EXPLORING RELATIONSHIPS BETWEEN STRESS AND
OLFACTION AS MEDIATED BY NEUROPEPTIDE Y

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ABSTRACT

The negative correlation between olfactory sensitivity and depression has been well documented, but the biological processes underpinning the relationship are not understood. This study explored a potential relationship between olfactory sensitivity, stress, and resilience to stress, specifically examining neuropeptide y (NPY) as a mediator. In phase I, 197 UTC students participated in a survey measuring stress and resilience among other factors. Of this sample, 25 students volunteered for phase II, in which they took an olfactory threshold test and gave blood. Serum levels of cortisol and NPY were analyzed from blood samples. Correlational analyses suggest a positive effect of stress (p=.013) and a negative effect of resilience (p=.003) on olfactory thresholds, while biological markers were inconclusive. Future studies should incorporate the diagnosis of stress disorders, as biological markers may not significantly fluctuate based on non-clinical levels of stress.
DEDICATION

To Eleanore, my wife: the inspiration behind all my undertakings.
ACKNOWLEDGEMENTS

Thanks first and foremost to my advisor and committee chair, Dr. Ozbek, as well as to Dr. Santiago and Dr. Foerder. I extend great gratitude to Jonathan Davidson, M.D., for his permission to use the CD-RISC to study relationships between resilience and olfaction. I would also like to thank Dr. Liz Culler with Blood Assurance of Chattanooga, along with Debbie Gentry and the rest of the Blood Assurance staff. Additional thanks to the olfaction team at UTC: Robert Gormley, Naomi Whitson, Katie Pendergast, Jessica York, and Justin Brown. Finally, I’d like to thank the students and faculty in the Research Master’s program, all of whom have contributed in their own way to my academic efforts.
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LIST OF ABBREVIATIONS

CESD-R, Center for epidemiologic studies depression scale revised

CD-RISC, Connor-Davidson resilience scale

CRF, Corticotropin-releasing factor

HPA, Hypothalamic-pituitary-adrenal axis

HPLC, High performance liquid chromatography

OBP, Olfactory-binding protein

PCL-C, PTSD checklist for civilians

PSS, Perceived stress scale

PTSD, Post-traumatic stress disorder

NPY, Neuropeptide y

UTC, University of Tennessee at Chattanooga

UV, Ultraviolet

WUTC, Wheeler-UTC odor threshold test
LIST OF SYMBOLS

Da, dalton, standard unit of atomic mass

g, g-force, a measure of acceleration

µg, microgram, designates 1x10^-6 grams

mL, milliliter, designates 1x10^-3 liters

pg, picogram, designates 1x10^-12 grams

°C, degrees Celsius, a unit of temperature

λ, lambda, designates wavelength
CHAPTER I

INTRODUCTION

Olfaction is an integral part of daily life. As one of the primary senses, it defines a great deal of human experience. Olfaction plays a role in many everyday situations: deciding what to eat (Kremer, Holthuysen, & Boesveldt, 2014), recalling autobiographical memories (Willander & Larsson, 2007), and even selecting a mate (Sergeant, Davies, Dickins, & Griffiths, 2005). In addition, olfactory research is also revealing relationships between the sense of smell and many aspects of psychology: memory, mood, and mental health to name a few (Weir, 2011). In order to engage in the study of olfaction and its effects, it is important to be able to measure it in a meaningful way.

Types of Measurement

Olfactory function varies in a number of aspects, and each one is important in measuring overall olfactory performance. (Lotsch, Reichmann, & Hummel, 2008). First of all, smell ability can be measured through odor thresholds. An odor threshold is calculated by offering a range of odorant concentrations to determine how sensitive the individual is to the odorant (C. U. M. Smith, 2008); the calculated value represents the concentration at which the person would theoretically smell the odorant half of the time. This is an important point of distinction: any concentration above this point is more likely to be smelled than not, and anything lower is more likely to not be smelled. Olfaction is also measured in regard to smell identification. This relies
on the participant to smell a given odorant and name it (Doty, Shaman, Kimmelman, & Dann, 1984). Other tests measure the ability to discriminate between different odors (Choudhury, Moberg, & Doty, 2003), as well as how people rate qualitative aspects of a given smell (Zarzo, 2011). As previously mentioned, each of these methods of olfactory analysis is useful for evaluating performance.

**Olfactory Sensitivity and Depression**

Researchers studying depression and olfaction have already utilized many of these analysis principles. In a study of women who sought in-patient treatment for depression, the patient group displayed a decreased ability to discriminate between odors as compared to a control group (Croy et al., 2014). As mood improved over the course of treatment, however, so did olfactory ability; there was no significant difference between groups in regard to odor discrimination once treatment was finished. Additionally, depression has been shown to have an effect on olfactory threshold. Individuals with severe depression display an increased odor threshold for multiple odorants in comparison to healthy controls (Lombion-Pouthier, Vandel, Nezelof, Haffen, & Millot, 2006). The researchers also found that the depressed group over-rated the quality of pleasant odorants (for example violet, orange, and cinnamon), finding them more pleasurable overall than the healthy group. Further studies have revealed more about this discrepancy in perceived smell quality for depressed individuals; Atanasova et al. (2010) found that unpleasant smells are rated as more unpleasant by patients with depression. Together with the previous finding, this suggests that depression causes a polarity in smell perception rather than causing all odors to shift in one direction (pleasant or unpleasant). Finally, Atanasova et al. (2010) also discovered a difference in intensity between good and bad odors. Depressed patients
reported a universally foul smell (butyric acid) as being much stronger than the universally pleasant smell (vanilla), even at odorant levels that healthy controls reported no difference in intensity. The combination of available data has led some researchers to conclude that olfaction is a reliable marker for depression (Croy et al., 2014).

**Olfactory Sensitivity and Stress**

The relationship between depression and olfaction has been substantially explored; stress, on the other hand, has not. The American Psychological Association (2014) reported that, for 2014, the top two causes of stress in the United States were job pressure and money. Seventy-seven percent of Americans report regular physical symptoms of stress, and 73% experience psychological symptoms. According to a joint poll by MtvU and the Associated Press (2009), 60% of college students have been so stressed that it impacted their work, and close to the same amount (53%) were too stressed to engage in social activity. Additionally, stress may be a correlate of collegiate obesity (Odlaug et al., 2015) and consideration of suicide (S. S. Smith et al., 2015) It is clear, therefore, that stress is a major issue facing college students.

Similar to depression, stress has been implicated in affecting olfaction through the quality of smells. Krusemark, Novak, Gitelman, and Li (2013) found that when participants were induced with anxiety by viewing unsettling pictures they reported smells as more unpleasant and responded stronger to these smells than when they were not anxious. Considering that anxiety and stress responses share many of the same physical characteristics, this same effect of odor unpleasantness could most likely result from stress as well. This assertion is supported by Croy, Schellong, Joraschky, and Hummel (2010), who studied chemosensory event-related potentials during olfactory processing in individuals with diagnosed PTSD. These individuals showed an
enhanced processing of unpleasant smells that correlated with the severity of their PTSD symptoms, whereas there was no effect on the processing of pleasant smells. The authors did not, however, find any meaningful difference in olfactory threshold levels between the PTSD and healthy groups. In another study, researchers did observe an increase in sensitivity for scents conditioned to be aversive (Ahs, Miller, Gordon, & Lundstrom, 2013). This could suggest a decrease in threshold for smells associated with traumatic memories in PTSD patients. More research is needed to determine whether olfactory sensitivity is indeed affected by levels of stress.

**Neurological Correlates of Stress and Olfaction**

One strategy that may be employed to elucidate such effects is to examine the underlying neural and hormonal processes of both stress and olfaction. One of the primary components of the body’s stress system is the hypothalamic-pituitary-adrenal axis (HPA), which mediates the response to physical and emotional stressors (Tsigos & Chrousos, 2002). Upon exposure to stress the hypothalamus sends corticotropin-releasing factor (CRF) to the pituitary gland, causing it to release adrenocorticotropic hormone. This hormone in turn activates the adrenal cortex, leading to the production of cortisol. Serum cortisol is frequently used to measure stress levels, as it correlates strongly with the amount of experienced stress (Oswald et al., 2006).

