrine, immunological, cognitive etc.) relevant for specific type of essential oil (e.g. sedating vs. stimulating) should be tested when aroma-specific effects are assessed.

Our study provided some evidence for beneficial aromatherapy effects on cognitive function following stress in adults, most of whom were in their 50s. The possibility of using aromatherapy, the fast and easy intervention with low side effects profile, for enhancing cognitive function in general and in the face of stress should be explored further.

Furthermore, to understand the exact mechanism of aromatherapy action future studies must evaluate active essential oil constituents and their properties including pharmacology, toxicology, and interactions. Using multiple control groups including both perceptible and not perceptible aroma controls is critical to assess complex relationships of pharmacological and psychological effects.

Additionally, using brain imaging techniques might be of value to determine brain areas and networks sub-serving different aspects of aromatherapy experience.

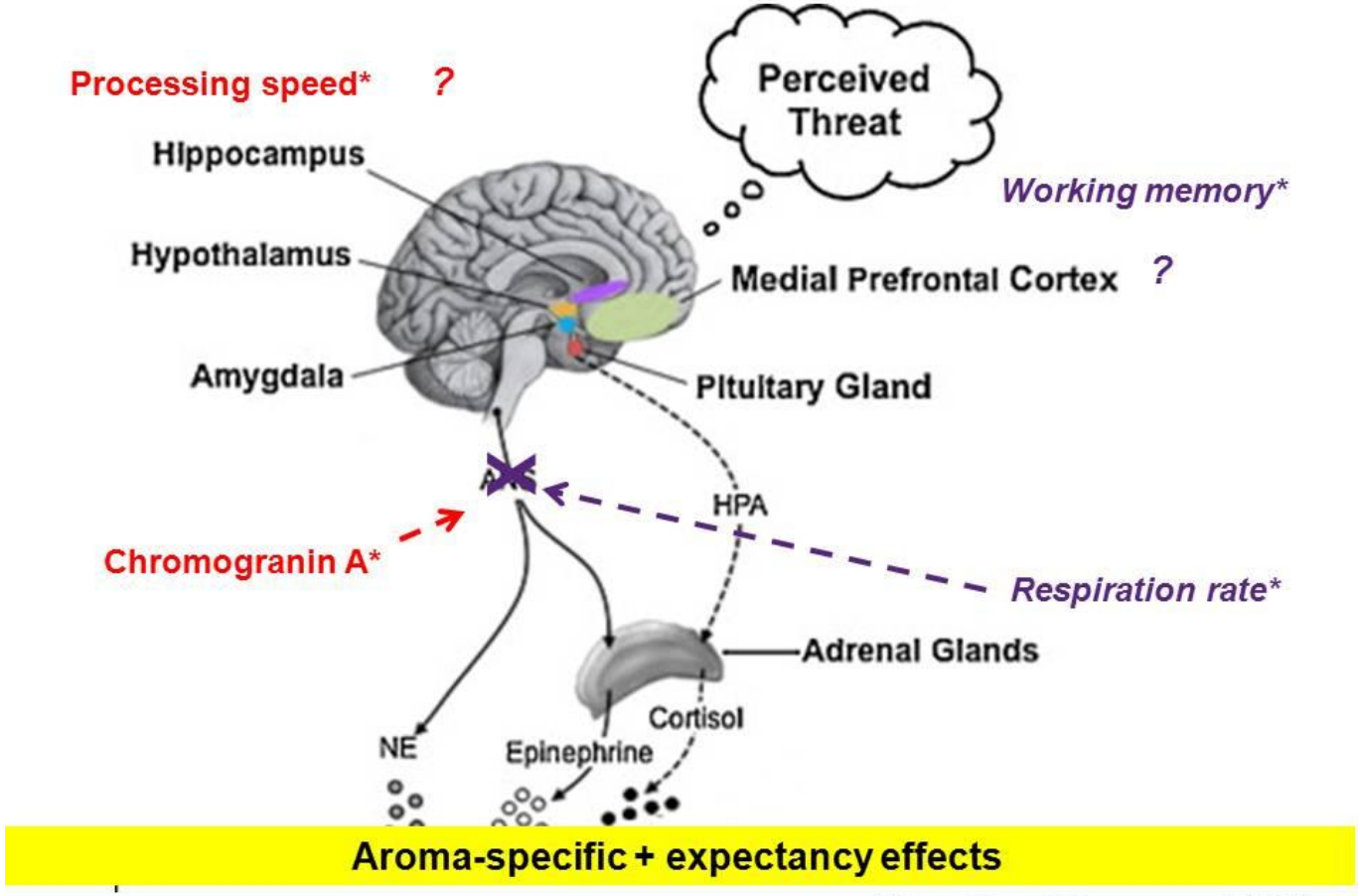
Finally, the current study did not aim to provide any in depth analyses of the personality and background characteristics of the participants that might have influenced the responses to the assigned aromas, but it is an important topic to evaluate in future investigations.

