

THE ROLE OF COLOR, CONGRUENCY, OBJECT SHAPE AND SIGNAL  
STRENGTH IN BIMODAL OLFACTORY AND VISUAL INTERACTIONS

A Dissertation

Presented to the Faculty of the Graduate School  
of Cornell University

In Partial Fulfillment of the Requirements for the Degree of  
Doctor of Philosophy

by

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May 2012

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# THE ROLE OF COLOR, CONGRUENCY, OBJECT SHAPE AND SIGNAL STRENGTH IN BIMODAL OLFACTORY AND VISUAL INTERACTIONS

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Cornell University 2012

Eating is a multisensory experience, input from sight, smell, sound, taste, and texture combine to create flavor perception. Evidence for crossmodal enhancement through olfactory and visual bimodal stimulation has been found in mammals and humans in the orbitofrontal cortex. However, the factors that result in a measurable bimodal enhancement in vision and olfaction have not been defined. A computerized methodology, involving visual stimulus presentation on a computer screen along with controlled odorant presentation through a specially designed puff-olfactometer, allowed for the psychophysical measurement of olfactory and visual crossmodal interactions.

There were two parts to this research. The first set of tests presented stimuli at calculated perithreshold levels and examined two different attention tasks. Visual stimuli were black and white outlines of fruit. One task evaluated the influence visual object shape on the detection of fruit odorants in an olfactory detection threshold, the other task evaluated whether an olfactory stimulus could alter detection of a visual object. Both attention conditions were tested using congruent and incongruent stimuli.

The second set of tests assessed if a color stimulus or the presentation

of a color along with a shape could alter the olfactory detection threshold. Results from the first set of tests suggest no crossmodal interaction when stimuli were presented at the perithreshold level, in either the olfactory attention task or the visual attention conditions when the stimuli were congruent or incongruent. The second set of tests demonstrated presentation of the congruent color at the level of recognition, well above the perithreshold region, strongly influenced the olfactory detection threshold. To a lesser extent object shape influenced the olfactory detection threshold.

The combined result from experiment 1 and experiment 2 reveals the ability of a recognizable visual stimulus to heighten olfactory detection performance. However, the overall impact of color in its ability to both increase olfactory detection performance and disrupt olfactory detection performance when presented in an incongruent condition demonstrates the ability of a visual signal to override olfactory processing mechanisms.

## BIOGRAPHICAL SKETCH

Anne J. Kurtz grew up in New York City and graduated from The Dalton School in 2002. From 2002 to 2006 she attended Hamilton College located in Clinton New York where she majored in chemistry and minored in psychology with a focus on cognitive psychology and neuroscience. During her undergraduate summers she conducted independent research in the Food Science Department at Cornell University under the guidance of Dr. Harry T. Lawless, where she learned about the importance of sensory evaluation methods. At Hamilton College she discovered a passion in rowing, where she joined the Hamilton College Varsity Women's Crew team her freshmen year and served as captain her senior year. She also learned how to scull, a hobby that she pursued through graduate school.

Upon graduation from Hamilton College, she enrolled in the Master's of Science program in Food Science at Cornell University where she worked under the guidance of Dr. Terry E. Acree from 2006 to 2008. For her Master's research, she focused on devising methods to study the perception of olfactory mixtures. In 2008 she became the head teaching assistant for FS430, Understanding Wine and Beer, winning the Outstanding Teaching Assistant Award for her hard work and dedication. Upon completing her Master's she worked from 2008-2009 at Unilever in Consumer Scientific Insights group studying Sensory, Perception, and Behavior as their Fragrance

Intern. While at Unilever she won the Values in Actions and Merits Award, and discovered her interest in crossmodal interactions shaping the direction of her dissertation research. In 2009 she returned to Cornell University to pursue her Ph.D. under the direction of Dr. Terry E. Acree in the Department of Food Science. She chose to study interactions between olfaction and vision in order to better understand how consumers interact with products.

