THE WUTC THRESHOLD TEST: A NEW PERSPECTIVE ON OLFACTORY ASSESSMENT

By

William Arthur Tewalt

Approved:

Irene N. Ozbek
Professor of Psychology
(Chair)

Michael Biderman
Professor of Psychology
(Committee Member)

Manuel Santiago
UC Foundation Professor of
Chemistry
(Committee Member)

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William Arthur Tewalt

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ABSTRACT

The WUTC Threshold Test is a new test of olfactory ability that focuses on the idea that deficits in olfactory ability are not necessarily generalizable to all odors. Though numerous diseases and disorders have been shown to lead to a loss of olfaction, tests of olfactory sensitivity have been limited to performance detecting a single odor. The WUTC is comprised of five odors that were selected based on differences in how they interact with the olfactory system and the chemical properties they possess. By utilizing a diverse odor profile, relationships between olfactory deficits to certain odors and specific diseases can be explored. The test also employs randomized, multiple presentation of odorants along with null-stimulus trials. Using this methodology, statistical measures of participant sensitivity, response-bias, threshold, and inter-rater reliability can be calculated with a single administration of the test. A pilot study, consisting of thirty three (N=33) participants, was conducted. Subject demographic data was also collected in order to conduct exploratory analyses and aid in the further development of the test. The reasoning and methodology of the WUTC Threshold test are discussed along with the analyses of the subject data. The results of this pilot study suggest that certain ailments do not have significant olfactory deficits to all odorants, only particular odor molecules. The principles behind the development of the WUTC Threshold Test may lead to the further understanding of links between olfaction and disease and an increase in the value of examining olfactory ability in a clinical setting.

DEDICATION

For Lauren and Starla, my two favorite people in the world.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

The testing of olfactory sensitivity is something that is seldom used in a clinical setting, yet it has provided very clear and measurable capability as a marker of numerous diseases. In some cases, tests of olfaction can predict future clinical diagnoses of disease better than more expensive and invasive measures. Many patients that are currently experiencing a loss of olfactory sensitivity due to a disease or disorder may not even be aware that any loss has occurred, making regular olfactory testing even more important. However, no olfactory test has yet been able to distinguish between centrally or peripherally caused deficits. Current olfactory threshold tests concentrate solely on how sensitive a participant is to a single odor but have not explored interactions between different types of odors and their ability to be detected by those with certain diseases. Both 'Sniffin' Sticks' and the Connecticut Chemosensory Clinical Research Center Test (CCCRC), popularly used tests of olfactory ability, employ threshold tests in their design that only test for the odor *n*-butanol (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997). By developing a test with odorants that are selected based on the diversity of how they interact with the physiology of the olfactory system, it may be possible to determine the pathological cause of the deficit instead of only identifying that a deficit exists.

According to N. A. Macmillan and Creelman (2004), "one way to characterize the shift in the attitude of psychologists toward their work that came with the cognitive revolution is as a decline in interest in "the stimulus"." This paper focuses on building a foundation for testing

odor sensitivity that centers on stimuli. First, a review of relevant literature concerning specific diseases and disorders characterized by olfactory deficits is presented along with the most commonly used tests of olfactory ability at the present. Secondly, by exploring the physiological changes that those with olfactory deficits undergo, an attempt is made to bring to light how the specific molecular properties of certain odors could cause them to be less detectable by individuals undergoing particular physiological changes. Next, a full assessment of the various methods of testing and measuring stimulus detection is completed. By using previous research as a basis, a new test of olfaction is offered that considers each of the reviewed topics in its construction and odor selection while more closely following standards of research methodology than currently available odor threshold tests. Finally, an analysis and discussion of the results of the initial pilot study of the WUTC threshold test is completed.

Causes of Olfactory Dysfunction

Olfactory impairment can come from a multitude of different sources. In fact, there are more than two hundred known conditions that can lead to changes in chemosensory ability. Table 1.1 shows that among these conditions, aging, exposure to toxic substances, obstructive nasal and sinus diseases, head trauma, respiratory infection, congenital, and psychiatric disorders are the most common to result in loss of olfaction, though causes can often be idiopathic.

Table 1.1 Reported Causes of Olfactory Loss

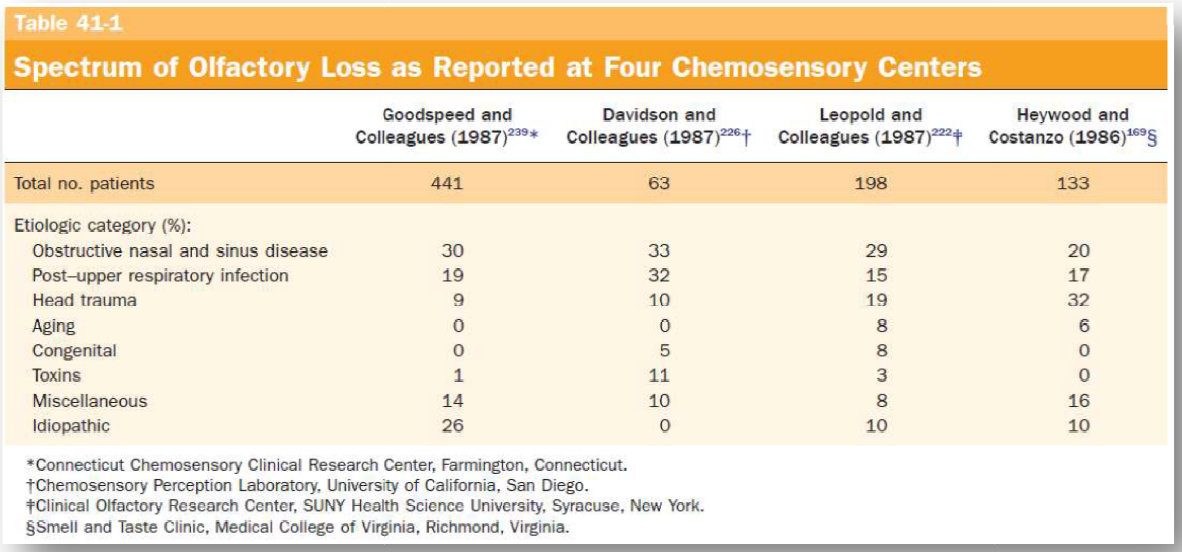


Table 41-1

Spectrum of Olfactory Loss as Reported at Four Chemosensory Centers

	Goodspeed and Colleagues (1987)[239]*	Davidson and Colleagues (1987)[226]†	Leopold and Colleagues (1987)[222]‡	Heywood and Costanzo (1986)[169]§
Total no. patients	441	63	198	133
Etiologic category (%):				
Obstructive nasal and sinus disease	30	33	29	20
Post–upper respiratory infection	19	32	15	17
Head trauma	9	10	19	32
Aging	0	0	8	6
Congenital	0	5	8	0
Toxins	1	11	3	0
Miscellaneous	14	10	8	16
Idiopathic	26	0	10	10

*Connecticut Chemosensory Clinical Research Center, Farmington, Connecticut.
†Chemosensory Perception Laboratory, University of California, San Diego.
‡Clinical Olfactory Research Center, SUNY Health Science University, Syracuse, New York.
§Smell and Taste Clinic, Medical College of Virginia, Richmond, Virginia.

Note: From Walton and Maeso (2012)

It has been known for many years that a person's ability to smell is directly related to his or her health. Diseases such as Alzheimer's (Dvand, Michaels-Marston, Liu, Pelton, Padilla, & Marder et al. 2000) (Murphey, Gilmore, Seery, Salmon, & Lasker, 1990), Parkinson's (Ross & Abbot, 2005), schizophrenia (Turetsky, Hahn, Borgmann-Winter, & Moberg, 2009) and depression (Negois, Croy, Gerber, Puschmann, Petrowski, Joraschky, & Hummel, 2010) have each been shown to have the reduction in olfactory ability as a comorbidity. Patients with kidney disease undergoing dialysis have also repeatedly been shown to have drastic decreases in their sense of smell. With End Stage Renal Disease (ESRD), patients commonly experience complete anosmia (the inability to detect odors) (Frasnelli, Temmel, Quint, Oberbaur, & Hummel, 2002). In addition, cases of concussion and various types of head trauma have shown to result in altered olfaction (MacCaffrey, 1997). For those who have experienced head injury, tests of olfaction have been shown to be the most sensitive measure of whether any residual neurological impairment exists (Ruff, Ruff, & Wang, 2008).

Additionally, complaints about olfactory ability often arise in patients with depression and schizophrenia as well as disorders characterized by hallucinations. These hallucinations experienced by patients can often be olfactory. This results in patients either believing that an odor is emanating from their own body (intrinsic) or from the environment (extrinsic) (Deems et al., 1991). These chemosensory distortions often lead to decreases in overall quality of life as they can be severe enough to cause disruptions to a patient's daily life and health.

Another known cause of loss of olfactory functionality is nutritional deficiency. In particular, a lack of vitamin A removes the body's ability to repair damage to the nasal epithelium. Duncan and Briggs (1962) have reported that over time, white rats will eventually become anosmic when fed a vitamin A deficient diet. Conversely, the supplementation of vitamin A has been shown to have the ability to partially restore lost olfactory ability (Duncan & Briggs, 1962).

Changes in olfaction emerge in diseases with very dissimilar pathologies. Though some suggest neurological origins of olfactory loss, others point to alterations in the mechanisms of olfactory function. However, little is known about the causes of smell disorders.

Oxidative Stress

A concept that, in many ways, unifies the theme of olfactory dysfunction and disease is "Oxidative stress". It has been linked to numerous diseases and disorders as well as aging and has similarly been shown to be related to olfactory dysfunction.

Oxidant stress occurs when there is an overabundance of free radical oxygen within the body. In part, this is a consequence of natural bodily functions. During the process of respiration, 80 to 90 percent of molecular oxygen (O_2) is transported to cellular tissue and utilized by the

mitochondria to create energy in the form of adenine triphosphate (ATP). However, as a natural byproduct of the reaction, small amounts of radical oxygen are produced. This oxygen naturally reacts with a hydrogen that is removed from a helper molecule known as nicotinamide adenine dinucleotide (NADH) during the process of respiration. As a result, water is produced within the cell. However, in addition to water, the oxygen intermediate products superoxide (O_2^{*-}), peroxide (O_2^-), Hydrogen Peroxide (H_2O_2), and hydroxyl radical ($^*OH$) are also produced (Halliwell, 1992). Radical oxygen within the body is known as a Reactive Oxygen Species (ROS) and these intermediate products are considered the primary forms they take on (Wu et al).

Although the mitochondria is the primary source of natural ROS production in humans (Wu & Cederbaum, 2003), additional sources of ROS include enzymatic processes within the liver and cells. However, not all oxidant stress is caused solely as a natural byproduct of respiration. External factors such as carbon monoxide exposure caused by smoking has been shown to significantly increase levels of oxidative stress (Lopez et al., 2009) as has alcohol abuse (Wu & Cederbaum, 2003). Additional contributors include radiation, UV light, and air pollution as well as certain types of medications. Increasingly, external causes of ROS are being discovered and researched.

ROS pose a danger to people due to the number of normal bodily and cellular processes they take part in and interact with. ROS within the body have the ability to react with various cellular molecules including deoxyribonucleic acid (DNA), proteins, and lipids. Often, ROS cause degradation to these molecules which, in turn, can lead to a change in or disruption of important cellular processes that take place within the body. Additionally, ROS-induced damage to DNA and mitochondrial DNA (mtDNA) has repeatedly been shown to occur. Oxidative damage to mtDNA has been linked to multiple diseases such as neuronal degeneration and

cardiovascular disease (Tritschler & Medori, 1993) as well as to increase with aging (Ames, Shigenaga, & Hagen, 1993).

Though oxidative stress damage has displayed the ability to degrade many types of proteins in the body, the protein apolipoprotein E (apoE) has gained interest due to its believed disruption of several important bodily functions such as cognitive processing and immunoregulation (Evola, Hall, Wall, Young, & Grammas, 2010). Deficiencies in apoE have been shown to lead to lower levels of cognitive performance in mice.

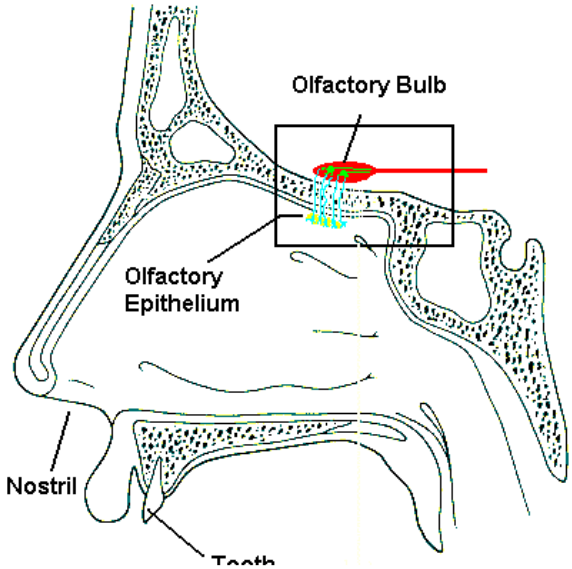
Importantly, levels of oxidative stress in the body have been shown to be significantly correlated with numerous diseases and disorders that are characterized by decreased olfactory ability such as Alzheimer's, Parkinson's, and uremia diseases. Higher than normal levels of oxidants "in vivo" have been linked to early onset dementia (Reutens & Sachdev, 2002) and also been shown to precede the principle pathologies of Alzheimer's disease (Perry, Cash, & Smith, 2002) as well as contribute to the creation of senile plaques (Misonou, Morishima-Kawashima, & Ihara, 2000), one of the hallmarks of Alzheimer's disease. Additionally, current research on the subject has found that oxidant stress can lead the creation of inflammatory proteins in the brain (Evola et al., 2010). Inflammation of these proteins causes destabilizing effects on cerebral circulation and blood-brain barrier leakage that can lead to the impairments of learning and memory (Evola et al, 2010). According to Himmelfarb, Stenvinkel, Ikizler, and Hakim (2002), oxidant stress may also be the concept that unifies the prevalence of cardiovascular disease in uremia, a condition marked by a high level of nitrogenous waste in the blood that accompanies renal failure as well as decreased olfactory ability. Those with kidney disease often experience anosmia, or a complete inability to detect the presence of any odor.