Olfaction has also been shown to affect the activity of the glands involved with the HPA. For example, the smell of compounds similar to sex hormones has been tied to certain hypothalamic activities (Savic, Berglund, Gulyas, & Per, 2001). Specifically, researchers found that the hypothalamus of each male participant was activated upon the olfactory uptake of an estrogen-like compound. Females had similar results when smelling compounds similar to male
androgens. Both of these compounds comprise steroid formations, making them structurally similar to cortisol. Another piece of the HPA stress pathway, the pituitary gland, has also been tied to olfaction. A commonly reported side-effect of pituitary surgery is anosmia, or the loss of the ability to smell; however, this link is not currently well-understood (Tam, Duggal, & Rotenberg, 2013).

**Resilience and Neuropeptide Y**

Olfaction and stress share aspects of their neural pathway. Resilience does as well as an agent of stress reduction. Resilience is the ability to deal with stressors in a way that enables the individual to minimize the negative consequences of the stress itself (American Psychological Association, 2016). This trait is often ascribed to people who persevere after a traumatic experience rather than succumbing to their stress (Iacoviello & Charney, 2016). This has led researchers to explore resilience as a potential preventative measure against traumatic stress in populations at risk for trauma, such as soldiers in foreign wars (Cacioppo et al., 2015). It is also being sought as a treatment for trauma that has already taken place, as learning to be resilient may counteract the stress response in PTSD. (Burton, Cooper, Feeny, & Zoellner, 2015).

Resilience is defined in terms of both behavioral and physiological adaptations to stressful events (Feder, Nestler, & Charney, 2009). Individuals who express behavioral resilience are those who engage in activities and thought practices that promote characteristics indicative of mental well-being. A few examples of these traits are adaptiveness, self-esteem, and strength in social structure (Connor & Davidson, 2003). People who express an increased tendency towards these types of behaviors regularly score lower on measures of stress and depression. These psychological aspects accompany a corresponding biological response in the activity of
neuropeptide y (NPY) (Sadryk, Fitz, & Shekhar, 2006). By counteracting the effects of CRF in the amygdala, NPY serves to reduce the magnitude and longevity of the stress response.

NPY has been shown to correlate closely with resilience and against stress through its behavior in the HPA (Reichmann & Holzer, 2016). One of the primary roles of NPY is the control of body weight and metabolism, which is achieved through its activity in the hypothalamus following a meal (Nguyen, Herzog, & Sainsbury, 2011). Increased levels of NPY in the paraventricular nucleus lead to an increased hunger response (Hanson & Dallman, 1995). Additionally, giving nasal doses of NPY to rats before stress-inducing tasks reduced the stress-related activation of the HPA axis (Sabban, Serova, Alaluf, Laukova, & Peddu, 2015). The link between NPY and resilience suggests that, on a surface level, NPY would improve olfaction by increasing stress resistance and thereby reducing the deleterious effects of stress on the sense of smell.

A similar effect can be seen with NPY and resilience against depression. For example, low NPY is very common among suicide attempters with depression. (Morales-Medina, Dumont, & Quirion, 2010). After taking antidepressants, patients’ NPY levels increase as symptoms improve. Additionally, Morales-Medina et al. (2010) have indicated a genetic polymorphism - Leu7Pro - that leads to enhanced production and faster processing of NPY. Individuals with this mutation have exhibited evidence of depression resistance compared to those with the normal gene. The potential mechanism of NPY’s effects on stress may be tied to the amygdala. PTSD is tied to amygdalar dysfunction, and NPY has been shown to be a marker for resilience in combat veterans (Yehuda, Brand, & Yang, 2006); soldiers with combat exposure but without PTSD have higher levels of NPY than those with PTSD, as well as veterans without combat exposure. When
injected directly into the amygdala of rats, NPY leads to a reduction in stress-conditioned behaviors (Flood, Baker, Hernandez, & Morley, 1989).

The amygdala has also been tied to olfactory function. People experience greater amygdala activity as the foulness of presented odors increases (Zald & Pardo, 1997). Damage to the amygdala results in significant impairment to odor memory and odor recognition (Buchanan, Tranel, & Adolphs, 2003). Similar to NPY, olfactory ability has also been shown to correlate negatively with depression (Croy et al., 2014) and PTSD (Croy et al., 2010); these effects on smell can also be traced to activity between the olfactory sensory relay and the amygdala (Krusemark et al., 2013). NPY may regulate this relationship as well. As found by Hansel, Eipper, and Ronnett (2001), NPY works as a neuroproliferative factor. In one specific example, it promotes the proliferation of olfactory neurons. NPY deficiencies in rats can cut the total amount of these neurons in half. This is particularly interesting considering the nature of the olfactory epithelium, as its neurons are being constantly renewed throughout adulthood (Yang, He, & Hao, 2015). A decrease in neuroproliferation would not stop the death of these cells but would certainly slow their regular replacement. This finding suggests that states coinciding with low NPY over time, such as stress and depression, would lead to olfactory deficits via relative reduction in olfactory neurons. In other words, NPY is tied to resilience against stress as well as against olfactory dysfunction.

**The Present Study**

The current literature is suggestive of a link between stress, resilience, and olfactory ability; however, this model has not been conclusively tested as a whole. Additionally, the studies mentioned above focused on comparing healthy populations with those with clinical
diagnoses, but do not compare olfactory performance against psychological measures within groups. These two issues comprise the focus of the current study.

**Hypotheses**

**Psychological Correlations**

1A) Stress, depression, and symptoms of traumatic experience will all correlate positively.

1B) Resilience will negatively correlate with stress, depression, and traumatic stress.

**Physiological Correlations**

2A) Serum NPY levels will correlate negatively with stress, depression and traumatic experience, but positively with resilience.

2B) Olfactory sensitivity will also correlate negatively with stress, depression, and traumatic experience, but positively with resilience.

2C) These correlations will be stronger for a bad smell than a good smell.
CHAPTER II

METHOD

Review Board

The current study was approved by the Institutional Review Board of UTC (Appendix A). As required by this approval, all electronic participant data has been stored in a manner consistent with ensuring confidentiality. Physical copies of collected data have been placed in locked storage and are inaccessible to individuals not involved in the current study.

Protection of Health Information

All investigators had current training as mandated by the Health Insurance Portability and Accountability Act (HIPAA), the National Institutes of Health (NIH), and the Collaborative Institutional Training Initiative (CITI) in regard to the handling of health information at the time of their contributions to the current study.

Method Overview

The current study comprised two phases. The first consisted of several self-report instruments to be described later in the method. This took place at the start of the spring 2016 semester at the University of Tennessee at Chattanooga (UTC). Subsequently, the second phase required participants to come in person to Blood Assurance of Chattanooga to participate in an
olfactory sensitivity test and give blood (Appendix B). Informed consent was obtained separately for both phases (Appendix C and D).

Participants

For phase I, 197 participants were recruited among the student body. Students ranged in age from 18 to 51 years old (M=20.9, SD=5.4), and females represented 81.7% of the sample. Of these students, 25 agreed to participate in phase II of the study. This sample’s age range was 18 to 33 years old (M=22.7, SD=8.3) and comprised 19 women (76% of this sample). Self-report demographic data for both phases are listed in tables 2.1 and 2.2.