Overall conclusions

Aromatherapy is a popular CAM approach for several conditions including stress-reduction. Recent reviews of aromatherapy stated that evidence for aromatherapy efficacy is inconclusive due to low quality of prior studies. Some suggested that aromatherapy might be an ineffective treatment but an effective placebo (Bent, 2000). The current work employing a rigorous RCT design and addressing many of the previous problems evident in prior research suggests that aromatherapy can be both: effective treatment with aroma-specific effects on some physiological and cognitive functions and a powerful placebo producing effects on a separate set of physiologic and cognitive functions in part through expectancies enhanced by aroma presence and verbal suggestion of aroma effects.

Figure 10 legend. Aroma-specific effects of lavender (presented in lavender color) were observed on respiration rate and a working memory task following acute stress. Aroma-mediated expectancy effects were evident on chromogranin A levels following stress battery, and verbally-mediated expectancy effects were observed on a processing speed task (effects due to expectancy are presented in red color). According to the results, lavender aroma effects on respiration rate and aroma-mediated effect on CgA levels occur through influencing ANS. The mechanism of action for lavender-specific effects on working memory and verbally-mediated expectancy effects on processing speed are not clear. Overall, responses to aromatherapy arise due to a combination of aroma-specific and expectancy effects.

Figure 10. The role of aroma-specific and expectancy effects in shaping response to aromatherapy



Adapted from O'Donovan et al., 2013

Appendices.

Appendix 1. Consent and authorization form for the project.

Oregon Health & Science University

Consent and Authorization Form

IRB#: 6890

Protocol Approval Date: 10/29/2012

OREGON HEALTH & SCIENCE UNIVERSITY
Consent and Authorization Form

TITLE: Effects of stress-reducing aromatherapy

PRINCIPAL INVESTIGATOR:	Barry Oken, MD	(503) 494-8873
CO-INVESTIGATORS:	Irina Fonareva, BA	(503) 494-9520

PURPOSE:

Aromatherapy is a complementary and alternative medicine method that uses aromatic oils or aromas that people inhale to get health benefits. Aromatherapy is often used to relieve stress. The purpose of this study is to understand how different aromas affect the body. We will test effects of several different aromas on physical responses in people who experience stress. You have been invited to be in this research study because you are healthy, you are 50-85 years old, and you feel stressed. This study requires one visit to a research laboratory and will take a single visit to complete. One hundred and ten participants will be enrolled into this study at OHSU.

Your study data including body and brain measures, saliva, cognitive test results and questionnaire data may be stored indefinitely in a research repository and may be used in future research studies. We will ask you to sign a separate consent form for the repository.

PROCEDURES:

The study includes a telephone interview to determine if you can continue in the study. After the interview, you will have a clinic visit. At this visit, you'll have a brief test that involves smelling several aromas.