The discussed side effects of oxidative stress are very important to olfaction as nearly all diseases with oxidative stress show olfactory impairment as a side-effect. Disruptions to normal bodily function by protein inflammation and/or cellular damage could cause olfactory dysfunction to manifest. Lavin et al. (2013) have shown that, in patients with high levels of inflammation in the olfactory neuroepithelium, decreases in olfactory sensitivity was found to be a better predictor of the inflammation than computed tomography (CT) and endoscopic observation. The cause of this may be linked to the inflammation of Odorant Binding Protein's (OBP's) that exist in the neuroepithelium.

Odorant Binding Proteins

OBP's are extremely important to the physiology of olfaction and disrupting their normal function would lead to a decrease in olfactory ability. However, the level of disruption would be dependent on the nature of the odorant that was being smelled as some molecules require OBP's more than others.

Every molecule can be described in terms of its hydrophobicity. This describes the degree to which a molecule is repelled by water. Molecules that are completely hydrophobic are completely insoluble in water, lacking the ability to mix in any proportion. On the other end of the spectrum, completely hydrophilic molecules are miscible, or soluble in water in all proportions. This is an extremely important concept in the biology of odor detection. Odorant molecules that are hydrophilic are able to pass through the water-soluble membrane of the nasal epithelium and move on to the odorant receptors (Vogt, Prestwich, & Lerner, 1991). However, hydrophobic molecules are unable to pass through the epithelium and require an Odorant Binding Protein to carry them across and to the receptor (Vogt et al., 1991). To do so, the OBP



Note: Adapted from (Murray, 2013)

Figure 1.1 Image of the Olfactory Epithelium and Olfactory Bulb. (Murray, 2013)

uses a method of facilitated diffusion where it essentially "solubilizes" molecules that are more hydrophobic.

Under situations (such as oxidative stress) where OBP's face inflammation and decreased functionality, a natural interruption of the transport of hydrophobic odors across the epithelium would occur and lead to a lowered ability to detect their presence.

Detection Theory

Signal Detection Theory (SDT), or simply "Detection Theory", was developed by David Green and John Swets as a psychophysical approach to the construction and analysis of detection experiments (N. A. Macmillan & Creelman, 2004). Though first developed to deal primarily with tests involving the ability to differentiate auditory stimuli from background noise, SDT has changed over time to incorporate a broader range of analyses. Modern Signal Detection Theory now includes a pool of information that encompasses tests of memory, cognition, and, of course,

sensory ability. In fact, this evolution from early SDT has led to the omission of the word "signal", leaving the collection of methods to be called simply *detection theory*.

One-interval Design

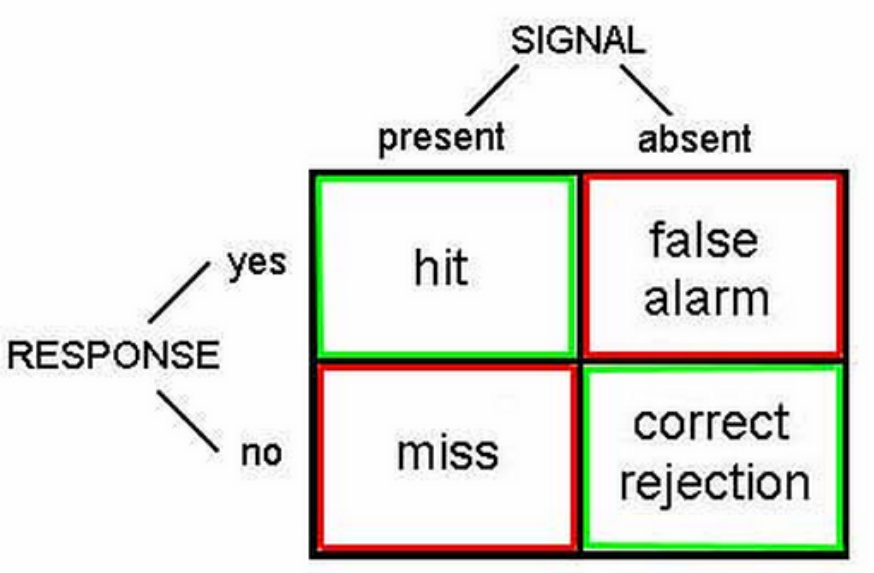
Though detection theory is responsible for the development of multiple design strategies for use in measuring sensory performance, the focus of this research is on the one-interval design. This type of design involves the presentation of a single stimulus to a subject on each trial of the test. The stimulus itself has the possibility of being one of a subset of differing stimulus types, depending on the design of the experiment (in this project, an odor). By utilizing variations of the one-interval design, an experiment can investigate drastically different measures of sensory performance. A use of this design is in measuring discrimination (N. A. Macmillan & Creelman, 2004). This describes the ability to distinguish a stimulus from another, different stimulus type. An example would be distinguishing a sweet odor from one that has a pungent scent. There are two types of discrimination tasks, the first of which is termed "detection". In SDT, detection task trials contain a stimulus as well as a null-stimulus and the participant must determine which they are currently being presented with. However, a discrimination task that does not contain a null-stimulus produces a performance measure termed "recognition" as a participant must attempt to recognize which of multiple stimuli is being presented. Finally, one-interval experiments can take the form of measuring the ability to identify/classify stimuli. In this task, stimuli differ from each other in only one characteristic which must then be "identified" by the participant upon the stimulus presentation.

One-interval designs can be used for diverse applications depending on the types and number of stimuli classes used in the experiment. While this type of experiment involves the

presentation of a single stimulus for each trial, there are other methods of evaluating discrimination available. In particular, a popular alternative choice to the one-interval design is the "Two-alternative forced choice" (2AFC) test (N. A. Macmillan & Creelman, 2004). While still a test of discrimination, participants in a 2AFC test are presented with two stimuli per trial that are randomly separated by time or position. Though it can be viewed as an extended one-interval design, this type of test is considerably different in that a participant is not being asked to discriminate between stimulus type, but instead by stimulus order. When increasing beyond the presentation of two stimuli in a single discrimination trial, the experimental design adopts the name or the *m*-alternative forced choice (*m*AFC) where the value of *m* represents the number of choices presented in each trial (N. A. Macmillan & Creelman, 2004).

Yes-No Trial

For the aforementioned one-interval design that is set up for the purpose of measuring stimulus detection ability, only one of two responses is possible for each trial. These responses are "yes" and "no". When responding to each trial in a detection experiment, a participant's answer can ultimately be categorized as one of four types of events. These include the hit, miss, false rejection, and correct rejection.



Note: Adapted from D. Heeger (1998)

Figure 1.2 Decision Making Outcomes

The goal of a yes-no experiment is two-fold: 1) to compare participant responses to the type/level/degree of the stimulus, and 2) determine the amount of bias present. The first of these goals focuses on what is termed *sensitivity*, or the measurement of a participant's ability to discriminate between stimuli. In terms of detection, a person with high sensitivity has a greater ability to detect stimuli than one with poorer sensitivity. The second of these goals, determination of bias, involves measuring a participant's inclination to answer "yes".

Yes-no experimental designs are unfortunately very susceptible to the effects of participant response-bias. Other experimental methods of measuring detection, such as the 2AFC and mAFC designs, have more recently become widely adopted due to their minimal response bias. However, these paradigms often take considerably longer to administer and have been found to be more statistically biased than yes-no experimental designs (Kershaw, 1985). This is due to 2AFC and mAFC having truncated ranges in their psychometric functions compared to the maximized range of a yes-no design.

Threshold

In addition to measures of sensitivity and response-bias, one-interval detection experiments also provide the ability to estimate a "threshold", or the "magnitude of the weakest detectable stimulus" (N. A. Macmillan & Creelman, 2004). Estimations of stimulus thresholds can be produced in a variety of ways and this variety is dependent in part on the method of stimulus administration used by the researcher. The order of stimulus presentation for each trial of a test often takes one of four possible forms; 1) Increase of stimulus level from lowest to highest, 2) Decrease stimulus level from highest to lowest, 3) a type of stair-case method that alternates between a high and low stimulus magnitude, and 4) randomized stimulus level.

The "threshold" is often defined as a stimulus which is detected, or responded *yes* to, on 50% of the trials given. One method for determining an individual's threshold for a stimulus is the use of logistic regression represented by the function (N. A. Macmillan & Creelman, 2004):

$$\lambda(x) = \frac{e^{x-\mu}}{\left[1+e^{x-\mu}\right]^2}$$

The use of the function can be used to determine predicted probabilities for each level of a stimuli which can then be used to find an estimated threshold value. The threshold is the stimulus deemed to have a value that corresponds to a p-value of .5 on the sigmoid curve (s-curve). A visualization of the s-curve is provided in Figure 1.3. When comparing thresholds, lower values indicate a better ability to detect stimuli.

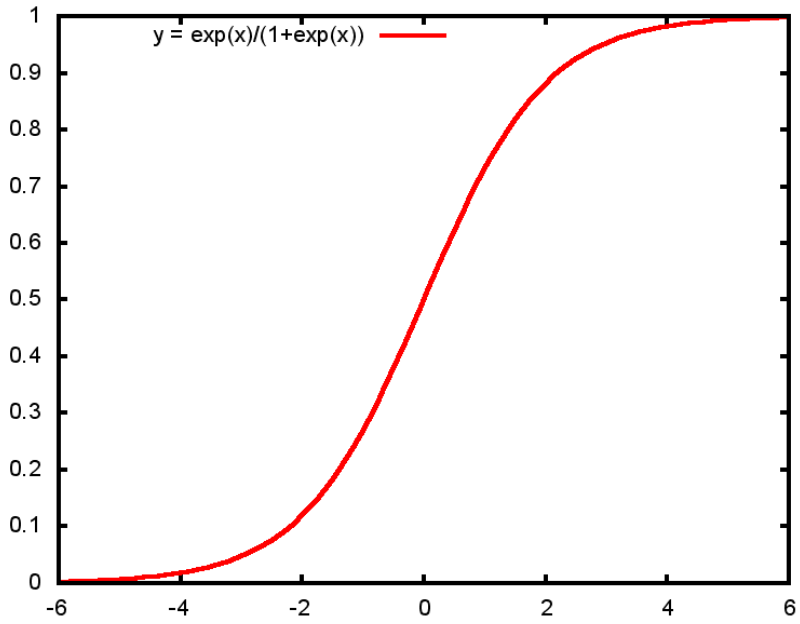


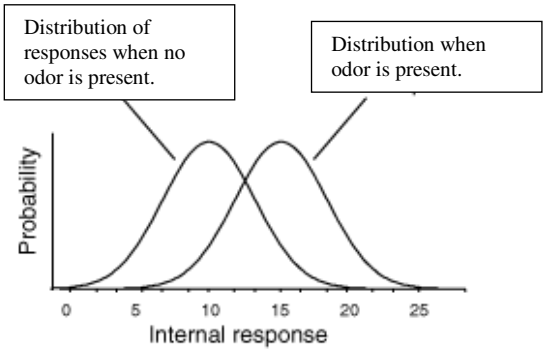
Figure 1.3 The Sigmoid Curve (s-curve)

A benefit of using a threshold model is that, unlike measures of sensitivity, it gives an actual calculated level of the lowest stimulus detectable.

Noise

The term "noise" represents anything that compromises the ability to detect a stimulus by introducing a level of uncertainty on whether or not the stimulus is present (N. A. Macmillan & Creelman, 2004). Two types of noise can ultimately contribute to a level of uncertainty. These are *internal* and *external* noise. External noise can come from many different sources depending on the nature of the discrimination test but are often factors that exist in the environment. Examples of noise could be static in a test of auditory detection or an odor in the testing area of an olfactory test. Internal noise, however, is a result of both cognitive and sensory components within the participant that lead to uncertainty or error.

In all detection tests, a stimulus trial always represents stimuli plus noise and a null-stimulus trial would be the occurrence of only noise. Figure 1.4 shows how the presence of noise can result in a lower signal to noise ratio.



Note: adapted from (D. Heeger, 2003)

Figure 1.4 Signal plus Noise

Due to the existence of noise in all trials of a test, it is extremely important to make all efforts to minimize any noise present and to attempt to keep testing conditions consistent across all trials and participants. For any test of stimulus detection, it is often the goal of the researcher to attempt to create a noise-free environment that results in the greatest signal-to-noise ratio possible, thereby enhancing the ability to measure signal discriminability.

Measures

There are multiple measures that can be used to describe olfactory ability.