Table 2.1 Age and Gender Demographics

<table>
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<th>Demographics</th>
<th>Mean</th>
<th>Std dev.</th>
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<tr>
<td>Gender (female)</td>
<td>81.7%</td>
<td>76.0%</td>
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<tr>
<td>Age (years)</td>
<td>20.92</td>
<td>22.72</td>
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Table 2.2 Ethnicity Demographics

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<tr>
<td></td>
<td>N</td>
<td>Percent</td>
</tr>
<tr>
<td>White</td>
<td>164</td>
<td>83.2%</td>
</tr>
<tr>
<td>Black</td>
<td>21</td>
<td>10.7%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>0.5%</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>2.0%</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>3.6%</td>
</tr>
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In addition to general demographic information, participants answered a number of questions regarding their personal health history. This primarily served to screen participants for potential allergies to odorants, and it also enabled the investigation of how medical health might relate to the variables listed in the hypotheses. Question topics included smoking habits, physical injuries, illnesses, and allergies, among other medical issues. For a full listing of health data surveyed, see figure 2.1.
Figure 2.1 Health History Data
Phase I Materials and Procedure

Perceived Stress Scale (PSS) (Appendix E)

Stress was measured using the Perceived Stress Scale (PSS) as developed by Cohen, Kamarck, and Mermelstein (1983). This scale consists of 14 questions regarding how often stressful and stress-related events have occurred within the past month. The focus is on the feelings and perceptions of stress rather than counting up potentially stressful events. Participants respond on a 0-4 scale with 0 meaning “never” and 4 meaning “very often.” In addressing net stress over the course of an entire month, this scale can ideally assess cumulative everyday stress over time rather than just a snapshot of the present moment.

Connor Davidson Resilience Scale (CD-RISC)

Resilience was measured by the Connor Davidson Resilience Scale (CD-RISC) (Connor & Davidson, 2003). This scale comprises 25 items designed to address multiple facets of psychological resilience, such as self-esteem, adaptability, and stability in relationships. The calculation of trait subscores has not been validated, so only one overall resilience score is measured. Each question gives respondents a 5-point response range, from 0 “not true at all” to 4 “true nearly all of the time.” A scale score was calculated for each participant by averaging the numerical value of the items. Additional items were added to address two additional theoretical factors of resilience: forgiveness and altruism. These items were included for the purpose of future scale development, and were not factored into scale scores or otherwise considered in the measurement of resilience for the current study.
Center for Epidemiologic Studies Depression Scale Revised (CESD-R) (Appendix F)

Depression was measured using the Center for Epidemiologic Studies Depression Scale Revised (CESD-R) (Eaton, Smith, Ybarra, Muntaner, & Tien, 2004). The CESD-R is a 20-item scale measuring nine aspects of depression. Responses are given by frequency of experience on a 0-4 scale with 0 meaning “not at all or less than one day” and 4 meaning “nearly every day for two weeks.” This scale is valuable in its ability to evaluate depression over a time frame rather than in a single moment. The CESD-R is commonly used as a clinical tool for diagnosing clinical depression, and has an established scoring system with this purpose in mind. The present study, however, only requires a quantitation of depression among participants. Therefore, scores were simply averaged into a scale score for each participant.

PTSD checklist for civilians (PCL-C) (Appendix G)

Participants also took the PTSD checklist for civilians (PCL-C) (Blanchard, Jones-Alexander, Buckley, & Forneris, 1996). This survey consists of responses to stressful experiences, and how often those responses have taken place in the last month. These questions are answered on a 1-5 scale (1=Not at all, 5=Extremely). The questionnaire was designed to be a self-report measure for use in clinical settings; the items are divided into categories based on diagnostic criteria for PTSD from DSM-IV. Now that the DSM-5 has been released, these criteria are no longer fully valid for diagnostic purposes. However, the scoring instructions also allow for a total severity scale score comprising all items, which is all that is required for the purposes of the current study. The PCL-C has been proven valid and reliable as a measure of response to traumatic stress in general, which is not contingent on being aligned with the current edition of the DSM.
Procedure

Participation in phase I was made available to students immediately upon IRB approval. The demographic, health, and psychological surveys were hosted on Qualtrics and offered through UTC’s research participation system website (SONA). Some surveys were also given in person at the UTC University Center in order to incorporate students outside of the psychology department. The entire survey took roughly 15 minutes to complete. Following the phase I surveys, participants were emailed with information about the on-campus counseling center. They were advised to seek aid there if they felt like they were having issues with stress, depression, or traumatic stress. Additionally, they were provided with a brief description of phase II and asked whether they were interested in participation. Each student who expressed interest was contacted via email to set up an appointment for phase II.

Phase II Materials and Procedure

Wheeler-UTC Odor Threshold Test (WUTC)

Olfactory performance was assessed with an odor threshold test, as threshold tests provide the greatest predictive value of overall performance among olfactory measures (Lotsch et al., 2008). The Wheeler-UTC odor threshold test (WUTC) was designed to provide olfactory threshold data on participants while also allowing researchers to measure response bias and task performance (Tewalt, 2013). Five odorants (vanillin, p-cresol, pinene, ethanol, and isoamyl acetate) were included in the original standardization of the WUTC. These were chosen based on the variability of their chemical structures in order to assess multiple odorant-binding categories.

For the current study only two odorants were chosen in order to reduce administration time: vanillin and p-cresol. While structurally similar, these odorants are perceived as qualitative
opposites by humans. Vanillin is almost universally regarded to have a good smell, theoretically due to its presence in breast milk (Edraki et al., 2013). p-Cresol, on the other hand, is widely regarded to be a foul odor. It is the primary compound associated with the scent of pig excrement (Borrell, 2009), and is also present in human waste as well (Hamer, Preter, Windey, & Verbeke, 2011). Each compound comprises a hydroxyl group attached to an aromatic ring. p-Cresol also possesses a methyl group para- to the hydroxyl group, whereas vanillin contains both an aldehyde and an ether group. Finally, vanillin and p-cresol both have fairly small molecular weights (152.15 and 108.13, respectively).

For each odorant, 20 concentrations were prepared. This was accomplished by first mixing a concentrated stock standard in water. These concentrations were .08 mg/mL p-cresol and .50 mg/mL vanillin. This constituted the most concentrated sample presented to participants; the remaining 19 steps were created following a 1:3 dilution scheme. In the end, each successive step had 1/3 the odorant concentration of the step that preceded it. Therefore, the lowest concentration step for p-cresol was .07 pg/mL and the lowest for vanillin was .43 pg/mL. In addition to the odorant-containing steps, 12 blanks were prepared that only included pure water. Each presentation contained 10 mL of solution, and was prepared in its own 20 mL amber glass vial and sealed with a screw-thread cap. Amber glass was chosen because vanillin is prone to degradation from exposure to light. Overall the test contained 52 vials: 20 steps of vanillin, 20 steps of p-cresol, and 12 blanks.

Administration of the WUTC

Participants taking the WUTC sat down in a comfortable chair. They were informed that a series of vials would be opened and presented to them, and they needed to smell the contents
and tell the researcher whether they smelled anything by answering “yes” or “no.” They were also told that some vials would have a scent while others would not, and that they should give the first response that occurs to them rather than trying to guess the correct answer. Finally, a modification was made in the participant instructions of the WUTC: participants were given specific instruction to consider the scent of water a lack of scent and given an example of a water blank to smell. This modification was added to reduce potential positive response bias. Each vial was then presented to the participant until they verbally responded to it, at which point the next vial was given five seconds later. Responses were recorded by the researcher administering the test and were hidden from the participant. Each vial was presented twice for a total of 104 presentations. The whole test was given in one of two randomized vial orders that alternated between participants. Each order is characterized by which odorant’s highest concentration appears first, in order to assess any effect it might have on smelling the other odorant. The test was concluded once a reply had been given for each vial.

Blood Sampling

Upon completion of the WUTC, participants were directed to the certified phlebotomists at Blood Assurance of Chattanooga for blood drawing. They were also given the option to donate to Blood Assurance at the same time. Each participant gave roughly 10 mL of blood stored in Vacutainer blood collection tubes, after which they received a $10 gift card. Each tube was labelled with the date and time of the collection, as well as the participant number used to match blood samples to phase I and WUTC data. Upon collection, samples were centrifuged at 2000 x g for 10 minutes to separate the serum from the rest of the blood. The serum was then removed and placed into a separate tube for storage. Centrifugation yielded 1-2 mL of serum from each
sample. Aprotonin was added to serum samples at 100µL per 1mL of serum to prevent protein degradation through proteolysis. Serum and supernatant for each sample were then stored at a maximum temperature of -20°C in a freezer in the UTC biochemistry lab. Once all blood samples had been taken, each serum sample was thawed and an aliquot was taken from each for further centrifugation. This time, samples underwent additional separation through the use of 30,000Da membrane filters. These samples were spun at 2000 x g for 30 minutes. Filtrate for each sample was collected, labeled, and stored under refrigeration for the duration of the study.