If the smelling test indicates you can continue in the study, you will be assigned to experience one of the study aromas at random—like tossing a coin. Three different aromas will be evaluated for their ability to relieve stress. This is a randomized study. You will not be able to choose which group you are in, and the research assistant giving the tests will not know which aroma you are experiencing. The study is done this way because knowing which aroma you experience can change the results of the study.

This clinic visit will take about 3-4 hours. There are three parts to this visit—baseline evaluation, laboratory tasks, and final evaluation.

Baseline Evaluation

First, we will attach electrodes, or small metal discs, to your head to record your brain waves. You will wear a cap with electrodes. We will use some gel so that the metal disks can connect with the scalp to measure the brain waves. We wipe the gel out at the end of the visit, but it will not come out completely until you take a shower. We will also attach some electrodes to your body to measure your body responses such as heart beat and skin response during different tasks you do at your visit. You will also have a blood pressure monitor on your finger to evaluate your blood pressure during the visit. We will record all these physiologic measures for the duration of the visit. Additionally, several times during your visit we will ask you to sit quietly with your eyes closed for 5 minutes so that we can record your brain activity when you are not doing any task.

Next, you will complete several questionnaires about your health, demographic information, previous aromatherapy use, emotions, and personality.

You will also take several brief tests of attention and memory. Some of these tests will be done using a computer.

Then you will be given your assigned aroma to smell for 5 minutes. After initial 5 minutes of inhaling the aroma you will continue to smell it during the rest of the visit while completing several laboratory challenge tasks.

Laboratory Challenges

While you continue smelling the aroma you will complete several tasks. After each of these tasks we will ask you to evaluate your stress level.

Physical challenge task: You will put your hand into a bucket with icy cold water and will keep it there for as long as you can tolerate the discomfort. We will give you a scale to rate you discomfort during the task.

Emotional challenge tasks: You will look at unpleasant images that might be upsetting or distressing.

Cognitive challenge task: You will continue with cognitive testing evaluating your attention, memory, and reaction time.

If any of these tasks seems too distressing or uncomfortable for you to complete you can stop at any time and move on to another task.

Final Evaluation

At the end of the laboratory challenge tasks you will complete several questionnaires about your emotions and the aroma you experienced. At the end of your visit we will take off all electrodes and other recording equipment from your head and body. You will also participate in a brief smell identification test. Before you leave a research assistant will talk with you about the aroma you experienced during the study.

During each part of the study we will collect several samples of your saliva to look at different substances your body releases in response to stress. We will also ask you to rate your stress level when your saliva sample is taken.

Laboratory visit activities

Activities	Screening	Initial evaluation	Laboratory tasks	Final evaluation
Screening and medical history	20 min			
Smell identification tests	3 min			7 min
Physiologic recording (brain waves, heart rate, blood pressure, skin response)		5 min	5 min	5 min
Saliva samples		5 min	5 min	5 min
Smelling assigned aroma		5 min	continuous	
Physical challenge: submerging hand in cold water			5 min	
Emotional challenge: Viewing unpleasant images			10 min	
Cognitive challenge: tests of attention and memory		25 min	30 min	
Questionnaires about emotions and personality		20 min		20 min
Information session with a research assistant				5 min
Total time	30 min	60 min	55 min	35 min

If you have any questions regarding this study now or in the future, contact Dr. Barry Oken at (503) 494-8873 or other members of the study team at (503) 494-5650 or (503) 494-9520.

RISKS AND DISCOMFORTS:

After the visit, your hair will have traces of gel used to connect electrodes to your scalp. We will wipe the gel after the visit, and the rest will be washed away when you take a shower.

There are no known medical risks to providing a sample of saliva (spit). You may choose not to provide this if you feel you cannot or if it would cause discomfort, due to dry mouth, for example.

You might experience some discomfort if you find the smell of your assigned aroma unpleasant.