Sensitivity

In SDT, several statistics are commonly used to describe different facets of a participant's detection ability. The first, a sensitivity measure index known as *d'*, is considered to be a pure measure of sensitivity that is unaffected by any response bias as long as the signal and noise

distributions are both normal (Swets, 1986). The calculation of *d'* provides a measurement of the difference between the signal and noise means in standard deviation units. Therefore, a *d'* value of zero (0) indicates an inability to distinguish between the signal and noise trials and positive values represent increasing levels of sensitivity. Participants that are unable to discriminate between stimuli and false positive and obtain identical hit (H) and false positive (F) rates, H=F, will therefore obtain a *d'* equal to zero. However, problems arise when H=1.0 as this causes *d'* values to become infinite, regardless of the proportion of false-positives they had. Fortunately, there are multiple methods of fixing hit-and-false positive rates to avoid this. One method, termed a "logilinear" approach, involves simply converting H and F proportions from values of 0 and 1 by adding 0.5 to both the amount of hits and false alarms and adding 1 to the total number of signal and noise trials (Hautus, 1995; Miller, 1996). Another approach involves the adjustment of extreme rates with the following conversions where n is the number of trials (N. Macmillan & Kaplan, 1985):

Rate of 0→ 0.5/n
Rate of 1.0→ (n-0.5)/n

Though this method of adjusting extreme rates is an accepted tactic, it is believed to yield sensitivity measures that are more biased than those of a *logilinear* approach (Miller, 1996). Additional solutions to the issue of extreme values in generating a *d'* measure of sensitivity exist, however their usefulness is highly debated as they involve combining data sets or the reliance on alternative statistical measures.

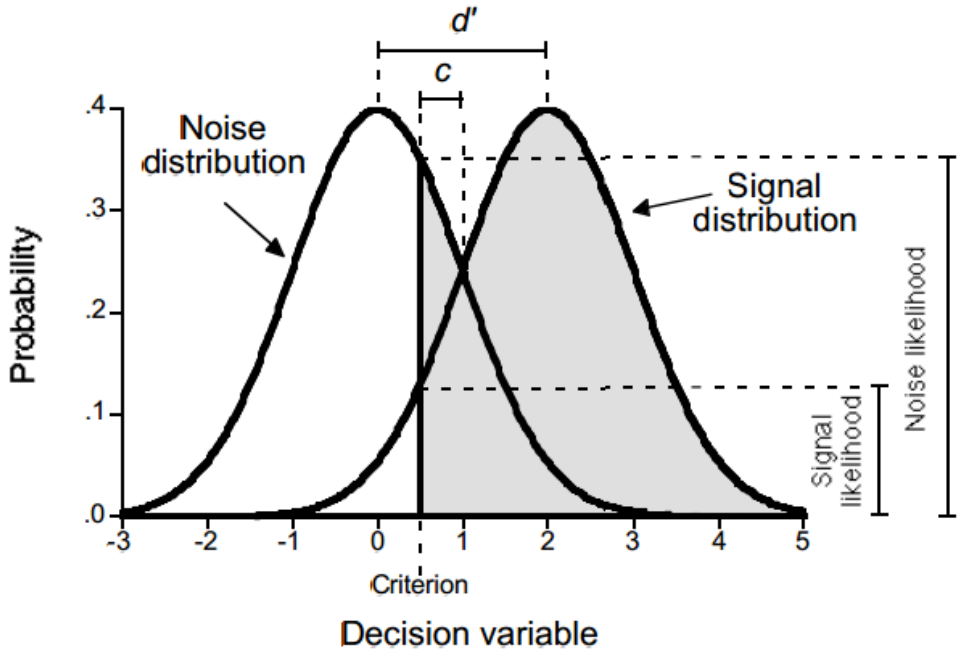
Another measure of sensitivity known as A' (A-prime) is also widely used and accepted as a measure of sensitivity (N. A. Macmillan & Creelman, 1996). A popular reason for this is its *non-parametric* nature meaning that there are no assumptions made about statistical parameters. A measure of this kind is often considered to be more statistically "robust", meaning that it

performs well in a variety of probability distributions. However, a downside to A' is that it requires more statistical power than d' to provide an accurate measurement of sensitivity.

Response Bias

In a yes-no experimental design, there is always a risk of participant response-bias, or the tendency to say "yes". The standard measurement of response bias is computed as β and is based on a likelihood ratio of either a "yes" or "no" response on a signal trial. A value of β=1 corresponds to a participant being effectively "neutral" in the tendency to respond either yes or no to a given trial. Those who have a tendency to respond *yes* have β values less than 1 whereas a value greater than 1 indicates a tendency to respond *no*. Being based on likelihood ratio, values of β are often represented instead by ln(β).

Though β has been most popularly used to measure response bias, there is growing support for the use of the statistic, criterion (*c*) (Banks, 1970); (Neil A. Macmillan & Creelman, 1990). In signal detection theory, *c* is the average of the z scores for both the hit and false alarm rates multiplied by negative one. The range of possible values for the *c* statistic extends from *c*=-2.33 to *c*=2.33. In a case where the false-alarm rate is larger than the miss rate, the criterion value will be negative. Negative values of *c* indicate that there is a bias towards responding "yes" during a trial. This also means that when values of *c* become smaller, there is an increase in the tendency of a participant to make "yes" responses. Alternatively, positive values of *c* correspond to a response-bias that is slanted towards responding "no". The primary benefit of using the statistic *c* instead of β as the primary measure of response-bias is that *c* is unaffected by changes in *d'* (Ingham, 1970). Visualization of the measures *d'* and *c* as they relate to the signal and noise distributions in a detection trial can be seen in Figure 1.5.



Note: Adapted from (Stanislaw & Todorov, 1999)

Figure 1.5 Distribution of the decision variable across signal and noise trials. *d'* and *c*

Reliability

Apart from sensitivity and response-bias, the fundamental measurements of signal detection theory, researchers are often concerned with the consistency of a measure. In psychometrics, estimates of consistency describe a measure's "reliability". If a detection test were to produce stable results across multiple trials to the same participant, it could be said that the test exhibits high reliability. Nunnally (1967) defined reliability as "the extent to which measurements are repeatable and that any random influence which tends to make measurements different from occasion to occasion is a source of measurement error". For a one-interval, yes-no detection test, a test-retest reliability measure can be made by measuring the consistency of participant sensitivity, bias, and responses across multiple administrations of the same test. Alternatively, a detection test can be divided into two equivalent halves and a measure of consistency can be assessed between them. Reliability of this type is known as "split-half" as it

involves the comparison of multiple, parallel forms of a test that are administered within the trials of a single test. When splitting a test in this way, it is incredibly important to attempt to create test halves that are as similar as possible.

One of the most widely used and important measures of reliability is known as the "coefficient alpha" or "Cronbach's alpha" (Cronbach, 1951). According to Cronbach (1951), alpha is the mean of all the split-half reliabilities. Though the essence of the theory behind Cronbach's alpha will not be described in depth here, its description is often one of a coefficient of equivalence. Acceptable values of alpha are displayed in Table 1.2.

Table 1.2 Values of Cronbach's alpha

Cronbach's	Internal
α ≥ .9	Excellent
.9 > α ≥ .8	Good
.8 > α ≥ .7	Acceptable
.7 > α ≥ .6	Questionable
.6 > α ≥ .5	Poor
.5 > α	Unacceptable

Note: Adapted from (Darren & Mallery, 2003)

Odor Threshold Tests

Currently, there are two odor threshold tests that are most popularly used in clinical settings. The first of these is 'Sniffin' Sticks', a chemosensory test that uses pen-like sticks to dispense an odor to participants during administrations. The threshold portion of the 'Sniffin'

Sticks' test is comprised of the presentation of *n*-butanol filled dispensers with various concentrations that are administered in a single staircase method. Each odor pen uses a propylene glycol solvent. During administration, the 'Sniffin' Stick is placed approximately 2cm from the participant's nose for around 3 seconds. Testing follows a triple-forced-choice, single staircase paradigm in which subjects are presented with a single odor concentration and two blanks and asked to respond *yes* or *no* as to whether they detect an odor. Upon the correct detection of the signal in two successive trials, the staircase is reversed for a total of seven reversals and the geometric mean of the last four is calculated and deemed the participant's "threshold" (Hummel et al., 1997). This threshold value is given as a test "score" as individual dispenser concentrations are not provided. However, the 'Sniffin' Sticks' test uses a top concentration of 4% n-butanol and a dilution factor of 1:2 (Hummel et al., 1997).

The second test commonly used to determine participants' odor threshold is the Connecticut Chemosensory Clinical Research Center Test (CCCRC). Similarly to the 'Sniffin' Sticks' test, the CCCRC uses *n*-butanol as its primary odorant. The odor is dispensed with the use of plastic squeeze bottles. The highest concentration of *n*-butanol used in the series is 4% in water with 11 additional geometric dilutions following a ratio of 1:3. Participants are tested with a 2AFC ascending model where each trial contains one signal and one null-stimulus in which subjects must attempt to identify the bottle containing the odorant. In the CCCRC, the threshold value is given as the concentration in which the participant was able to succeed in identifying the signal and the 5 successive trials that preceded it.

Problematic for each of the two tests is the use of *n*-butanol as the single odorant being tested. According to (Brand, 2006), *n*-butanol produces more activation of the trigeminal nerve than molecules that have a larger olfactory component, such as floral or sweet odors. Activating

the trigeminal nerve(or 5th cranial nerve) results in feelings of pain that can be detected even in the absence of odor detection. Though the molecular concentration of *n*-butanol in an odor trial also plays a large part in whether it leads to stimulation of the trigeminal system, irritation has been found to be caused by concentrations of approximately 200ppm, considerably less than what is found in the CCCRC and 'Sniffin Sticks' tests. Instead of isolating the olfactory system, this nerve activation can lead to changes in olfactory information processing (W. Silver, 1991).

Though the CCCRC and 'Sniffin' Sticks' threshold tests both employ the use of blanks in determining an individual's threshold, they are not used to measure additional statistics such as participant response-bias or sensitivity.

The Present Study

The present study involved the creation and pilot study of a new test of olfactory ability, deemed the Wheeler University of Tennessee, Chattanooga (WUTC) Odor Threshold Test. This test was developed building upon the methodology behind signal detection theory as it allowed for multiple measures of olfactory ability to be calculated from a single test. These measures are sensitivity, response-bias, and threshold. Unlike currently available threshold tests, the WUTC Odor Threshold Test utilizes a randomized, multiple odor administration along with the presentation of blank concentrations.

By using multiple odors, the WUTC can look for relationships between odor property and its ability to be detected by those with different diseases or disorders. The odors used were selected based on the diverse properties they possess. Odors differ in their descriptive quality (sweet, pungent, etc.) as well as their molecular classification. Additionally, odorants have varying levels of hydrophobicity which lead to different Odorant Binding Protein (OBP) usage to be detected. Due to the inflammation and degradation of proteins observed in many diseases, the

lowering of the detectability of odors requiring OBP's may be seen. By presenting the odors to participants in random sequence, an attempt can be made to decrease levels of olfactory fatigue to individual odors.

Finally, the WUTC closely adheres to research methodology by employing a double-blind design along with multiple administrations of each odor to participants, allowing for inter-rater reliability to be determined for each odor. These reliability measures can lead to additional comparisons to be made with demographic data as well as aid in the selection of odors that have higher reliability between multiple administrations.

CHAPTER II

METHOD

Participants

A total of thirty three participants (N=33), collected from the UTC campus, were administered the WUTC threshold test. Subject ages ranged from 18 to 46 years old (M=23.69, SD=7.917) for the 32 participants who provided their age. The subjects' consisted of 12 (36.4%) male and 21 (63.6%) female. Out of this sample, 23 (69.7%) of tested individuals were Caucasian and 10 (30.3%) were African American. Information on current education was collected from each subject and is shown in Table 2.1.

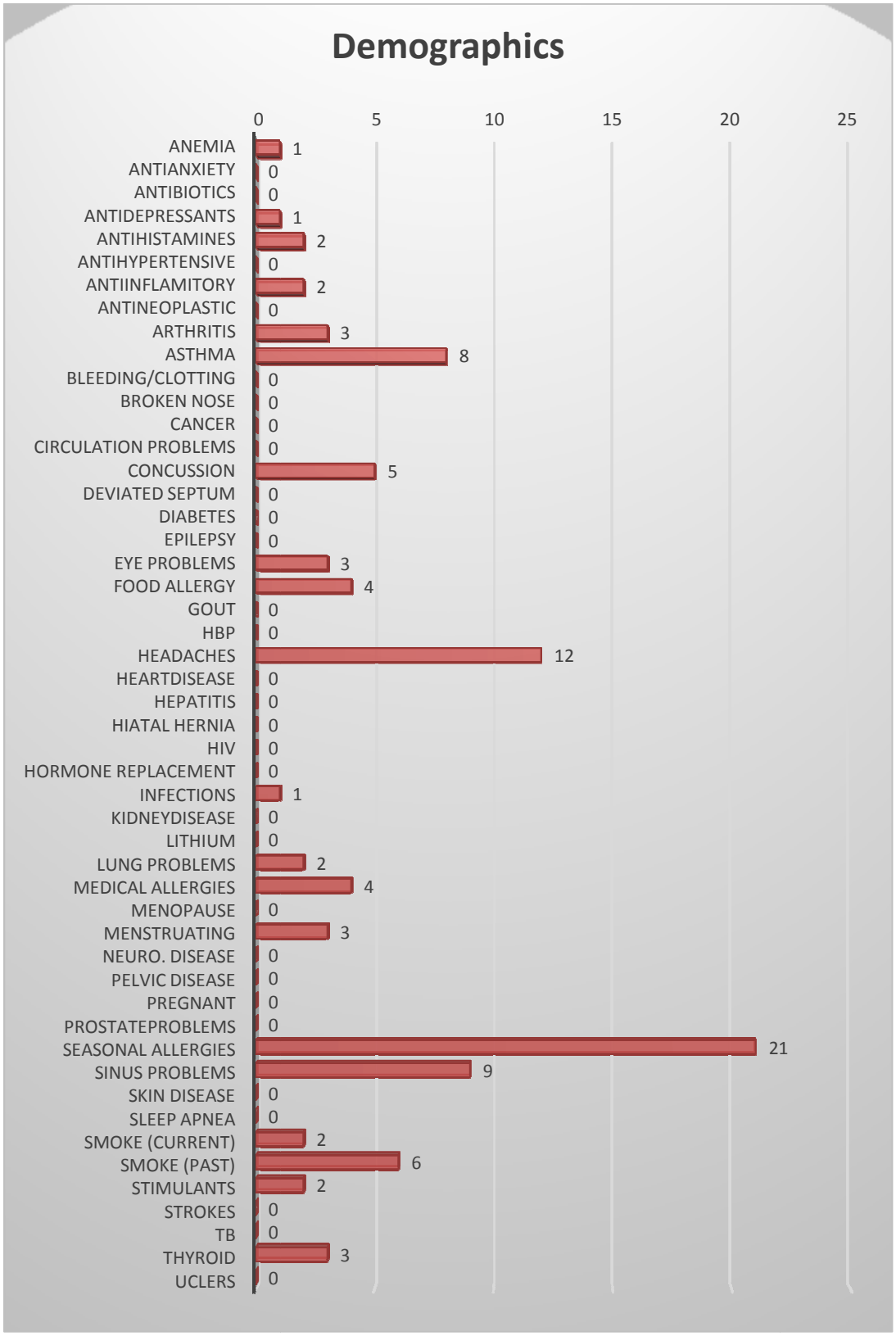
Table 2.1 Participant Data on current Educational Status