Protein Analysis

After filtration, the presence and concentration of protein was measured for each sample using the Bradford assay technique (Bradford, 1976). Five protein standards of known concentration were prepared, and 100µL of each was mixed with 4mL of Bradford dye. This solution was then vortexed and transferred to a plastic cuvette for analysis. The absorbance of these solutions was determined with the protein analysis function of a Shimadzu Biospec-1601©. The values obtained for each standard were used to calculate a standard curve that enables the calculation of unknown protein concentrations in blood samples. Once the standards had been analyzed, each sample was analyzed the same way after mixing 30µL of sample with 4mL of Bradford dye.

UV/Fluorimeter

In order to quantify cortisol and NPY concentrations using advanced instrumentation, the absorbance wavelength (λ) of each compound was ascertained. Molecular ring structures are highly absorbent, and are typically representative of the absorbance of the molecule as a whole.
This applies to the steroid base of cortisol. Proteins can be much more complex due to the variance of amino acids with ring structures that absorb at different wavelengths. Of these amino acids, NPY contains only tyrosine. This erases the potentially increased difficulty inherent in scanning UV absorbance in proteins. Standards for these compounds were analyzed using the UV spectrometry function of a Shimadzu Biospec-1601© in order to determine those values. Preparations of each standard were transferred to quartz cuvettes and scanned for UV absorbance, indicated by a peak in the output of the instrument. This analysis yielded $\lambda=252\text{nm}$ for NPY and $\lambda=290\text{nm}$ for cortisol.

Using the values from the UV scan, these wavelengths were refined using an Agilent Cary Eclipse Fluorescence Spectrophotometer©. By scanning at a particular absorbance, the excitation of the molecule can be determined. Scanning at this excitation will yield an absorbance value, and continuing the process back and forth will eventually single out a specific excitation wavelength. For NPY, this was 249nm and for cortisol this was 285nm. By recording the peak size for numerous different standard concentrations, a standard curve was established for both compounds. This enabled quantitation of both NPY and cortisol utilizing the fluorimeter.

High Performance Liquid Chromatography (HPLC)

Standard solutions at known concentrations of each compound were prepared in order to establish a standard curve using high performance liquid chromatography (HPLC). Each standard was run through a Zorbax Poroshell© column, which separates compounds by hydrophobicity. The UV detector of the HPLC was programmed to detect UV absorbance at the
wavelengths determined from fluorimeter analysis. Instrument settings for the determination of each compound are listed in Appendix H.
CHAPTER III

RESULTS

Psychological Measures

For each measure given in the survey, a scale score was calculated by averaging the individual scores of each item. Correlations were calculated between every pair of measures for all 197 phase I participants. Among scores for the CESD-R, PSS, and PCL-C, all pairs exhibited significant positive correlations (CESD-R/PSS: \( r = .641, p < .000 \); CESD-R/PCL-C: \( r = .779, p < .000 \); PSS/PCL-C: \( r = .644, p < .000 \)). Conversely, scale scores for the CD-RISC correlated negatively with all three of the aforementioned measures (CESD-R: \( r = -.455, p < .000 \); PSS: \( r = -.579, p < .000 \); PCL-C: \( r = -.360, p < .000 \)).

Among the 25 phase II participants, all pairs of scores for the CESD-R, PSS, and PCL-C yielded significant positive correlations (CESD-R/PSS: \( r = .751, p < .000 \); CESD-R/PCL-C: \( r = .700, p < .000 \); PSS/PCL-C: \( r = .525, p = .007 \)). As with the overall phase I scores, the CD-RISC correlated negatively with the CESD-R and PSS within the phase II sample (CESD-R: \( r = -.636, p = .001 \); PSS: \( r = -.644, p = .001 \)). Unlike phase I, however, the correlation between CD-RISC and PCL-C scale scores in phase II participants was not significant (\( r = -.258, p = .214 \)). Finally, from phase I to phase II there is a roughly one integer reduction in the maximum score attained for the CESD-R, PSS, and PCL-C. An account of these scores can be found in Table 3.1. Using regression analysis to account for all four psychological measures, stress was found to be a
significant predictor of phase II participation (p = .05). This was an inverse relationship, wherein lower stress increased the probability of volunteering for phase II.

Table 3.1 Psychological Measure Scale Scores

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>CESD-R</th>
<th>PSS</th>
<th>PCL-C</th>
<th>CD-RISC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase I</td>
<td>Phase II</td>
<td>Phase I</td>
<td>Phase II</td>
</tr>
<tr>
<td>N</td>
<td>197</td>
<td>25</td>
<td>197</td>
<td>25</td>
</tr>
<tr>
<td>Mean</td>
<td>1.77</td>
<td>1.90</td>
<td>2.84</td>
<td>2.75</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.72</td>
<td>0.78</td>
<td>0.60</td>
<td>0.57</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.00</td>
<td>1.00</td>
<td>1.50</td>
<td>1.71</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.75</td>
<td>3.70</td>
<td>4.64</td>
<td>3.86</td>
</tr>
</tbody>
</table>

Biological Measures

All 25 phase II samples were initially analyzed for protein concentration using protein analysis. Protein was present in all samples (M = 39.5ug/mL, SD = 14ug/mL). NPY was quantified using fluorimeter analysis; sample peak areas were converted to ug/mL using the equation derived from the standard curve (M = 114.3ug/mL, SD = 36.18). A protein itself, NPY nevertheless does not correlate with measured protein levels (r = .165, p = .429). Finally, the concentration of cortisol in the samples was determined using HPLC. The relatively small concentration of cortisol in the samples necessitated the use of an internal standard. Aliquots of each sample were diluted with stock standard to reach an internal standard concentration of .6 ug/mL. The peak area of pure standard at this same concentration was subtracted from the peak areas of the ensuing sample runs. The concentration in ug/mL was calculated from sample peak areas using the equation of the standard curve (M = .093ug/mL, SD = .055ug/mL).
**Olfactory Thresholds**

Olfactory thresholds were calculated using logistic regression to determine the concentration at which each participant has an equal chance of smelling or not smelling the odorant. Nagelkerke’s $R^2$ values were calculated for each threshold. Similar to the $r^2$ statistic calculated for linear correlations, Nagelkerke’s $R^2$ measures the goodness-of-fit of the data points to a logistic regression (Peng, Lee, & Ingersoll, 2002). Thresholds with corresponding $R^2$ values less than .25 were discarded, as such a low score indicates that the data is a poor fit for calculating logistic regressions. Five of the subjects’ vanillin thresholds and three of the subjects’ p-cresol thresholds were removed from further analysis for this reason.

An additional statistic was calculated to assess response bias. If a participant has a tendency towards one answer over the other, it makes it more difficult to attribute results purely to olfactory function. The statistic used to check this is $B^*$ (Stanislaw & Todorov, 1999). Response bias is a function of two probabilities: how likely a participant is to answer yes when it is correct to do so, and how likely that participant is to say yes when it is incorrect. A measure of task performance was also calculated, called Youden’s $J$ (Youden, 1950). This statistic measures how “successful” a participant was at the threshold test by comparing the rate of correct yesses to the rate of correct noes. A perfect $J$ score would involve answering yes to every vial with odorant, and no to every blank. In this sense it theoretically provides a different, less direct assessment of olfactory performance. Threshold values for each odorant, accompanied by the aforementioned statistics, can be found in Table 3.2.
Table 3.2 Statistical Measures for Olfactory Threshold Test

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Threshold</th>
<th>Nag R2</th>
<th>B''</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vanillin</td>
<td>p-Cresol</td>
<td>Vanillin</td>
<td>p-Cresol</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>22</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0228</td>
<td>0.0277</td>
<td>0.67</td>
<td>0.70</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.0379</td>
<td>0.0462</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.28</td>
<td>0.37</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.1748</td>
<td>0.1983</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The order of presentation was altered between administrations; 13 participants had order A and 12 participants had order B. The vial order did not significantly affect threshold values for vanillin (p = .134) or for p-cresol (p = .777). There was also no effect of order on participants’ combined B” (p = .563) or J (p = .109) scores.