Our questionnaires may cause some anxiety. Some of the questions may seem personal or embarrassing. You may refuse to answer any of the questions that you do not wish to answer.

Our cognitive tests may cause fatigue and some anxiety about performance. This may upset you. You can stop any test at any time if you do not wish to continue with it.

Our laboratory challenge tasks may make you upset, physically uncomfortable, or feeling pressured for time for short period of times. Looking at the upsetting images might make you uncomfortable. If you do not want to continue with any of the study tasks you can stop the task at any time.

Although we have made every effort to protect your identity, there is a small risk of loss of confidentiality. If the results of these studies were to be accidentally released, it might be possible that the information we will gather about you as part of this study could become available to an insurer or an employer, or a relative, or someone else outside the study. Even though there are discrimination protections in both Oregon law and Federal law, there is still a small chance that you could be harmed if a release occurred.

BENEFITS:

You will not benefit from being in this study. However, by serving as a subject, you may help us learn how to benefit people experiencing stress in the future.

ALTERNATIVES:

You may choose not to be in this study.

CONFIDENTIALITY AND PRIVACY OF YOUR PROTECTED HEALTH INFORMATION:

We will not use your name or your identity for publication or publicity purposes.

If you sign this form, you are agreeing that OHSU may use and disclose protected health information collected and created in this research study. The specific health information and purpose of each use and disclosure are described in the table below:

Health Information	Purpose(s)
THE FOLLOWING CHECKED ITEM(S) WILL BE GENERATED/COLLECTED DURING THE COURSE OF THIS STUDY:	
☒ History and physical examinations	a, d, e
☒ Bioelectric Output (e.g., EEG, EKG, EDR)	a, d, e
☒ Questionnaires, interview results, focus group survey, psychology survey, behavioral performance tests (e.g., memory & attention)	a, d, e
☒ Other: Saliva	a, d, e

Purpose Categories

a. To learn more about the condition/disease being studied
b. To facilitate treatment, payment, and operations related to the study
c. To comply with federal or other governmental agency regulations
d. To bank for future research
e. Other To analyze research results

The persons who are authorized to use and disclose this information are: Oregon Clinical and Translational Research Institute (OCTRI) staff, all investigators listed on page one of this form and others at OHSU who are participating in the conduct of this research protocol, and the OHSU Institutional Review Board.

The persons who are authorized to receive this information are representatives of the Office for Human Research Protections, and the National Center for Research Resources.

We may continue to use and disclose protected health information that we collect from you in this study indefinitely.

While this study is still in progress, you may not be given access to medical information about you that is related to the study. After the study is completed and the results have been analyzed, you will be permitted access to any medical information collected about you in the study.

You have the right to revoke this authorization and can withdraw your permission for us to use your information for this research by sending a written request to the principal investigator listed on page one of the research consent form. If you do send a letter to the principal investigator, the use and disclosure of your protected health information will stop as of the date he receives your request. However, the principal investigator is allowed to use and disclose information collected before the date of the letter or collected in good faith before your letter arrives. If you withdraw any tissue or blood samples that were collected from you, they either will be destroyed or stored

without any information that identifies you. Revoking this authorization will not affect your health care or your relationship with OHSU.

The information about you that is used or disclosed in this study may be re-disclosed and no longer protected under federal law.

The information you give us will be kept confidential. We will not use your name or your identity for publication or publicity purposes.

COSTS:

There will be no cost to you for participating in this research. We will reimburse you for your time spent participating in this study at a rate $10.00 per hour.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Dr. Barry Oken at (503) 494-8873.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

It is not the policy of the federal funding agencies to compensate or provide medical treatment for human subjects in federally funded studies.

PARTICIPATION:

If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

The investigator, Dr. Barry Oken (503) 494-8873, has offered to answer any other questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

If in the future you decide you no longer want to participate in this research, we will destroy your saliva sample. However, if your samples are already being used in an on-going research project and if their withdrawal jeopardizes the success of the entire project, we may ask to continue to use them until the project is completed.