College Education	Frequency	Percent	Valid Percent	Cumulative Percent
Freshman	9	27.3	27.3	27.3
Sophomore	6	18.2	18.2	45.5
Junior	11	33.3	33.3	78.8
Senior	3	9.1	9.1	87.9
Five or more years	4	12.1	12.1	100
Total	33	100	100	

Participants were also asked to complete a demographic form with detailed questions about their personal health. These included questions about smoking habits, any current diseases or disorders, medications, menstruation, and pregnancy (Table 2.2). The most frequently

reported demographic information between all subjects were seasonal allergies (N=21, 63.6%), persistent headaches (N=12, 36.4%), sinus problems (N=9, 27.3%), and asthma (N=8, 24.2%). A total of 8 participants (24.2%) circled “yes” to smoking on the demographic form though six of those had not smoked for greater than one month.

Table 2.2 Demographic Data for all Participants



Review Board

This study was approved by the University of Tennessee at Chattanooga Institutional Review Board (IRB). These approval forms can be found in Appendix B of this paper. All test responses and demographic data collected in this study were kept confidential and in encrypted data files.

Materials and Procedure

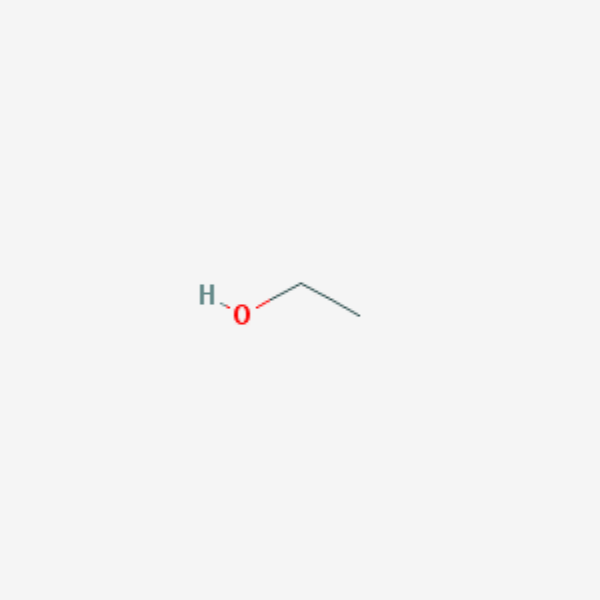
In creating the test, the five odorants ethanol, para-cresol, isoamyl acetate, L-α-pinene, and vanillin were chosen because of various factors. Properties for each odor molecule are included in the same order as the Figures 2.1 through 2.5. First, the two molecules ethanol and α-pinene were used based on their hydrophobicity characteristic and need for Odorant Binding Protein (OBP) interaction in crossing the nasal epithelium. Their inclusion allows for the ability to determine whether there is any damage to these proteins present in an individual.

Ethanol is completely miscible, meaning that it is completely mixable in water in all proportions. This hydrophilic nature allows it to cross the water-soluble membrane of the epithelium and reach the odor receptors. Research conducted by (W. L. Silver, Mason, Russell, Michael, & Smeraski, 1986) determined that the degree to which an alcohol is an irritant is directly related to the length of its carbon chain with increasing irritability as the number of carbons increased. With only two carbons, ethanol does not produce irritation in the trigeminal nerve until it is encountered in concentrations over 1000 ppm. Because methanol (an alcohol containing only one carbon) has been shown to have wildly fluctuating threshold values based on purity, ethanol was deemed the more suitable choice for use in the WUTC.

L-α-pinene, unlike ethanol, is extremely hydrophobic. This results in a need for an OBP to transport the molecule across the water-soluble membrane of the nasal epithelium (Pevsner & Snyder). Though pinene is a known irritant and usually stimulates the trigeminal nerve, it has been shown that the stereospecificity of the molecule plays a large role in its potency (Kasanen et al., 1998) with L-α-pinene being nearly inactive as an irritant.

The odorant vanillin was chosen to be used in the threshold test due to the known differences in ways that it is processed by infants. Vanillin has been shown to significantly prevent apnea in premature newborn infants (Edraki et al., 2013). Being one of the first odors recognizable and preferred by infants, vanillin detectability may prove to be related to infant and childhood development.

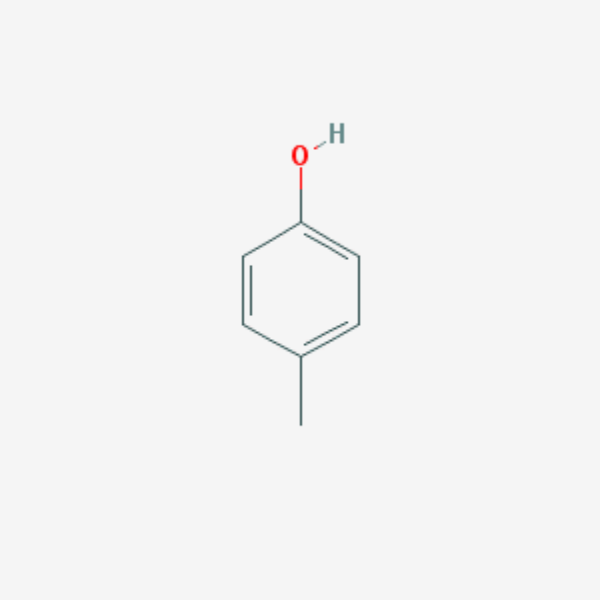
The final two odors are isoamyl acetate and para-cresol. Isoamyl acetate, which has the fruity smell of bananas is vastly different from the pungeunt, tar-like odor of para-cresol. Additionally, para-cresol has been identified as a uremic toxin (Vanholder et al., 2003). By adding these final two odors, the odorant quality profile of the test is very diverse. Also diverse is the compound class of the molecules with the odors containing varied functional groups and structures. The inclusion of this variety of molecules in a single test can allow for the exploration of olfactory deficits to specific odor properties to be explored.



Molecule: Ethanol
Classification: Alcohol
Odor Quality: Sweet, Wine-like
Purity: 99.8%
Lit. Threshold: 49-716
Solubility (in Water): miscible

Note: Adapted from PubChem Substance Database CID: 702

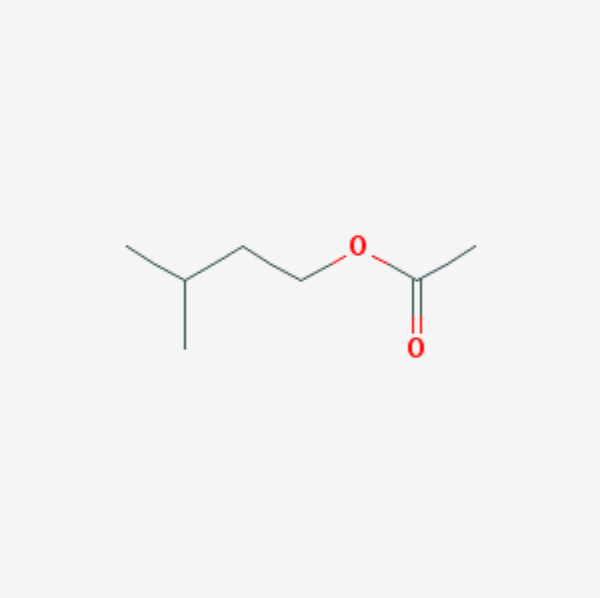
Figure 2.1 Ethanol



Molecule: para-cresol
Classification: Cresol
Odor Quality: Pungent, Tar-like
Purity: 99+%
Lit. Threshold: ~1
Solubility (in Water): 2.4 g/100 ml

Note: Adapted from PubChem Substance Database CID: 2879

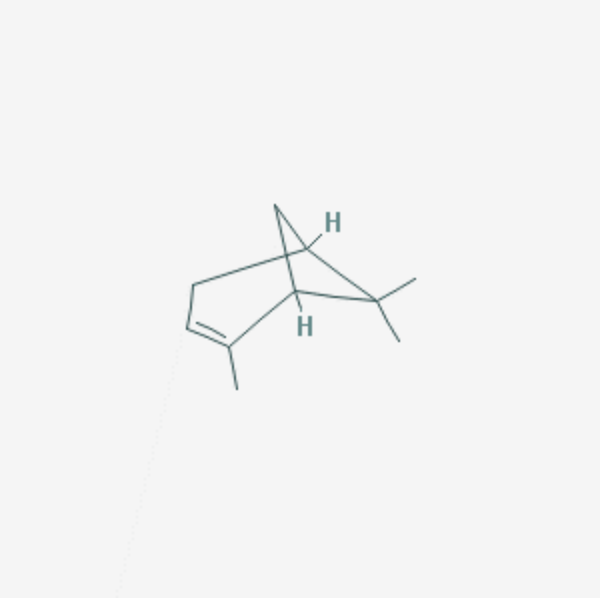
Figure 2.2 para-cresol



Molecule: Isoamyl Acetate
Classification: Ester
Odor Quality: Fruity, Banana
Purity: 99+%
Lit. Threshold: 49-716ppm
Solubility (in Water): 2000mg/L

Note: Adapted from PubChem Substance Database CID: 31276

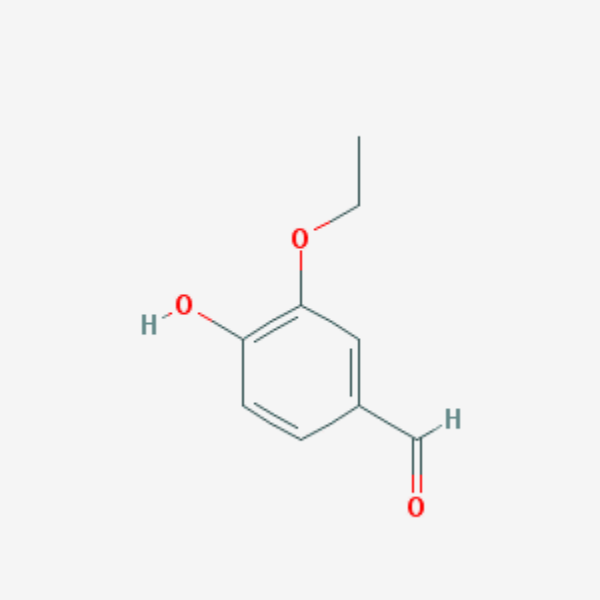
Figure 2.3 Isoamyl Acetate



Molecule: α-pinene
Classification: Turpene, Alkene
Odor Quality: Pine, Turpentine
Purity: 97%
Lit. Threshold: ~2.1
Solubility (in Water): 2.49 mg/L at 25 deg C

Note: Adapted from PubChem Substance Database CID: 6654

Figure 2.4 α-pinene



Molecule: Vanillin
Classification: Phenolic Aldehyde
Odor Quality: Sweet, Pleasant
Purity: 99%
Lit. Threshold: ~2ppm
Solubility (in Water): 1g/mL

Note: Adapted from PubChem Substance Database CID: 8467

Figure 2.5 Vanillin

Odorant Dilutions

To make the test, odorant molecules were first dissolved in a purified H_2O solvent following their individual levels of solubility to create standard solutions. These standards were the highest concentration for each odorant and the base from which all successive dilutions were made. Liquid odorants ethanol, pinene, and isoamyl acetate were diluted by volume whereas para-cresol and vanillin were diluted by mass. Each standard was rounded to the nearest µL. A total of nine concentrations were made from each standard solution and diluted at a ratio of 1:2. The highest concentration for each odor as well as concentration ranges were chosen based on literature threshold values and a small, preliminary testing period. Solutions (10mL) of each odorant were diluted and contained in sterilized and dried glass vials with black, screw-top lids (see Figure 2.6).