Combined Results

Psychological Measures and Olfactory Thresholds

Correlations with vanillin were insignificant for all psychological measures. Thresholds for p-cresol were positively correlated to PSS score, and negatively correlated to CD-RISC score. Correlations for both odorants can be found in Table 3.3.

Table 3.3 Psychological Measures Correlated to Thresholds

<table>
<thead>
<tr>
<th>Correlations</th>
<th>CESD-R</th>
<th>PSS</th>
<th>PCL-C</th>
<th>CD-RISC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Vanillin</td>
<td>-0.157</td>
<td>0.507</td>
<td>-0.237</td>
<td>0.314</td>
</tr>
<tr>
<td>p-Cresol</td>
<td>0.367</td>
<td>0.093</td>
<td>0.520</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Regressions were also run to determine the combined ability of threshold, response bias, and performance for each odorant in predicting scores on the psychological measures. When accounting for the variability in all three, these analyses implicated the threshold of p-cresol to be uniquely predictive of depression \((p = .045)\), stress \((p = .02)\), and resilience \((p = .002)\). The relationship was direct for depression and stress, and inverse for resilience. The response bias of p-cresol was also uniquely predictive of depression \((p = .021)\) and stress \((p = .015)\) in an inverse relationship. As opposed to p-cresol, the collection of olfactory measures revealed much less when using vanillin. Only the response bias was predictive of any of the psychological measures, and only for stress \((p = .041, \text{ an inverse relationship})\).

Biological Measures and Olfactory Thresholds

Protein concentration did not significantly correlate with the thresholds for vanillin \((r = -.256, p = .275)\) or p-cresol \((r = .134, p = .552)\). Similarly, NPY levels exhibited no significant relationship with vanillin \((r = -.137, p = .564)\) or p-cresol \((r = .021, p = .927)\). Finally, neither vanillin nor p-cresol correlated significantly with serum cortisol concentration \((r = .024, p = .919; r = .058, p = .796 \text{ respectively})\).

Psychological Measures and Biological Measures

The two measures of stress in the current study, PSS and cortisol, do not correlate with one another \((r = .063, p = .766)\). The measured concentration of the theoretical marker for resilience against stress, NPY, correlates with neither resilience \((r = -.118, p = .575)\) nor stress \((r = .218, p = .295)\). PCL-C score did correlate positively with protein levels \((r = .447, p = .025)\).
Relationships with Health Status

Upon analysis of data from the health questionnaire, a number of interesting relationships were identified. Depression scores were positively correlated to lung disease ($r = .154$, $p = .033$), which was also positively correlated with traumatic stress ($r = .231$, $p = .001$) and protein concentration ($r = .497$, $p = .011$). Also of note, NPY concentration was positively correlated with a history of traumatic brain injury ($r = .537$, $p = .006$). Three health issues that are typically implicated in olfactory dysfunction (asthma, seasonal allergies, and sinus issues) were not observed to share any significant relationships.
CHAPTER IV
DISCUSSION

In the current study, stress was examined as a potential dampener of olfactory performance. Resilience was measured as a counter-actor to the effects of stress: a trait beneficial to the sense of smell. Depression was assessed in regard to olfactory performance as well, in addition to traumatic stress. These psychological constructs were measured via survey. Additionally, stress and resilience were measured biologically. This was accomplished by determining concentrations of cortisol and NPY, respectively, in serum. This study constitutes the first attempt to use resilience (via CD-RISC) and olfactory threshold data (via WUTC) to clarify the relationship between olfactory sensitivity and stress. Ultimately, stress and resilience are indeed correlated to olfactory performance; however, their respective biological markers were inconclusive. A summary of the support for each stated hypothesis can be found in Table 4.1.
Table 4.1 Hypothesis Support Summary

<table>
<thead>
<tr>
<th>Hypotheses</th>
<th>Supported?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Stress, depression, and traumatic stress correlate positively</td>
</tr>
<tr>
<td>1B</td>
<td>Resilience correlates negatively with the above three</td>
</tr>
<tr>
<td>2A</td>
<td>NPY will have a negative relationship with stress, depression, and traumatic stress. It will have a positive correlation with resilience</td>
</tr>
<tr>
<td>2B</td>
<td>Olfactory threshold will have correlations with stress, depression, traumatic stress, and resilience in the same pattern as expected for NPY</td>
</tr>
<tr>
<td>2C</td>
<td>Relationships will be stronger for the bad smell, p-cresol</td>
</tr>
</tbody>
</table>

The relationships among stress, traumatic stress, depression, and resilience came out as hypothesized in the full phase I sample. This was consistent with previous literature, and indicated that the constructs involved are closely related; people who have high stress will tend to be more depressed, and people who have high resilience will be resistant to both stress and depression. The means and standard deviations of these measures were almost unchanged from the 197 phase I participants to the 25 phase II participants. The ranges, however, got smaller in phase II for the CESD-R, PSS, and PCL-C. This change took place specifically at the maximum of these measures, where each phase II maximum was roughly one whole point less than its phase I counterpart. Given that each was measured on a scale of 1-5, this is a considerably large drop off. Ultimately, there was no representation within the phase II analyses of people who fall
within the highest levels of stress, depression, and traumatic stress. As a clear illustration of this effect, high stress was determined to be a significant predictor of nonparticipation in the second phase. It may be necessary to restrict future studies to one phase in order to prevent this effect.

Among the psychological scales that were measured, stress and resilience did have significant relationships with the olfactory threshold for p-cresol. This was not the case with vanillin. Additionally, the combination of threshold, response bias, and task performance was much more predictive of psychological measures when using p-cresol instead of vanillin. This may suggest that foul smells are more vulnerable to the effects of stress-related loss of olfactory sensitivity than other smells, possibly due to an unconscious aversion to further negative stimuli in a highly stressed state. Furthermore, the good smell of vanillin may be particularly robust against alterations. Vanillin is present in human breast milk and amniotic fluid (Edraki et al., 2013), so its detection may be prioritized through natural selection. Additionally, the observed outcome may be attributed to the relationship of each odorant to olfactory-binding proteins (OBPs). These proteins reside within the mucus membrane of the nasal cavity and aid in the transport of odorant molecules to the olfactory bulb (Goldberg, Turpin, & Price, 1979). Previous studies on bovine OBPs indicate that vanillin does not bind with them (Pevsner, Hou, Snowman, & Snyder, 1990), while p-cresol does quite strongly (Karthikeyan et al., 2014). This suggests that the effects of stress and resilience may specifically affect OBP function. As a result, olfactory performance for odorants that rely on OBPs for uptake into the olfactory epithelium could be much more vulnerable to stress than for those that do not. This ultimately suggests that odor selection is vastly important when assessing olfactory function; the use of a single odorant cannot represent olfactory performance as a whole.
Biological compounds measured in blood serum samples consisted of cortisol and NPY, as well as the level of overall protein present. NPY, a protein itself, did not correlate with the total protein concentration. This was due most likely to the multitude of other proteins present in human blood; at least 325 different proteins have been identified in the serum portion alone (Pieper et al., 2003). Therefore, the variance in NPY does not have enough influence to noticeably alter the overall concentration of serum protein. Additionally, there is no observed relationship between NPY and cortisol. As the biological indicators of resilience and stress (respectively), they should theoretically share an inverse relationship as opposed forces. The fact that they do not suggests that there is a more complicated set of circumstances governing their concentrations. In its role as a hunger regulator, NPY may fluctuate based on an individual’s satiety at a given time (Kalra, Dube, Sahu, Phelps, & Kalra, 1991). This is compounded by the fact that cortisol drops considerably over the course of the day (Wust et al., 2000).