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

At his discretion, the investigator may terminate your participation prior to study conclusion. This termination could be for your health and safety, or for lack of compliance (for example, if you do not follow instructions).

The participation of OHSU students or employees in OHSU research is completely voluntary and you are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course.

We will give you a copy of this form.

SIGNATURES:

Your signature below indicates that you have read this entire form and that you agree to be in this study.

_____
Subject's Printed Name

_____ _____
Subject's Signature **Date**

_____ Place your initials here if you are willing to be contacted by research staff for future study opportunities.

_____ _____
Signature of Person Obtaining Consent **Date**

Appendix 2. Debriefing information for the study participants.

eIRB# 6890, P.I. Barry Oken, MD

Study information

Thank you for volunteering to be in this study. We need to inform you that we were not completely honest about the details of this study. In this study in addition to evaluating effects of aromatherapy on stress we also assessed whether *expectancy* or *placebo* effects could play a role in aromatherapy actions for reducing stress.

You may have heard of the "placebo effect" or "expectancy effect." A placebo is a fake treatment or a pill, like a sugar pill. The placebo effect occurs when a fake treatment is given to a person, and that person has a response to the fake treatment similar to the response expected from a real treatment.

Sometimes people's health improves simply because they expect their health to improve. Scientists have shown that there are brain changes produced by giving placebos. These brain changes include brain cells making specific chemicals or increasing their activity. If we can understand how the placebo effect can make changes in your body, it may lead to improved ways to study effects of different drugs and treatments such as aromatherapy. This knowledge can also help us better understand the relationship between mind and body, and it may suggest ways people can improve their own health.

In order to measure the placebo effect for reducing stress after aromatherapy, it is necessary for us to tell some of our participants that they would get a potent stress-reducing aroma when they really got a placebo aroma in order to maximize their expectancy. We then compare the difference between people who inhaled a stress-reducing aroma and those who inhaled a placebo aroma. Unfortunately, if we were to inform you about the true intent of the study at the start, the experiment would not be possible because people's awareness of the intention of the study would change the study results.

The issue of honesty with research participants is taken very seriously. We have been doing these types of studies in people with Alzheimer's disease and in healthy people for several years. Although aromatherapy may be helpful in stress management, we want to learn if the placebo effect might play a part in its effectiveness.

We very much appreciate your willingness to participate in research and thank you for your time and cooperation. We feel you have contributed to the advancement of scientific knowledge in an important clinical area. We hope that you feel your participation has been worthwhile and we apologize for any ill feelings you may have regarding the deception. As mentioned in the consent form, you may choose to withdraw from this study at any time, and your data will

be excluded from the study. If you have any questions or concerns about your participation in the study, please contact the lead investigator, Barry Oken, MD, at (503) 494-8873, or the OHSU IRB at (503) 494-7887. If you are distressed about this study, there is a patient advocate available to discuss any further concerns you may have regarding your participation in this research.

Please do not tell anyone who might be a prospective study participant that this is a placebo study, or any of the details.

Appendix 3. Analytical certificate for lavender essential oil used in the study provided by the vendor, Mountain Rose Herb (Eugene, OR, USA)

ANALYTICAL CERTIFICATE

LAVENDER OIL ORGANIC

PHYSICO-CHEMICAL PROPERTIES ACCORDING BULGARIAN STATE STANDARD

1. Appearance	Fluid transparent liquid
2. Colour	Pale yellow
3. Odour	Characteristic of lavender

4. **Characteristic components**
(by GC – analysis), %

- Linalyl acetate	37,48
- Linalool	26,60
- cis- beta – Ocimene	5,67
- Terpinen – 4 – ol	3,86
- Lavandulyl acetate	2,69
- trans – beta – Ocimene	3,15
- Lavandulol	0,51
-1,8 – Cineole	0,61
- Camphor	0,22

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