Figure 2.6 Tubes Used in the WUTC Threshold Test

Each vial was left unmarked and liquids were visually clear and characterless. Blanks were made using 10 mL of the same purified H_2O used as a solvent for the other odorants. The final test contained 45 vials with odorant concentrations and nine blanks for a total of 54 vials. Tests were remade after either one month or ten administrations had been reached to avoid any amount of loss of odor strength that could result from extended shelf time or exposure to air during administrations. Before reproducing the test, vials underwent sterilization and drying procedures.

Administration

After being explained the nature of the research and acknowledging their informed consent (Appendix C) to participate in the study, each subject was then instructed to complete the provided demographic form (Appendix A). These preliminary steps were helpful not only in the collection of important data for this study, but also in allowing subjects time to adapt to any olfactory stimuli that may have been present in the testing location, despite efforts to minimize such stimuli. Each participant was then seated in a cushioned, high backed chair facing away

from a table where each testing vial was placed. In a brief tutorial to the test, subjects were instructed that they would be presented with a number of vials, some containing odors and some not, one at a time. Subjects were then told that they would only be required to smell the contents of the vial and verbally give a "yes" or "no" answer as to whether they detected anything. Continuing the tutorial, a capped tube was held by tongs and placed approximately 1cm below the center of the participant's nose, demonstrating that this would allow for both nostrils to have equal opportunity to smell the liquid inside. Once the subject felt comfortable with the instructions presented in this tutorial, the actual test was started.

Following a randomly generated number sequence for each subject, a seated test administrator would select the correspondingly numbered test vial for each trial, place it in tongs, and hand it to a second administrator that, like the participant, was facing away from the administration table. The second administrator would then remove the top to the vial and place the tube under the subject's nose, as previously demonstrated in the tutorial. The "yes" or "no" response given by the subject was recorded by the first administrator and the vial and tongs were returned to him/her by the second administrator. This procedure was repeated for each of the odorant tubes in the test with each tube presented to the participant twice. Throughout the entirety of the testing, only the seated administrator was aware of the vial being presented for each trial as well as the number of trials remaining in the test. By doing so, the test followed a double-blind procedure. Both administrators utilized non-latex, medical gloves during each test administration to keep the vials as clean as possible and free from oils or residue that may have been present on the administrator's hands. At the conclusion of the test, participants were debriefed and any questions they may have had were answered. Administration time varied

depending largely on subject response time, normally taking between 35 and 45 minutes from the time they entered the room until they were finished and departed.

Analysis

Multiple statistical tests were used to define the value of the WUTC as a measure of olfactory sensitivity. Unlike other olfactory sensitivity testing methods, the inclusion of multiple administrations and a random presentation of trials in the WUTC garners a much deeper pool of statistical information that permits for a wider breadth of relationships to be explored. This allows the WUTC to describe each participant's olfactory ability in four different ways for each odor. These are: 1) Sensitivity, 2) Response-bias, 3) Threshold, and 4) Inter-rater Reliability.

To measure olfactory sensitivity, the standard SDT measure *d'* was calculated. This statistic gave clear representation of the differences in sensitivity to different odors participants had due to values being on the same scale. The index was calculated as follows (Neil A. Macmillan, 1993):

$$d' = z(H) - z(F)$$

The calculation for the *d'* sensitivity measure involves z-transformations of both the hit and false alarm rates, converting them to z-scores. The resulting difference between these z-scores then becomes the measure of accuracy, *d'*. Values of the *d'* statistic range from zero to 4.65, what is considered to be its "ceiling" (N. A. Macmillan & Creelman, 2004), with low values corresponding to lower sensitivity and higher values to high sensitivity to a stimulus. In order to compute values of *d'* in the presence of Hit or False Alarm rates being equal to 1 or 0, a *logilinear* (Miller, 1996) approach was used.

In addition to *d'*, the sensitivity index A' was calculated due to its non-parametric nature. A' was calculated with the equation (Snodgrass & Corwin, 1988):

$$A' = \begin{cases} .5 + \dfrac{(H-F)(1+H-F)}{4H(1-F)} & \text{when } H \geq F \\ .5 - \dfrac{(F-H)(1+F-H)}{4F(1-H)} & \text{when } H < F \end{cases}$$

Values for A' range from 0 to 1 with a value of .5 indicating that the signal trials were unable to be distinguished from noise.

Both *d'* and A' were calculated from a combination of all participant data (n=33). This provided mean sensitivity statistics for each concentration of the five odors of the test. Therefore, there were a total of forty-five calculated values of both d' and A' (nine concentrations of each of the five odors).

Response-bias was computed as a way to measure the tendency of participants to answer either "yes" or "no" during both signal and noise trials. The value *c* is defined in the equation (Neil A. Macmillan, 1993):

$$c = -\tfrac{1}{2}[z(H) + z(F)]$$

The statistic *c* was used in place of the standard response-bias measure β due to its independence from changes in *d'*. Measurements of response-bias were calculated for each participant and also for all participant data combined.

The estimated odor threshold of each participant for the individual odors of vanillin, pinene, ethanol, isoamyl acetate, and para-cresol were also obtained. To calculate the threshold values, yes/no responses were analyzed using logistic regression and a set of predicted values were generated based on those responses. The estimated threshold value was designated as the odor concentration that corresponded to a p-value of .5 on the sigmoid curve. Graphical

representation of concentration was shown on a logarithmic scale to better represent and avoid skewing of data.

To determine inter-rater reliability, each participant's data was first split into their first and second administrations of each odor. The reliability statistic cronbach's alpha (α) was then computed to determine the reliability of participant yes/no responses. Additionally, the reliability of the response-bias (*c*), along with estimated threshold concentrations for each odor, were calculated. As an additional measure of reliability, the correlation coefficient Pearson's *r* was also determined for each measure.

Calculations of sensitivity, response bias, threshold, and reliability for the combination of all participant administrations represent mean normative data for the specific population tested.

Demographics collected from each participant were analyzed for the existence of relationships with all calculated measures.

CHAPTER III

RESULTS

Yes-no data for all 33 subjects taking the WUTC were combined to give single measures of mean response-bias and threshold for each of the five odors administered. These statistics are presented in Table 3.1a.

Alternatively, estimated thresholds were calculated for each individual participant and then used to provide means and standard deviations for each odor (Table 3.1b). Mean estimated threshold values differ slightly from those found in Table 3.1a due to some individual estimated thresholds being too high or low to be discernible by the test and are therefore calculated from a lower number of participants. Ethanol (*M*=251.808, SD=246.041), Pinene (M=251.225, SD=248.933), and Vanillin (M=119.978, SD=105.570) had highest estimated threshold concentrations with those of isoamyl acetate (M=13.935, SD=16.790) and para-cresol (M=1.340, SD=1.645) being considerably lower. However, due to differences in odor strengths, threshold values were expected to differ. Standard deviations for each estimated odor threshold were also expectedly varied as concentration ranges were different for each odorant. Estimated thresholds for ethanol and para-cresol were at or within literature values. However, the estimated isoamyl acetate threshold was below its literature value and vanillin and pinene were greater than values found.

The sensitivity measures *d'* and A' were calculated from all participant data for each concentration of each odor. Each statistic is the mean across all participant trial for that odor concentration. Values for d' are listed in Table 3.2a and A' values are found in Table 3.2b.

The reliability between calculated test measures for each test half was assessed with the use of Cronbach's alpha (α) and linear regression (r). The results of the analyses are presented in tables. 3.3a and 3.3b. The measure of response-bias(c) for each odor had high reliability as measured by Cronbach's alpha and were significantly correlated at $p<.01$. Both vanillin and para-cresol estimated threshold values for each test half had high levels of reliability with $\alpha=.750$ and $\alpha=.856$, respectively. Their estimates thresholds were also significantly correlated for vanillin ($r=.623$) and para-cresol ($r=.749$) at $p<.01$. Estimated thresholds between test halves for isoamyl acetate, pinene, and ethanol were found to have low reliability as measured by Cronbach's alpha and regression analysis showed that correlations were not significant as well.

Demographics data were analyzed to determine if any significant correlations existed between them and measures of sensitivity, response bias, and estimated threshold for each odor. Mean estimated vanillin threshold values were compared between those with (N=8, M=207.137, 207.137) and without (N=18, M=86.084, SD=86.084) asthma. Those without asthma were found to have significantly lower ($p<.01$) vanillin odor thresholds than those with asthma. This relationship is presented in Figure 3.1.

A significant difference in means was also found to exist between estimated ethanol thresholds based on subject self-report of headaches (Figure 3.2). Participants with (N=8, M=396.631, SD= 332.365) headaches were found to have significantly higher ethanol thresholds than those without (N=12, M=155.260, SD=94.039) headaches. This relationship was significant at $p<.05$.

A significant relationship was also found to exist between ethnicity and mean estimated threshold values for ethanol. Caucasians were found to have higher thresholds for ethanol (M=344.968, SD=276.694) than African Americans (M=112.069, SD=82.529) significant at $p<.05$. The relationship can be seen in Figure 3.3.

Response bias for both para-cresol and pinene were found to be significantly related to age (see Figures 3.4 and 3.5). For each odor, bias to respond "yes" was found to increase significantly as participants' age increased. The relationship was significant for pinene at $p<.05$ ($t= -2.125$) and for para-cresol at $p<.05$ ($t=-2.250$) as well.

Table 3.1a Statistical Measures of Mean Response-Bias and Threshold for Combined Trials

Odorant	***c***	**Threshold (ppm)**
Ethanol	0.198	263.750
Isoamyl Acetate	0.31	38.274
para-cresol	0.217	1.058
Pinene	0.194	275.598
Vanillin	0.381	112.426

Table 3.1b Descriptive Statistics for Odorant Thresholds

Odorant	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Ethanol	20	1.057	942.747	251.808	246.041
Isoamyl Acetate	22	0.513	65.102	13.935	16.790
para-cresol	26	0.005	5.28	1.340	1.645
Pinene	24	0.778	795.338	251.225	248.933
Vanillin	25	2.619	339.932	119.978	105.570

Table 3.2a Mean Odorant *d'* Values at Each Concentration for Combined Participant Trials

	Lowest				**Concentration**				*Highest*
Odorant	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ethanol	0.898	0.704	0.035	0.819	0.16	0.941	0.513	1.066	1.154
Isoamyl Acetate	-0.249	-0.198	-0.198	-0.053	0.704	0.704	1.11	1.299	1.024
para-cresol	-0.416	0.16	0.32	0.32	0.475	1.154	1.458	1.403	2.246
Pinene	-0.249	0.898	0.16	0.035	0.513	0.742	1.458	2.097	1.72
Vanillin	-0.302	-0.053	0.077	0.359	0.16	0.55	1.024	1.11	1.885

Table 3.2b Mean Odorant A' Values at Each Concentration for Combined Participant Trials

	Lowest				**Concentration**				*Highest*
Odorant	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ethanol	0.757	0.716	0.514	0.741	0.561	0.766	0.668	0.789	0.804
Isoamyl Acetate	0.407	0.424	0.424	0.478	0.716	0.716	0.796	0.826	0.781
para-cresol	0.356	0.561	0.614	0.614	0.313	0.804	0.847	0.84	0.908
Pinene	0.407	0.757	0.561	0.514	0.668	0.724	0.847	0.901	0.875
Vanillin	0.39	0.478	0.531	0.625	0.561	0.678	0.781	0.796	0.888

Table 3.3a Reliability Measure between Test Halves (Cronbach's α)

Odorant	*c*	Threshold (ppm)
Ethanol	0.917	0.299 (N=17)
Isoamyl Acetate	0.972	0.182 (N=18)
para-cresol	0.965	0.856 (N=22)
Pinene	0.945	0.06 (N=22)
Vanillin	0.971	0.750 (N=23)

Table 3.3b Correlation between Test Halves (Pearson's *r*)

Odorant	yes-no	*c*	Threshold (ppm)
Ethanol	.423*	.865*	.19 (N=17)
Isoamyl Acetate	.547*	.946*	.105 (N=18)
para-cresol	.585*	.932*	.749 (N=22)*
Pinene	.539*	.908*	.031 (N=22)
Vanillin	.542*	.945*	.623 (N=23)*