Further still, these two compounds were not found to correlate with their corresponding psychological measures. This may be a result of the nature of the scales; both the PSS and the CD-RISC ask participants to indicate how well the statements have applied to them in the past month. On the other hand, NPY and cortisol measured in the blood are emblematic of the state of the body at the exact time the blood was drawn. Momentary stress, such as that associated with phlebotomy needles, may have a disproportionately large effect on the levels of these compounds. It is possible that biological markers are not good indicators of long-term physiological trends, but this would run contrary to the aforementioned literature on stress and resilience. It is more likely that this observed result is another consequence of the reduced range in phase II participants’ psychological scale scores. Long-term stress may not have a noticeable effect on the normal variability of cortisol concentration unless that stress is very high. Likewise,
individuals with normal levels of stress may not exhibit NPY levels according to their psychological resilience; NPY levels are highest after exhibiting resilience against a recognized stressor (Yehuda et al., 2006). Ultimately, this incongruence between psychological and biological measures suggests that olfactory dysfunction at the hands of stress is accumulated over time rather than being subject to acute loss.

This conclusion lends credence to the proposed mechanism of loss of olfactory sensitivity. Stress-related olfactory loss was theorized to arise out of a gradual reduction in olfactory neurons. This reduction was assumed to occur not from excess neuronal destruction, but rather from a lack of neuroproliferation. Since the replenishment of the olfactory bulb is so rapid, interrupting the creation of new neurons could significantly deplete the population of neurons in a matter of weeks. Despite the fact that NPY did not share a relationship with olfactory function in this study, it is not unreasonable to suggest that the NPY level on the day of blood collection may not be indicative of the overall trend in NPY over a period of time when stress is high.

Additional relationships were discovered through the analysis of health information. Most notable among these is the correlation between NPY and traumatic brain injury. As previously mentioned, the rise in NPY corresponding with resilience is contingent upon needing to utilize that trait. Physical trauma is capable of creating psychological trauma (deRoon-Cassini, Mancini, Rusch, & Bonanno, 2010), therefore a traumatic brain injury could trigger the need for psychological resilience. Also of note is the relationship between lung disease and traumatic stress. This makes sense, as individuals diagnosed with PTSD have a greater lifetime prevalence of many types of disease (Weisberg et al., 2002), including lung disease. This does not explain, however, why the many other diseases on the health questionnaire did not also correlate with
traumatic stress. Smoking may be another culprit here. Although smoking history did not correlate with lung disease in the present data, it did correlate with stress. When considering this, it is important to point out that a large majority of the participants were between the ages of 18-22. Many of the diseases listed in the survey do not arise for most people until later in life, such as high blood pressure, stroke, and heart disease. Finally, it is also interesting that olfactory function was not affected by asthma, seasonal allergies, or sinus problems. All three of these health issues were widely represented in the phase II sample. It may be that these illnesses all have acute effects on olfactory ability. The questions on the survey simply ask for a history, so they are not necessarily representative of the current health state as it would apply to olfactory performance.
CHAPTER V
CONCLUSION

The evidence gathered in the current study suggests that, in the end, stress leads to and resilience protects from the loss of olfactory ability. However, without the confirmation of their biological correlates (cortisol and NPY, respectively) it is impossible to determine whether each factor is acting independently or if they are simply opposite ends of the same process.

Limitations

One primary limitation of the present study was the number of participants in phase II. With only 25 individuals, there was not enough statistical power to ensure that all existing relationships were identified. This also means the effects that were found to be significant were more vulnerable to the skewing nature of extreme values. With a larger number of subjects there would be more certainty with regard to the accuracy of the given results. Additionally, the small sample did not include any individuals who scored in the highest quarter of the CESD-R, PSS, or PCL-C. This limits the generalizability of the present findings, as it fails to account for people with extreme levels of stress and depression. Finally, the overabundance of females as opposed to males may have affected the results. Women have lower olfactory thresholds than men on average (Diamond, Dalton, Doolittle, & Breslin, 2005). The hormonal fluctuations brought on by the menstrual cycle also alter olfactory performance (LeMay, 2014). Participants were not asked about their menstrual cycle though, so any variability resulting from it is unaccounted for.
There were a number of limitations with the measurement of NPY and cortisol as well. The ideal method for analyzing levels of NPY is by radioimmunoassay. This lies outside the possibilities of the UTC biochemistry lab, so the concentration of serum NPY was quantified using a fluorimeter. This instrument reads the amount of UV absorbance of each sample for a given wavelength. This is the limit of specificity for the fluorimeter; any proteins or other compounds that absorb at the set wavelength will add to the measured absorbance. Therefore, the calculated values for NPY are vulnerable to overstating the amount of NPY actually present. Also, there are a number of factors contributing to the variability of cortisol and NPY that were not controlled for. For example, cortisol levels undergo a sharp increase after waking up in roughly 75% of individuals (Wust et al., 2000). NPY levels fluctuate based on hunger (Kalra et al., 1991). To properly account for these effects, participants would need to submit to strict dietary instructions and multiple blood draws at different times of day.

Another limiting factor was the utilization of only one type of smell test. While threshold is the most telling measurement, smell identification and discrimination also make up a sizeable portion of overall olfactory performance (Lotsch et al., 2008). Not only could these measures help form a more comprehensive picture of each participant’s ability to smell, but they could also help to reveal odor-specific and test-specific olfactory relationships similar to those suggested in the present study. The threshold test is also limited itself in that 10% of scores had to be discarded due to a lack of fit with the logistic regression analysis (as measured by Nagelkerke’s $R^2$). While this is beneficial for ensuring the accuracy of relationships between threshold and other variables, it exacerbates the issue of having a small sample size. Having additional measures of olfactory performance could help alleviate this problem. Finally, stress may affect olfactory threshold measurements in unexpected ways. Response bias was predictive of stress
with both odorants; people with higher stress were more likely to answer “no” to any given odorant presentation. This suggests that highly stressed individuals could have a predilection towards not being wrong, viewing “no” as the safer choice when they are unsure of their answer. This would artificially increase their measured threshold as a result.

**Future Directions**

In the future, the present study should be replicated with an added focus on recruiting more and more varied participants for olfactory and blood analyses. The fluctuations in NPY and cortisol due to resilience and stress respectively may not be evident when levels of stress are only mild/moderate. The results can be confirmed along the entire range of scores by specifically targeting individuals who score very high on depression, traumatic stress, and stress. This will enhance the generalizability of the study to cover people with all levels of stress and depression.

Additional work needs to be done with other odorants as well. Future experiments should incorporate compounds that bind with OBPs as well as those that do not. This will help to elucidate the specific interactions that underlie how mood may affect olfactory ability. Aside from this dichotomy, there are also odorants with middling affinity for OBPs (Pevsner et al., 1990). These should be tested as well to assess the properties of stress and olfaction over the whole range of binding activity.

In regard to taking blood samples for physiological measures, there are a number of steps that should be taken in future studies to ensure the utility of such analyses. In addition to cortisol and NPY, serotonin could also be quantified as the marker for depression (Tamatam, Khanum, & Bawa, 2012). To correct for the variance in concentrations of these compounds (both within and between subjects), participants would need to give blood multiple times throughout a given test
day. Their eating habits would also need to be strictly regulated to control for the fluctuations in NPY due to hunger. Finally, assessing heart rate would also be prudent for ensuring accurate measurements; a change in heart rate immediately before giving blood (due to arousal from seeing the needle, for example) would indicate a likely increase in cortisol level that is not otherwise related to the target variables of the current study.

Ultimately, the current study joins the previous literature in suggesting a link between stress and the sense of smell, even when stress is rated as mild to moderate. The mechanism behind this interaction, however, has still not been definitively confirmed. This therefore should be the primary goal in future research into olfaction and psychological well-being. As the relationship between the two becomes better comprehended, the focus of study should broaden to incorporate the diagnostic capabilities of this knowledge. Given that the threshold and response bias for p-cresol were predictive of general stress levels, it is exciting to consider extending the utility of measuring olfactory ability towards diagnostic purposes. Specifically within the purview of the current study, the theorized relationship between olfactory performance and traumatic stress could aid in the diagnosis of PTSD. This disorder is uniquely difficult to adequately diagnose due to the wide array of symptoms and causes that may present (Rosen, Spitzer, & McHugh, 2008). Having a reliable, noninvasive diagnostic measure that includes a measure of response bias would therefore be highly beneficial for practitioners. This study provides strong evidence for a relationship among stress, resilience, and olfaction, which should help guide future research into the intersection of psychology and olfaction.
REFERENCES


LeMay, C. C. (2014). *Hormones rule the roost: Fluctuations of olfactory functioning throughout the menstrual cycle and during pregnancy*. (Masters Thesis), The University of Tennessee at Chattanooga, Chattanooga, TN.