## ACKNOWLEDGMENTS

First and foremost this research would not have been possible without the dedication of my loyal panelists. Furthermore the dedication and help of Dr. Terry Acree and Edward Lavin, contributed greatly to the success of these studies. None of this would have been possible without the support of my major advisor. Dr. Terry Acree's steadfast support of pursuing new and novel ways of studying olfaction enabled me to be a better researcher and thinker. The time I spent working in his lab, has been both extremely challenging but has forced me to grow into a more inquisitive scientist.

I would like to thank my minor committee members Dr. Harry Lawless and Dr. Brian Wansink, for their insightful advice and encouragement. They both heightened the quality and objectives of this research. A big thank you to John Barnard, who assisted in the statistical analysis of this research as well as many other projects I undertook. His advice has always been extremely clear and helpful.

The people I have met while at Cornell were a truly amazing group of supportive friends. I feel incredibly lucky to have been able to interact and become close with people from such unique backgrounds.

Lastly I would like to acknowledge the encouragement of my parents, their belief in my abilities and potential has been integral to my success.

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## LIST OF ABBREVIATIONS

- ACIII — adenylyl cyclase III
- AFC — alternative forced choice
- ATP — adenosine triphosphate
- B — banana
- BENZ — benzaldehyde
- c — congruent
- cAMP — cyclic AMP (adenosine monophosphate)
- cBOF — congruent banana olfactory task
- cBVF — congruent banana visual task
- Ch — cherry
- cChOF — congruent cherry olfactory task
- cChVF — congruent cherry visual task
- CNG — cyclic nucleotide gated channel
- G<sub>olf</sub> — G-protein olfactory
- GKA — G protein-coupled receptor kinase
- GPCR — G-protein coupled receptors
- GRK — G protein-coupled receptor kinase
- i — incongruent
- IA — iso-amyl acetate
- iBOF — incongruent banana olfactory task
- iBVF — incongruent banana visual task
- iChOF — incongruent cherry olfactory task
- iChVF — incongruent cherry visual task
- ISI — interstimulus interval

IT — inferotemporal  
LGN — lateral geniculate nucleus  
M-Cells — magnocellular ganglion cells  
MS — masking stimulus  
OB — olfactory bulb  
OF — olfactory focus task  
OFC — orbital frontal cortex  
OR — olfactory receptor  
OSN — olfactory sensory neuron  
P-Cells — parvocellular ganglion cells  
PEG — polyethylene glycol  
PKA — phosphorylated kinase A  
SE — standard error  
TS — test stimulus  
TM — transmembrane  
VF — visual focus task

# CHAPTER 1

## INTRODUCTION

The olfactory and visual systems are separate; however, information from each of system contributes to the perceptual flavor experience. The color and shape of a fruit enable the individual to assess what it should taste like, even without eating it. Once the food is consumed, all of the sensory systems engage, taste, smell, touch, and the somatosensory systems contribute to that overall perception. However, how these sensory inputs combine to create the multimodal synthetic experience, and how expectations influence the overall perception is not well understood.

Perceptions result from the seamless integration of input from multiple sensory systems. The anatomical structures critical to the flow of sensory information have been studied extensively; however, the pathways in which the information is combined are not understood. Both biological cellular and neuro-imaging studies suggest higher-order sensory processing occurs in the orbitofrontal cortex (Abdi 2002, Calvert 2001, Gottfried 2010, Kadohisa, Wilson 2006, Plailly et al. 2008, Wolfe 2001). Imaging studies have enabled researchers to localize regions in the orbitofrontal cortex believed to integrate signals (Calvert 2001, Fulbright et al. 1998, Gottfried, Winston and Dolan 2006, Rolls, Baylis 1994, Small, Prescott 2005, Thesen et al. 2004, Zelano et al. 2005, Verhagen, Engelen 2006). However, there are still many unanswered

questions addressing both the processing and implications of multimodal sensory integration.