*p<.01

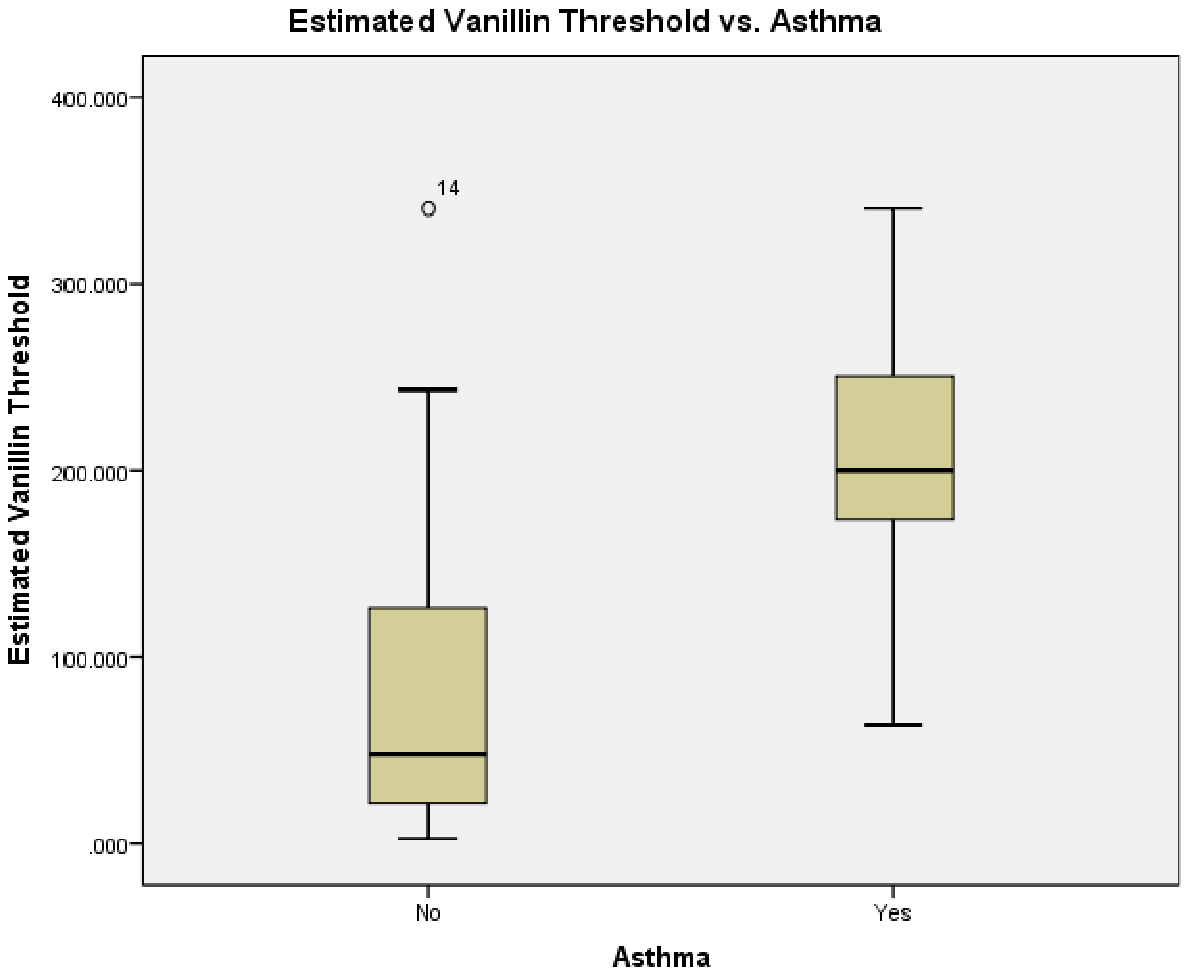


Figure 3.1 Comparison of Estimated Vanillin Thresholds for subjects with and without Asthma.

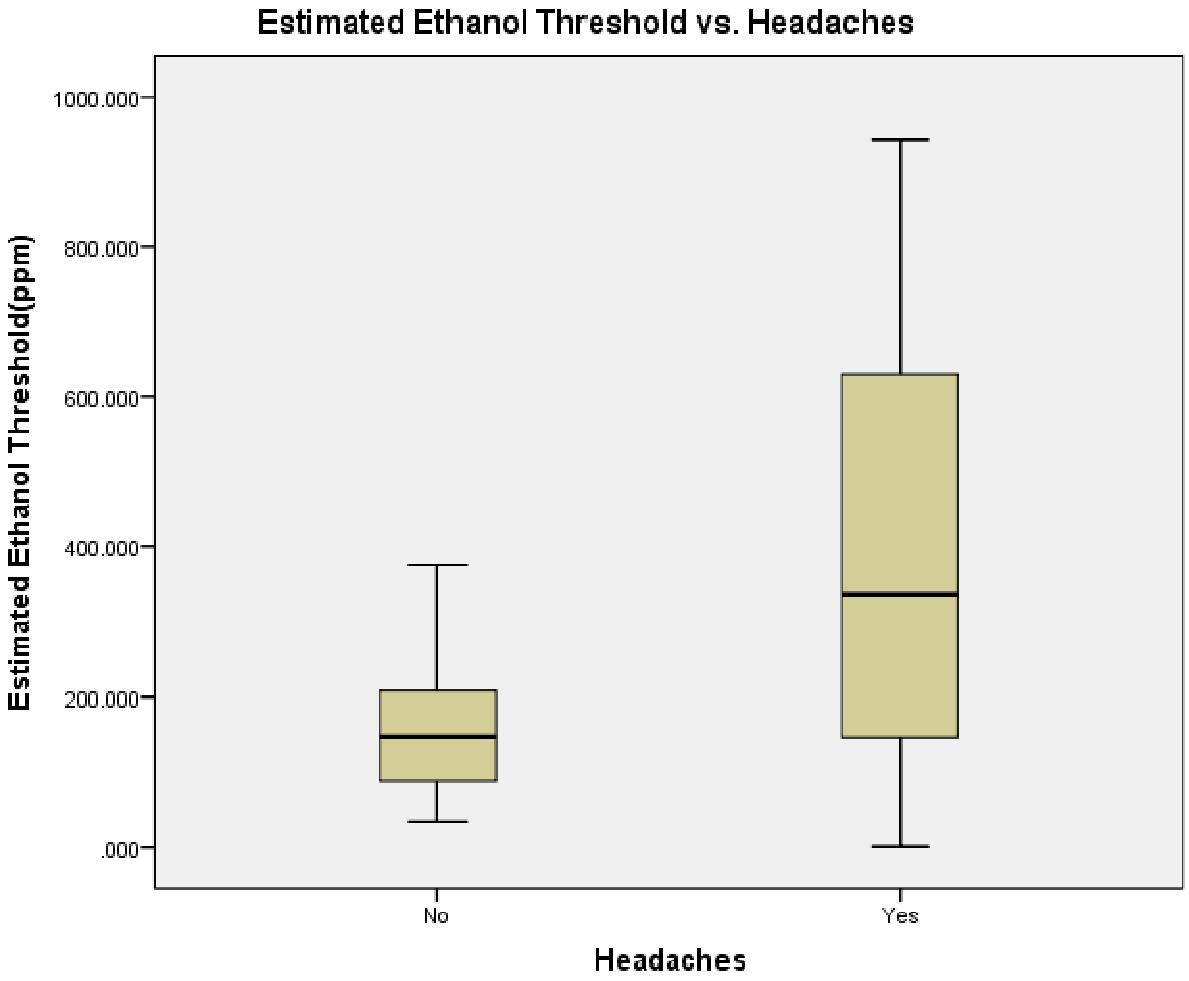


Figure 3.2 Comparison of Estimated Ethanol Thresholds for subjects with and without persistent Headaches

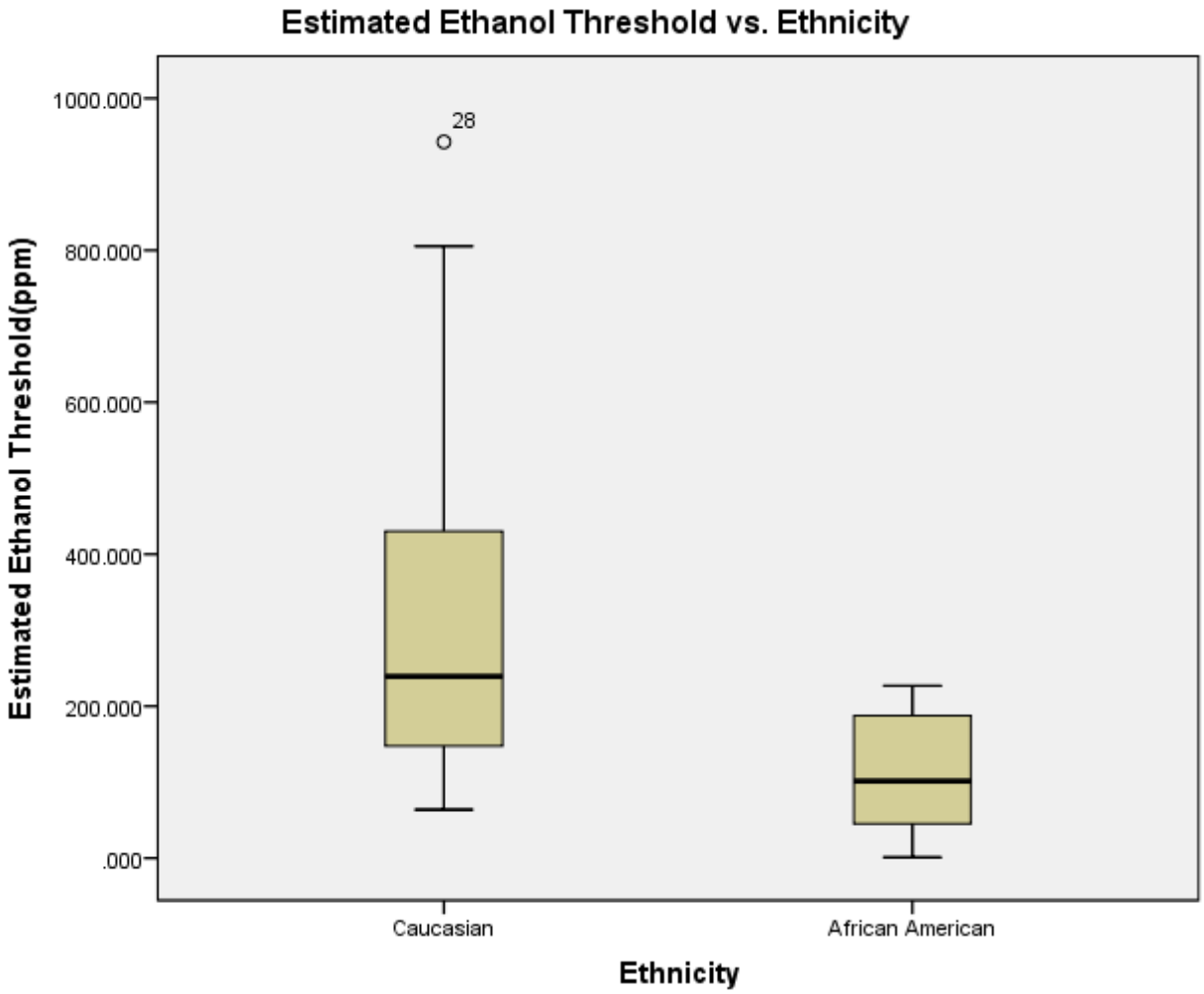


Figure 3.3 Comparison of Estimated Ethanol Thresholds between Caucasians and African Americans.