APPENDIX A

IRB APPROVAL
MEMORANDUM

TO: William J. Heaton  IRB # 15-185
    N. Whitson
    K. Pendergast
    J. York
    S. Gagliano
    M. Santiago
    I. N Ozbek

FROM: Lindsay Pardue, Director of Research Integrity
      Dr. Amy Doolittle, IRB Committee Chair

DATE: January 27, 2016

SUBJECT: IRB #15-185: Exploring Relationships between Stress and Corticosteroids
         as Mediated by Neuropeptide Y

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

The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004148) has
approved this research project # 15-185.

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
instpdb@utc.edu

Best wishes for a successful research project.
APPENDIX B

BLOOD ASSURANCE SUPPORT LETTER
MEMORANDUM

To: UTC IRB
From: Liz Cullier, MD - Medical Director
Date: 12/30/2015
Subject: Blood Assurance permission letter for Exploring Relationships between Stress and Olfaction as Mediated by Neuropeptide Y

Blood Assurance has reviewed the IRB application for "Exploring Relationships between Stress and Olfaction as Mediated by Neuropeptide Y". We have worked with Dr. Ozbek in the past in a similar capacity. Please let this communication serve as Blood Assurance's permission to collect blood to support this research project once the project is approved by the UTC IRB.

We appreciate our relationship with UTC and are excited to be a part of this important project. Please let me know if you have any questions. I may be contacted via email at eec@bloodassurance.org or on my direct line at (423) 752-5901.
APPENDIX C

PHASE I INFORMED CONSENT
SUBJECT INFORMATION AND CONSENT FORM

Your participation in this research study is strictly voluntary, meaning that you may or may not choose to take part.

The purpose of the study is to determine whether the sense of smell may be tied to stress through the action of certain blood-borne proteins. Once you have provided your consent to participate in the study, information about you and your health history will be collected and you will be given a self-report survey to complete. This constitutes phase I of the study; if you are interested in taking part in Phase II you can give your contact information to us following the survey.

POTENTIAL BENEFITS: There is no guarantee of personal benefit as a result of your participation. Your participation in this study may provide information that may help people with anosmia (loss of taste and smell) and people suffering from depression or stress disorders.

COSTS TO YOU: There is no cost to you for participation in this study.

COMPENSATION TO YOU: You will receive no tangible compensation from the investigators for your participation in Phase I of this study. Completion of Phase I does qualify you for Phase II, in which $10 gift certificates are given as compensation.

WITHDRAWAL AND TERMINATION: Your participation in this study is completely voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits.

CONFIDENTIALITY: As a part of this research, we will collect and use records that contain information or data about you and your health. Under the privacy laws, you have the right to decide who can use your personal health information (called PHI). When you sign this consent form, you are agreeing that you will allow the use or your personal health information for this study. The information that will be collected about you as a part of this research includes: Name, Birth date, race, allergies, medications, etc. Information collected about you for the study will be kept in a research file that is separate from your medical chart and all information about you will be kept confidential. You will not be able to see your research file until after the end of the study.

AUTHORIZATION FOR USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES: You have agreed to participate in a research study that has been described to you in the research consent form above. To do this research, we need to collect health information that identifies you. There is a federal law called the Health Insurance Portability and Accountability Act of 1996 (HIPAA). This law protects the confidentiality of your protected health information. Protected health information is information about you that could be used to find out who you are.
HOW WILL MY INFORMATION BE USED? The researchers may use your health information to analyze and evaluate the results of the study. Study data does not directly identify you. The study may be published in psychology journals or may be shared with others as part of scientific discussions. Your name will not be included in any summary of this study.

WHO CAN SEE YOUR RECORDS? By signing this consent form, you are letting the researchers at the University Of Tennessee at Chattanooga and the Southeast Renal Research Institute see your PHI collected in this study. All Personal Information and Health Information will be kept confidential.

PATIENT CONSENT: I hereby freely and voluntarily consent to participate in the study described above. This consent is given based on the verbal and written information provided to me, and the understanding that I am medically and physically qualified to participate in this study. I am free to ask questions at any time. I understand I have the option to choose not to participate or to withdraw from the study at any time without incurring any penalty or loss of benefits otherwise available to me. I understand that by consenting to participate in this study, I am responsible for carrying out instructions and that I must relate to the study personnel any information that might be pertinent to the study, such as side effects of the treatment or procedure. I understand that any significant new findings or discoveries during the course of this research which may be related to my willingness to continue participation will be provided to me. I have read, or have had read to me, the above statements, and understand them as they apply to me. I further understand that I may revoke this consent at any time, except to the extent that action has already been taken in accord with this consent. I have been told that, if I have additional questions about the study, I may contact William Heaton (principal investigator for this study; hxw451@mocs.utc.edu) or I. N. Ozbek, Ph.D., Professor of Psychology at UTC; nicky-ozbek@utc.edu, either of whom can be contacted through the UTC Psychology office at 423-425-4262.

________________________________________
Printed name of participant

________________________________________
Signature of participant                     Date/Time

________________________________________
Research Assistant Obtaining Consent        Date/Time
APPENDIX D

PHASE II INFORMED CONSENT
SUBJECT INFORMATION AND CONSENT FORM

Your participation in this research study is strictly voluntary, meaning that you may or may not choose to take part.

The purpose of the study is to determine whether the sense of smell may be tied to stress through the action of certain blood-borne proteins. This phase requires your presence at Blood Assurance of Chattanooga. Once you have provided your consent to participate in the study, you will take a smell test that involves smelling a series of vials. Following this test, you will give a small amount of blood (taken by professional phlebotomists at Blood Assurance) that will be analyzed for certain protein levels. You will also be given the option to donate blood to Blood Assurance at that time.

POTENTIAL BENEFITS: There is no guarantee of personal benefit as a result of your participation. Your participation in this study may provide information that may help people with anosmia (loss of taste and smell) and people suffering from depression or stress disorders.

COSTS TO YOU: There is no cost to you for participation in this study.

COMPENSATION TO YOU: Participants who complete Phase II of the study will receive a $10 gift card. For your information, the Internal Revenue Service (IRS) has ruled that a research participant can ‘earn’ up to $600.00 a year as a participant in research studies, before monies (gift cards) are considered income and subject to tax.

WITHDRAWAL AND TERMINATION: Your participation in this study is completely voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits.

CONFIDENTIALITY: As a part of this research, we will collect and use records that contain information or data about you and your health. Under the privacy laws, you have the right to decide who can use your personal health information (called PHI). When you sign this consent form, you are agreeing that you will allow the use or your personal health information for this study. The information that will be collected about you as a part of this research includes: Name, Birth date, race, allergies, medications, etc., Information collected about you for the study will be kept in a research file that is separate from your medical chart and all information about you will be kept confidential. You will not be able to see your research file until after the end of the study.

AUTHORIZATION FOR USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES: You have agreed to participate in a research study that has been described to you in the research consent form above. To do this research, we need to collect health information that identifies you. There is a federal law called the Health Insurance Portability and Accountability Act of 1996 (HIPAA). This law protects the confidentiality of your protected health information. Protected health information is information about you that could be used to find out who you are.
HOW WILL MY INFORMATION BE USED? The researchers may use your health information to analyze and evaluate the results of the study. Study data does not directly identify you. The study may be published in psychology journals or may be shared with others as part of scientific discussions. Your name will not be included in any summary of this study.

WHO CAN SEE YOUR RECORDS? By signing this consent form, you are letting the researchers at the University Of Tennessee at Chattanooga and the Southeast Renal Research Institute see your PHI collected in this study. All Personal Information and Health Information will be kept confidential.