In 1935 a simple test developed by, John Stroop, revealed the complexity of sensory integration by asking subjects to name the ink color of typed words; however, the words were names of colors (Stroop 1992, MacLeod 1991). The differing ink-color delayed the reaction time of the participant during the naming task, creating a disruption to the otherwise seamless process of naming. In 1976, Harry McGurk and John MacDonald discovered the McGurk effect, demonstrating the ability of a visual representation to alter an auditory sensory input (McGurk and MacDonald 1976). Researchers are still examining the mechanisms underlying these two classic examples of multisensory integration.

Sensory integration is part of the everyday food experience. Two studies in Garber et al (2001) illustrate real-world examples of subjects who were served food where the colors had been . Subjects in both accounts report off-odors and off-flavors and feelings of nausea (Garber et al. 2001). These examples allude to the ability of visual information to inform flavor perceptions (the integration of taste and smell). The multisensory integrative process occurs whether or not the individual is conscious of it. The visual experience is as much part of the eating as smell or taste; however, the ability of visual information to influence flavor perception is not understood. Undoubtedly experience influences expectations, and forms our understanding of

congruency effects, meaning we associate the color red with cherry, strawberry, and raspberry. For a comprehensive examination of how appearance influences our expectations please see Hutchings (2003) and Spence et al (2010). The extent to which olfactory information can be altered by visual information has yet to be determined. There are countless approaches to examining the impact of visual information on another sense. It is possible to examine luminance, contrast, patterns, curvature, timing of the presentation, color, motion and many other types of visual changes. When examining olfaction it is possible to test liking, preference, mixtures, intensity, timing, attention, again the different types of odor evaluations are endless.

This dissertation investigates signal enhancement resulting from crossmodal olfactory and visual input by using psychophysical detection threshold methods. The research explores the factors that need to be present in order to result in a crossmodal enhancement and by using highly controlled forms of stimulus delivery. The interactions of both vision on olfaction and olfaction on vision are studied, as well as the roles of object shape, object color, and object intensity.

## **1.1 The Underestimated Power of Crossmodal Interactions**

### *1.1.2 The Indirect Impact of Odor Cues on Overall Perception*

This section serves as a brief overview of the influence of olfactory and visual information in the context of consumer research. The following studies outline

how olfactory cues lead to measurable change in the perceived quality of a product. A review by Bone et al (1992), outlines several examples of how olfactory is capable of altering consumer perception of products (Bone and Jantrania 1992). Laird (1932) conducted one of the earliest studies demonstrating the power of an olfactory cue on consumer behavior.

Housewives were asked to evaluate the quality of four identical pairs of silk hosiery; three of the four pairs of hosiery were scented. A pair of unscented hosiery served as the control. Housewives were not informed of the scented hosiery; however, based on the results, the scent strongly influenced the perception of quality. Housewives rated the hosiery scented with narcissus as having the highest quality, even alluding to differences in texture . The housewives were unaware the perceived differences in quality they were linked with the different scents of the hosiery, not differences in silk quality.

A similar study conducted in 1967 by Cox, reported orange-scented hosiery sold better than unscented hosiery, demonstrating the strong link between olfaction and quality. Similarly, Miller et al (1991) reported scenting a room, resulted in higher liking ratings of Nike shoes when compared to the an environment with ambient air.

Churchill et al (2009) evaluated changes in consumer response due to the addition of fragrance to a fixed shampoo base. The subjects were asked to evaluate several textural aspects of their hair. Panelists shampooed with both the scented and unscented products. Results revealed that panelists

associated certain fragrances with positive textural attributes, such as silky, soft, smooth, and conditioned. While other fragrances imparted negative textural attributes such as sticky, tacky, slimy, brittle, and tangled. All fragrances in the study were considered to be pleasant, the authors state the differences in textural perception were not due to the unpleasant nature of the stimuli, but the fragrance characteristics were quite varied. Churchill et al (2009) suggest liking of a fragrance is likely important in influencing the consumer perception of texture.