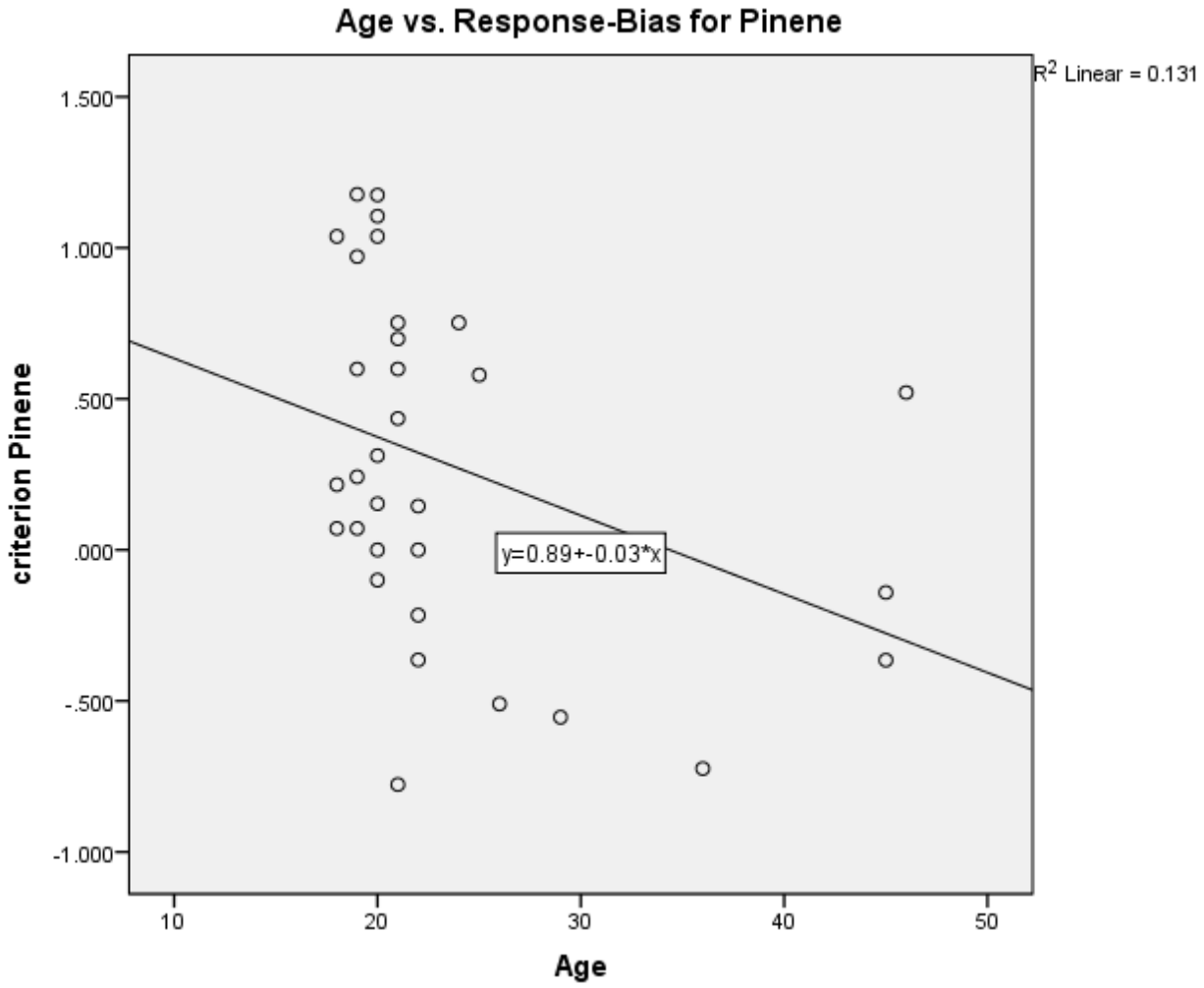


Figure 3.4 Age vs. Response-Bias for Pinene

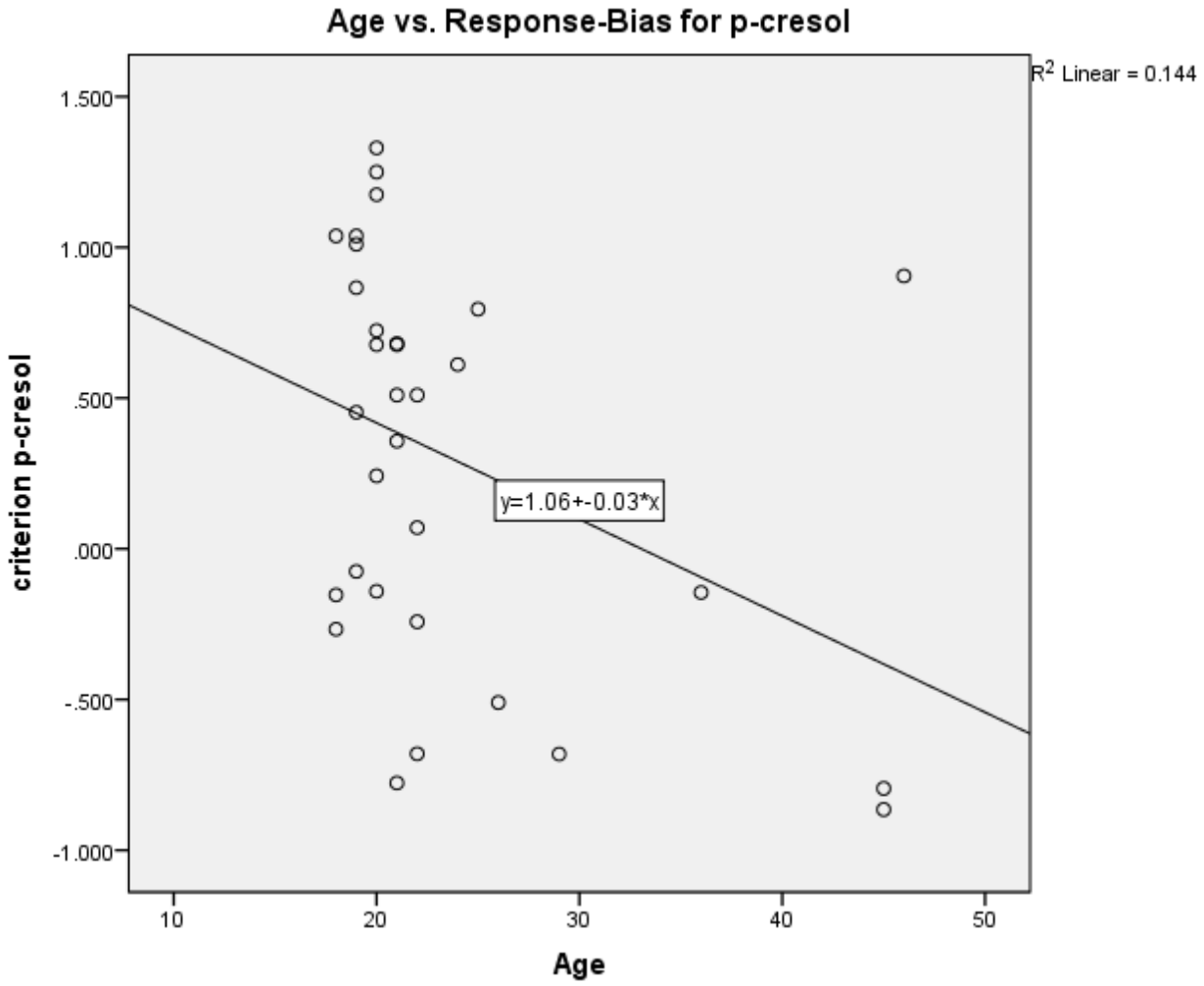


Figure 3.5 Age vs. Response Bias for para-cresol

CHAPTER IV

DISCUSSION

The purpose of this study was to develop a methodology of testing participants' ability to detect multiple odors. Each odor was chosen to provide a more robust and varied odor profile so that more specific relationships between disease and olfaction can be explored. With this in mind, the physiological interactions between binding proteins and odor transport, odor molecule classification, and scent type were incorporated into the development of the WUTC Threshold Test.

Along with the creation of the test, an initial pilot study was completed with N=33 participants to observe the inter-rater reliability for measures of response bias and estimated threshold for each odor. The five odorants para-cresol, ethanol, isoamyl acetate, α-pinene, and vanillin were administered in the test alongside null-stimulus trials which made these multiple measures possible. Unexpectedly, only vanillin and para-cresol thresholds were found to be reliable ($p<.01$ and $\alpha=.750$ and .856, respectively). This could be due to both odors having aromatic structures that are very different from those of the other odors included in the test. This similarity in structure may cause the odors to be processed similarly by the olfactory system, leading to high reliability for both. The aromatic nature of the odorants may also cause them to have higher stability, allowing more of the molecule to reach the olfactory sensory than the other odors used in the WUTC Threshold Test. The lack of reliability for ethanol, isoamyl acetate, and α-pinene thresholds may also be a result of uncertainty in test methodology or inappropriate

concentration range leading to higher levels of participant uncertainty as to whether a stimulus was detected.

By combining all participant trials for each odor, the mean sensitivity for each odor concentration was obtained. These values of *d'* and A' should exhibit a pattern of increasing as the concentration of the odorant increases. This signifies an increase in the sensitivity, or ability to detect, an odor stimulus as the magnitude of the stimulus increases. However, this pattern is not seen across all odors. This may be due to the randomized nature of the trials or to differences in how each odor interacts with the olfactory system. Vanillin and para-cresol have the most consistent pattern of sensitivity increase as concentration increases. This is reflected by their high reliability across test halves. The relationship between inter-rater reliability and odor concentration should be investigated further to further develop olfactory testing methodology.

Participants taking part in this study provided answers to a demographic form which made correlational analyses possible. Among the data analyzed, correlations were found to exist between response-bias for both para-cresol and pinene and participant age ($p<.05$). Since the relationship was negative, the tendency of a participant to guess "yes" to a trial increased with age. This may be explained by the known decrease of olfactory ability as age increases yet should be investigated further with a larger and more varied sample pool in regards to participant age.

An analysis between ethanol threshold and headaches found a significant interaction ($p<.05$). Those with headaches had higher thresholds than those without. Due to ethanol being a hydrophilic molecule, it may be possible that it encounters difficulty crossing the mucus membrane surrounding the nasal epithelium when a common cause of headaches, dehydration, occurs.

Ethanol was also found to be correlated with ethnicity with Africans Americas having significantly lower mean ethanol thresholds than Caucasians ($p<.05$). A possible reason for this is unknown though may be related to small differences in the olfactory system of Caucasians and African Americans that lead to higher levels of ethanol reaching the olfactory receptors.

A final significant interaction ($p<.01$) was found to exist between mean estimated vanillin thresholds and a self-report of asthma. Participants with asthma had significantly higher thresholds than those without the disease. This may be due to lowered levels of airflow in those with asthma that leads to less of the odorant reaching the nasal epithelium. Because a similar relationship is not present between asthma and the other odors in the test, the specific deficit to vanillin threshold may be due to the chemical properties of vanillin or the way that it interacts with the olfactory system. This interaction needs to be further investigated in future studies.

Interestingly, there was a lack of significant interaction between participant threshold values based on gender despite previous research supporting gender differences in olfaction. This may be due to the nature of the test measuring only a small subsection of olfactory ability.

CHAPTER V

CONCLUSION

Limitations

Though the main goal of this study was to develop a new threshold test, multiple interactions were analyzed using demographic data. These analyses were limited by the small sample size. Only thirty three subjects were recruited for the study due to the large time commitment needed for participation. The number of samples makes the interactions found in need of further investigation and greater sample size.

Another limitation was the concentration range used for each odor. Though concentration ranges were built around literature thresholds and an initial testing period, some participant thresholds were unable to be calculated due to being outside (either too low or too high) the range of the test.

Due to the range of temperature throughout the year and fluctuating air quality, the environmental conditions were not consistent for all participants. This factor was attempted to be controlled for by using an indoor testing space that was kept at a consistent temperature. However, it remains as a limitation.

Finally, the shelf-life of the WUTC Threshold Test is unknown. This is limiting as it is unknown whether the strength of odor concentrations diminished over time. To attempt to combat this possibility, the WUTC was remade approximately after ~1mo. of use or 10 administrations. However, the nature of what level of odor strength is lost over time is unknown.

Directions for Future Research

This study provides a foundation for the WUTC threshold test through the development of its test methodology and concentration on stimuli used. Though there were several limitations to the study, there are also many strong points.

The test focused on the use of the odors ethanol, pinene, para-cresol, vanillin, and isoamyl acetate. However, researchers could expand on this by adding or replacing odors on the test that they believe may be better linked to specific diseases than those used. Odors selected based on theories of evolutionary survival or social functioning could be used to search for differences in detection. The adaptable nature and developed methodology of the WUTC could allow for multiple tests to be made to test particular populations as new links to olfactory deficits are discovered.

With the randomized presentation of odors in the WUTC, the possibility exists that there are cross-effects between odorants. Analyses could be completed to determine if any relationship exists between the order of the odor trials and olfactory ability. Doing so would lead to information that could further enhance the usability of the test.

There were also several relationships found between the individual measures determinable by the WUTC and demographic data. These olfactory deficits were found to exist not with all odors but with only specific odors used in the test. This further strengthens the argument that tests of olfaction benefit from the use of diverse stimuli as diseases may not cause global deficits to all types of odors.

The development of the methodology behind the Wheeler UTC Threshold Test represents a shift in olfactory testing archetypes. By including double-blind testing, randomization of stimuli, and multiple presentations of each odor, the WUTC conforms to standards of research

methodology more than other, currently available tests. Additionally, the WUTC adopts a paradigm that focuses highly on the nature of the stimulus. Odors were chosen for molecular diversity and differences in how they interact with the olfactory system. By using this kind of odor profile, complex relationships between olfactory ability for specific odors and certain diseases can be identified. Doing so may lead to the WUTC becoming a valid predictor of particular ailments within individuals, an accomplishment that no other test of olfaction can do at this time.

Importantly, the nature of this research also attempts to reinforce the need for multidisciplinary study and partnership in order to confront complex scientific inquiries.

REFERENCES

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A, 90*(17), 7915-7922.

Banks, W. P. (1970). Signal detection theory and human memory. *Psychological bulletin, 74*(2), 81.

Brand, G. (2006). Olfactory/trigeminal interactions in nasal chemoreception. *Neuroscience & Biobehavioral Reviews Neuroscience & Biobehavioral Reviews, 30*(7), 908-917.

Cronbach, L. J. (1951). Coefficient alpha and the internal structure of tests. *Psychometrika, 16*(3), 297-334.

Darren, G., & Mallery, P. (2003). *SPSS for Windows step by step: a simple guide and reference, 11.0 update*: Allyn and Bacon.

Deems, D. A., Doty, R. L., Settle, R. G., Moore-Gillon, V., Shaman, P., Mester, A. F., . . . Snow, J. B., Jr. (1991). Smell and taste disorders, a study of 750 patients from the University of Pennsylvania Smell and Taste Center. *Arch Otolaryngol Head Neck Surg, 117*(5), 519-528.

Duncan, R., & Briggs, M. (1962). Treatment of uncomplicated anosmia by vitamin A. *Archives of otolaryngology (Chicago, Ill. : 1960), 75*, 116-124.

Edraki, M., Pourpulad, H., Kargar, M., Pishva, N., Zare, N., & Montaseri, H. (2013). Olfactory stimulation by vanillin prevents apnea in premature newborn infants. *Iranian journal of pediatrics, 23*(3), 261-268.

Evola, M., Hall, A., Wall, T., Young, A., & Grammas, P. (2010). Oxidative stress impairs learning and memory in apoE knockout mice. *Pharmacology Biochemistry and Behavior Pharmacology Biochemistry and Behavior, 96*(2), 181-186.

Halliwell, B. (1992). Reactive Oxygen Species and the Central Nervous System. *JNC Journal of Neurochemistry, 59*(5), 1609-1623.

Heeger, D. (1998). Signal detection theory handout. from http://www-psych.stanford.edu/~lera/psych115s/notes/signal/

Heeger, D. (2003). Signal detection theory. from http://www.cns.nyu.edu/~david/handouts/sdt/sdt.html

Himmelfarb, J., Stenvinkel, P., Ikizler, T. A., & Hakim, R. M. (2002). The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia.

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chemical senses, 22*(1), 39-52.

Ingham, J. G. (1970). Individual differences in signal detection. *Acta psychologica, 34*(1), 39-50.

Kasanen, J., Pasanen, A. L., Pasanen, P., Liesivuori, J., Kosma, V. M., & Alarie, Y. (1998). Stereospecificity of the sensory irritation receptor for nonreactive chemicals illustrated by pinene enantiomers. *Archives of toxicology, 72*(8).

Kershaw, C. D. (1985). Statistical properties of staircase estimates from two interval forced choice experiments. *BMSP British Journal of Mathematical and Statistical Psychology, 38*(1), 35-43.