PATIENT CONSENT: I hereby freely and voluntarily consent to participate in the study described above. This consent is given based on the verbal and written information provided to me, and the understanding that I am medically and physically qualified to participate in this study. I am free to ask questions at any time. I understand I have the option to choose not to participate or to withdraw from the study at any time without incurring any penalty or loss of benefits otherwise available to me. I understand that by consenting to participate in this study, I am responsible for carrying out instructions and that I must relate to the study personnel any information that might be pertinent to the study, such as side effects of the treatment or procedure. I understand that any significant new findings or discoveries during the course of this research which may be related to my willingness to continue participation will be provided to me. I have read, or have had read to me, the above statements, and understand them as they apply to me. I further understand that I may revoke this consent at any time, except to the extent that action has already been taken in accord with this consent. I have been told that, if I have additional questions about the study, I may contact William Heaton (principal investigator for this study; hxw451@mocs.utc.edu) or I. N. Ozbek, Ph.D., Professor of Psychology at UTC; nicky-ozbek@utc.edu, either of whom can be contacted through the UTC Psychology office at 423-425-4262.

________________________________________
Printed name of participant

________________________________________
Signature of participant Date/Time

________________________________________
Research Assistant Obtaining Consent Date/Time
APPENDIX E

PERCEIVED STRESS SCALE (PSS)
INSTRUCTIONS

The questions in this scale ask you about your feelings and thoughts during THE LAST MONTH. In each case, you will be asked to indicate your response by placing an “X” over the circle representing HOW OFTEN you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don’t try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Fairly Often</th>
<th>Very Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In the last month, how often have you been upset because of something that happened unexpectedly?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2. In the last month, how often have you felt that you were unable to control the important things in your life?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3. In the last month, how often have you felt nervous and “stressed”?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4. In the last month, how often have you dealt successfully with day to day problems and annoyances?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6. In the last month, how often have you felt confident about your ability to handle your personal problems?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>7. In the last month, how often have you felt that things were going your way?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>8. In the last month, how often have you found that you could not cope with all the things that you had to do?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>9. In the last month, how often have you been able to control irritations in your life?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>10. In the last month, how often have you felt that you were on top of things?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>Almost Never</td>
<td>Sometimes</td>
<td>Fairly Often</td>
<td>Very Often</td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>--------------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>11. In the last month, how often have you been angered because of things that happened that were outside of your control?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>12. In the last month, how often have you found yourself thinking about things that you have to accomplish?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>13. In the last month, how often have you been able to control the way you spend your time?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>14. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
Appendix F

CENTER FOR EPIDEMIOLOGICAL STUDIES DEPRESSION SCALE REVISED (CESD-R)
## Center for Epidemiologic Studies Depression Scale – Revised (CESD-R)

Below is a list of the ways you might have felt or behaved. Please check the boxes to tell me how often you have felt this way in the past week or so.

<table>
<thead>
<tr>
<th></th>
<th>Not at all or Less than 1 day</th>
<th>1 - 2 days</th>
<th>3 - 4 days</th>
<th>5 - 7 days</th>
<th>Nearly every day for 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>My appetite was poor.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I could not shake off the blues.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I had trouble keeping my mind on what I was doing.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I felt depressed.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>My sleep was restless.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I felt sad.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I could not get going.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Nothing made me happy.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I felt like a bad person.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I lost interest in my usual activities.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I slept much more than usual.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I felt like I was moving too slowly.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I felt fidgety.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I wished I were dead.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I wanted to hurt myself.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I was tired all the time.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I did not like myself.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I lost a lot of weight without trying to.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I had a lot of trouble getting to sleep.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I could not focus on the important things.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Appendix G

PTSD CHECKLIST FOR CIVILIANS (PCL-C)
PTSD Checklist-Civilian Form (PCL-C)

Below is a list of problems and complaints that people sometimes have in response to stressful life experiences. Please read each one carefully, and then fill in the circle of the response to indicate how much you have been bothered by that problem **IN THE PAST MONTH**. Please fill in ONE option only for each question.

<table>
<thead>
<tr>
<th></th>
<th>Response</th>
<th>Not at all (1)</th>
<th>A little bit (2)</th>
<th>Moderately (3)</th>
<th>Quite a bit (4)</th>
<th>Extremely (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Repeated, disturbing memories, thoughts, or images of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Repeated, disturbing dreams of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Suddenly acting or feeling as if a stressful experience were happening again (as if you were reliving it)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Feeling very upset when something reminded you of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Having physical reactions (e.g., heart pounding, trouble breathing, or sweating) when something reminded you of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Avoid thinking about or talking about a stressful experience from the past or avoid having feelings related to it?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Avoid activities or situations because they remind you of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Trouble remembering important parts of a stressful experience from the past?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Loss of interest in things that you used to enjoy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Feeling distant or cut off from other people?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Feeling emotionally numb or being unable to have loving feelings for those close to you?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Feeling as if your future will somehow be cut short?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Trouble falling or staying asleep?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Feeling irritable or having angry outbursts?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Having difficulty concentrating?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Being “super alert” or watchful on guard?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Feeling jumpy or easily startled?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix H

HPLC INSTRUMENT SETTINGS
Injection Location: Als

Data File: C:\Chem32\DATA\20160523_cortisolsamples_WJH\071-0101.D
Acq. Method: 20160518_CORTISOLSAMPLES_WJH.M

The Acq. Method's Instrument Parameters for the Run were:

Grad. Pump

Flow: 1.000 mL/min
Low Pressure Limit: 0.00 bar
High Pressure Limit: 600.00 bar
Maximum Flow Gradient: 100.000 mL/min
Primary Channel: Automatic

Stroke
Automatic Stroke Calculation: Yes

Compress
Compressibility Mode: Compressibility Value Set
Compressibility: 8.3 x 10^-6/bar

Stop Time
Stop Time Mode: Time set
Stop Time: 16.00 min

Post Time
Post Time Mode: Time set
Post Time: 5.00 min

Solvent Composition
Channel Name 1 Used Percent %
A MeOH Yes 75.0
B 0.1% FA Water Yes 25.0

Timetable
Time A % B Flow ml/min Pressure bar
2.00 70.0 30.0 1.000 600.00
4.00 65.0 35.0 1.000 600.00
6.00 60.0 40.0 1.000 600.00
8.00 55.0 45.0 1.000 600.00
10.00 50.0 50.0 1.000 600.00

61
16.00  75.0  25.0  1.000  600.00

Instrument Curves
Store Pressure: Yes
Store Flow: Yes
Store Direction of Piston A: Yes
Store Solvent Ratio A: Yes
Store Solvent Ratio B: Yes

Sampler
Sampler [G4294B]

Stop Time
Stoptime Mode: As pump/No limit
Post Time
Posttime Mode: Time set
Posttime: 5.00 min
Auxiliary
Draw Speed: 200 µL/min
Eject Speed: 200 µL/min
Draw Position Offset: 0.0 mm
Injection
Injection Mode: Standard injection
Injection Volume: 20.00 µL
High throughput
Overlapped Injection
Enable Overlapped Injection: No
Timetable
VITA

William “Joe” Heaton was born in Hickory, NC, to Greg and Maggie Heaton. He is the first of three children, with two younger brothers. He attended elementary school in Richmond, KY, and completed middle and high school in Seymour, TN. After finishing his secondary education, he attended the University of Tennessee at Knoxville, where he earned a Bachelor’s degree in December 2010 in Biology with a concentration in Biochemistry and Cellular and Molecular Biology. Initially intending to pursue a career in medicine, his career plan shifted when he realized his interests aligned more with the mind than with the body. To refocus on a career as a mental health practitioner he enrolled in the Psychology Research Master’s program at the University of Tennessee at Chattanooga. It was here that he began to accrue research experience in psychology with the Olfaction Lab of Dr. Nicky Ozbek, which also exposed him to interdisciplinary work under the guidance of Dr. Manuel Santiago in the UTC chemistry department. He completed his Master of Science degree in Psychology in August 2016 and is working towards acceptance into a clinical psychology Ph.D. program.