Lavin, J., Meen, E., Lam, K., Kato, A., Huang, H., Norton, J., . . . Tan, B. (2013). The Impact and Nature of Inflammation in the Olfactory Cleft On Olfaction in Patients with Chronic Rhinosinusitis. *Journal of Allergy and Clinical Immunology, 131*(2, Supplement), AB59.

Lopez, I., Acuna, D., Beltran-Parrazal, L., Lopez, I. E., Amarnani, A., Cortes, M., & Edmond, J. (2009). Evidence for oxidative stress in the developing cerebellum of the rat after chronic mild carbon monoxide exposure (0.0025% in air). *BMC neuroscience, 10*.

MacCaffrey, T. V. (1997). *Rhinologic diagnosis and treatment*: Thieme.

Macmillan, N., & Kaplan, H. L. (1985). Detection theory analysis of group data: estimating sensitivity from average hit and false-alarm rates. *Psychological bulletin, 98*(1), 185-199.

Macmillan, N. A. (1993). Signal detection theory as data analysis method and psychological decision model. *A handbook for data analysis in the behavioral sciences: Methodological issues*, 21-57.

Macmillan, N. A., & Creelman, C. D. (1990). Response bias: Characteristics of detection theory, threshold theory, and" nonparametric" indexes. *Psychological bulletin, 107*(3), 401.

Macmillan, N. A., & Creelman, C. D. (1996). Triangles in ROC Space: History and Theory of "Nonparametric" Measures of Sensitivity and Response Bias. *Psychonomic bulletin & review., 3*(2), 164.

Macmillan, N. A., & Creelman, C. D. (2004). *Detection Theory: A User's Guide*: Taylor & Francis.

Miller, J. (1996). The sampling distribution of d'. *Perception & psychophysics, 58*(1), 65-72.

Misonou, H., Morishima-Kawashima, M., & Ihara, Y. (2000). Oxidative stress induces intracellular accumulation of amyloid beta-protein (Abeta) in human neuroblastoma cells. *Biochemistry, 39*(23), 6951-6959.

Murray, M. (2013). *Our chemical senses: olfaction*. http://faculty.washington.edu/chudler/chems.html

Nunnally, J. C. (1967). *Psychometric theory*: McGraw-Hill.

Perry, G., Cash, A. D., & Smith, M. A. (2002). Alzheimer Disease and Oxidative Stress. *Journal of Biomedicine and Biotechnology Journal of Biomedicine and Biotechnology, 2*(3), 120-123.

Pevsner, J., & Snyder, S. H. Odorant-binding protein : odorant transport function in the vertebrate nasal epithelium. *15*(Oxford University Press), 217-222.

Reutens, S., & Sachdev, P. (2002). Homocysteine in neuropsychiatric disorders of the elderly. *Int J Geriatr Psychiatry, 17*(9), 859-864.

Ruff, R. L., Ruff, S. S., & Wang, X.-F. (2008). Headaches among Operation Iraqi Freedom/Operation Enduring Freedom veterans with mild traumatic brain injury associated with exposures to explosions. *J Rehabil Res Dev, 45*(7), 941-952.

Silver, W. (1991). Physiological factors in nasal trigeminal chemoreception. *Chemical senses, II*, 21-37.

Silver, W. L., Mason, J., Russell, A., Michael, A., & Smeraski, C. A. (1986). Nasal trigeminal chemoreception: Responses to n-aliphatic alcohols. *BRES Brain Research, 376*(2), 221-229.

Snodgrass, J. G., & Corwin, J. (1988). Pragmatics of measuring recognition memory: applications to dementia and amnesia. *Journal of Experimental Psychology: General, 117*(1), 34.

Stanislaw, H., & Todorov, N. (1999). Calculation of signal detection theory measures. *Behavior research methods, instruments, & computers, 31*(1), 137-149.

Swets, J. A. (1986). Form of empirical ROCs in discrimination and diagnostic tasks: implications for theory and measurement of performance. *Psychological bulletin, 99*(2), 181-198.

Tritschler, H. J., & Medori, R. (1993). Mitochondrial DNA alterations as a source of human disorders. *Neurology, 43*(2), 280-288.

Vanholder, R., De Smet, R., Glorieux, G., Argilés, A., Baurmeister, U., Brunet, P., . . . Deppisch, R. (2003). Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney international, 63*(5), 1934-1943.

Vogt, R., Prestwich, G. D., & Lerner, M. R. (1991). Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *Journal of neurobiology, 22*(1), 74-84.

Walton, B., & Maeso, P. (2012). can you smell that? anatomy and physiology of smell. from Walton, B., & Patricia, M. (January, 2012 30). Can you smell that? anatomy and physiology of smell. Retrieved from https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCwQFjAA&url=http://www.utmb.edu/otoref/grnds/anosmia-Walton-2012-0130/smell-pic-2012-0130.ppt&ei=9WltUv7aMszQkQe_74G4AQ&usg=AFQjCNFSIYy1mKBYkm4-nVWthTqEal7pug&sig2=ou8mDzYtRmN-NcbT8QVoVA&bvm=bv.55123115,d.eW0

Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism, 27*(4), 277-284.

APPENDIX A

DEMOGRAPHIC QUESTIONNAIRE

Age (in years): _____

Gender (circle one); Male Female

If female, please answer the questions located on the next page. ***

Ethnicity (circle one): Caucasian African American Asian American Hispanic

Bi-Racial Other (please indicate): _____

Do you currently smoke (circle one): Yes No

If yes; How many cigarettes per day?____; Cigars per day?___

What type of cigarettes do you smoke? _____

How many years have you smoked? _____

If not currently smoking, have you ever smoked? (Circle one): Yes No

If yes, how long ago did you stop?_____

How many cigarettes did you smoke per day?____; Cigars per day?____

Did your ability to smell change after you stopped smoking? (Circle one): Yes No

If yes; How?

_____

_____

What is your occupation: _____

Highest grade completed? (Circle only one number):

6 7 8 9 10 11 12 College 1 2 3 4 5 +

Please indicate if you have had past history of the following medical Illnesses. (Circle Yes or No):

High blood pressure	Yes	No	Diabetes	Yes	No
Arthritis	Yes	No	Heart disease	Yes	No
Thyroid disorder	Yes	No	Headaches	Yes	No
Lung trouble	Yes	No	Gout	Yes	No
Epilepsy	Yes	No	Circulation problems	Yes	No
Broken nose	Yes	No	Anemia	Yes	No
Strokes	Yes	No	Eye problems	Yes	No
Asthma	Yes	No	Cancer	Yes	No

Please indicate if you have had past history of the following medical Illnesses. (Circle Yes or No):

Hepatitis	Yes	No	Ulcer	Yes	No

Hiatal hernia	Yes	No	Kidney disease	Yes	No
Pelvic disease	Yes	No	Skin disease	Yes	No
Prostate problems	Yes	No	Infections	Yes	No
Bleeding/clotting disorder	Yes	No	HIV	Yes	No
TB	Yes	No	Neurological disease	Yes	No
Deviated septum	Yes	No	Sinus problems	Yes	No
Concussion/head trauma	Yes	No	Medical allergies	Yes	No
Food allergies	Yes	No	Seasonal allergies	Yes	No

Other: _____

Please indicate if you are currently taking any of the following types of medications. (Circle Yes or No):

Antibiotics	Yes	No	Antidepressants	Yes	No
Hormone replacements	Yes	No	Antihistamines	Yes	No
Antihypertensive	Yes	No	Antianxiety	Yes	No
Lithium	Yes	No			
Anti-inflammatory†	Yes	No			

†Including ibuprofen

Antineoplastic†† Yes No

††Examples of Antineoplastics are *Elspar* (asparaginase), *Alkeran* (melphalan), floxuridine, lomustine, procarbazine, thioguanine, thiotepa

Stimulant medications††† Yes No

†††Examples of Stimulant medications are *Adderall* and *Vyvanse*

Have you ever been diagnosed with Sleep Apnea? (Circle one): Yes No

***Females (optional, But VERY BENEFICIAL to answering research questions)**

If **FEMALE**; Are you currently on your menstrual cycle? (Circle one): Yes No

If **FEMALE**; Are you currently pregnant? (Circle one): Yes No

If **FEMALE**; Are you in menopause or post menopause? (Circle one): Yes No

APPENDIX B

INSTITUTIONAL REVIEW BOARD APPROVAL

MEMORANDUM

TO: William Tewalt **IRB # 12- 121**
Jessica McKinney
Hannah Tumlin
Dr. Nicky Ozbek

FROM: Lindsay Pardue, Director of Research Integrity
Dr. Bart Weathington, IRB Committee Chair

DATE: June 19, 2012

SUBJECT: IRB # 12-121: Collection of Normative Data for an Odor Threshold Test

The Institutional Review Board has reviewed and approved your application and assigned you the IRB number listed above. You must include the following approval statement on research materials seen by participants and used in research reports:

The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project #12-121.

Please remember that you must complete a Certification for Changes, Annual Review, or Project Termination/Completion Form when the project is completed or provide an annual report if the project takes over one year to complete. The IRB Committee will make every effort to remind you prior to your anniversary date; however, it is your responsibility to ensure that this additional step is satisfied.

Please remember to contact the IRB Committee immediately and submit a new project proposal for review if significant changes occur in your research design or in any instruments used in conducting the study. You should also contact the IRB Committee immediately if you encounter any adverse effects during your project that pose a risk to your subjects.

For any additional information, please consult our web page http://www.utc.edu/irb or email instrb@utc.edu

Best wishes for a successful research project.

THE UNIVERSITY of TENNESSEE CHATTANOOGA

Institutional Review Board
Dept. 4915
615 McCallie Avenue
Chattanooga, TN 37403-2598
Phone: (423) 425-5867
Fax: (423) 425-4052
instrb@utc.edu
http://www.utc.edu/irb

MEMORANDUM

TO: William A. Tewalt **IRB # 12-121**

FROM: Lindsay Pardue, Director of Research Integrity
Dr. Bart Weathington, IRB Committee Chair

DATE: October 22, 2013

SUBJECT: IRB #12-121: Collection of Normative Data for an Odor Threshold Test

The Institutional Review Board has reviewed and approved your application for Annual Renewal for the IRB project listed above.

You must include the following approval statement on research materials seen by participants and used in research reports:

The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project #12-121.

Please remember that you must complete a form for completion when the project is completed or provide an annual report if the project takes over one year to complete. The IRB Committee will make every effort to remind you prior to your anniversary date; however, it is your responsibility to ensure that this additional step is satisfied.

Please remember to contact the IRB Committee immediately and submit a new project proposal for review if significant changes occur in your research design or in any instruments used in conducting the study. You should also contact the IRB Committee immediately if you encounter any adverse effects during your project that pose a risk to your subjects.

For any additional information, please consult our web page http://www.utc.edu/irb or email instrb@utc.edu

Best wishes for a successful research project.

APPENDIX C

INFORMED CONSENT FORM

INFORMED CONSENT FORM

COLLECTION OF NORMATIVE DATA FOR AN ODOR THRESHOLD TEST.

Please read this consent document carefully before you decide to participate in this study. This research has been approved by the University Institutional Review Board.

Purpose of the research study:

The purpose of this study is to collect data on a new odor threshold test.

What you will be asked to do in the study:

You will initially be asked to complete a brief questionnaire. During the test, a researcher will present you with a test tube filled with clear liquid beneath your nose for 5 seconds. After that initial 5 seconds, you will have another 10 seconds to tell the researcher either "yes"- you did detect an odor or, "no"- you did not. The test consists of 108 tubes of various odors and concentrations. Some of the tubes contain odors and some do not.

Time required:

~30-40 minutes

Risks and Benefits:

You may experience some temporary nasal dryness from prolonged smelling. We do not anticipate that you will benefit directly by participating in this experiment. However, your participation is appreciated as your efforts contribute to a body of knowledge that we hope will eventually be on benefit to others

Confidentiality:

Your identity will be kept confidential to the extent provided by law. Your information will be assigned a code number. The list connecting your name to this number will be kept in a locked file cabinet and office. Your name will not be used in any report. The questionnaire is not HIPPA protected. As stated previously, your name is separated from the information you have provided.

Voluntary participation:

Your participation in this study is completely voluntary. There is no penalty for not participating.

Right to withdraw from the study:

You have the right to withdraw from the study at anytime without consequence.

Whom to contact if you have questions about the study:

Dr. Nicky Ozbek (nickyozbek@gmail.com)
William Tewalt (wtewalt@gmail.com).

Agreement:

I have read the procedure described above. I voluntarily agree to participate in the procedure and I have received a copy of this description.

Participant: (signed)_____ Date: _____

Participant: (printed)_____

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact Dr. Bart Weathington, Chair of the Institutional Review Board, at 423-425-4289. Additional contact information is available at www.utc.edu/irb

VITA

William Tewalt was born in Front Royal, Virginia to the parents of Michael and Rebecca Tewalt. He completed a BS in Psychology with a minor in Philosophy from the University of Tennessee in 2007. After working in the greater Knoxville area for three years, William moved to Chattanooga, TN and returned to academia. William will earn his MS in Research Psychology from the University of Tennessee at Chattanooga in December 2013. He currently works as a Graduate Assistant conducting olfactory research through a grant awarded by the William H. Wheeler Center for Odor Research. In the spring of 2013, William's research on olfactory test development was accepted into the International Association for Chemoreception Sciences (AChemS) and he was awarded a Student Travel Award to attend. William looks forward to teaching or continuing to conduct research in the Chattanooga area after graduation.