The studies described above allude to the ability of an odor to influence the consumer's hedonic preference of a product. Authors have examined the role of pleasantness and unpleasantness of odorants in their ability to influence a product perception. Bone and Ellen (1999) review scent's influence on consumer behavior. They suggest the traditional view of scent altering sales of items has not been properly tested. The traditional tenets used to explain the role of olfaction in product purchasing suggest: 'approach avoidance', 'mood shifts', and 'cognitive elaboration'. Bone and Ellen (1999) propose these three tenets are not supported by the current studies that have been conducted, they challenge whether an olfactory cue is capable of altering a mood state thus changing a consumer behavior or whether it is possible for odor to change a consumer's approach and avoidance behaviors. The authors imply congruency may influence a consumer's interaction with a product, but prior experience most likely has the greatest impact on consumer judgment.

### *1.1.3 The Indirect Impact of Visual Cues on Overall Perception*

Visual cues can also influence the consumer experience. In 1993 Pepsi Cola launched Clear Pepsi, traditionally, a brown product, with the goal of gaining two percent of the cola market (approximately one billion in sales) within the first year; however, Pepsi only sold a reported \$335 million. Triplett (1994) and Garber et al (2008) suggest the failure of Clear Pepsi is due to the underestimated importance of color to a product. Without the brown color cue consumers did not automatically associate the product with cola flavor. Tennet (1993) elaborates that the trend in the 90s toward clear products may have only worked for a select few products, such as the malt beverage Zima, due to its novelty (Triplett 1994). The consumer has no prior expectations when presented with a Zima, the consumer did not already have an idea of a pre-existing flavor concept.

Garber et al (2001) suggests different approaches to successfully integrating color into new products. First, creating novelty in both the product and color thus make incongruence intentional, whether the consumer understands the intention, thus the marketer controls flavor perception, for example Gatorade's Blue Raspberry beverage. Another example of this is Gatorade's line of Frost beverages, where neither the colors nor the flavor names 'Glacier Freeze' are understood to have a preconceived flavor. In another example of understood incongruity, Heinz introduced a line of green ketchup, playing off of its traditionally red ketchup in order to attract a different

portion of the market (Garber, Hyatt and Starr 2001). Another strategy is to dissociate the product packaging from the color of the product, in this particular case the product is not visible inside of the packaging (thus the package is opaque), therefore the consumer can make a flavor judgment based on the packaging information rather than the color of the beverage.

Research has also shown the power of labeling to inform the overall judgment and expectations of a product. Studies have shown how a product labeled in two different ways, one implying an upscale-product while another suggesting a budget-conscious product will create different flavor experiences for the consumer (Shankar et al 2009, Yeomans et al. 2008, Wansink et al 2005, Herz and von Clef 2001, Wansink et al. 2000, Cardello et al. 1985). The visual appearance of a food is the first attribute the consumer can use to assess a product and will set up certain expectations for flavor all based on models from prior experience.

Several scientific studies have tried to examine the role of color in creating our perceptions of a product. These studies will be discussed in greater depth in the section on crossmodal interactions. The examples described thus far are scenarios in which there is a direct connection between the sensory attributes of a product and its influence on consumer perception. These studies illustrate how information from vision and olfaction can impart greater meaning on a product. This dissertation critically examines how the combined signals from olfaction and vision are able to convey a stronger

signal. The following sections will describe the physiology of both olfaction and vision, and then the scientific studies that have been conducted in the areas of both olfaction and vision.

## **1.2 Olfaction**

An odorant can enter through the nose (orthonasally) or the mouth (retronasally). The odorant binds to odorant receptors (ORs) located on the olfactory epithelium (OE). An odorant is defined as a small volatile organic compound, whose molecular weight is  $\sim 300$  daltons or less (Touhara and Vosshall 2009). Odorants are most commonly found in plants, insects, animals, and microbes. Most odorants are organic; however, there are known inorganic odorants such as  $\text{SO}_2$ , which smells of rotten eggs. The olfactory epithelium is located in the upper nasal cavity and measures  $\sim 1 \text{ cm}^2$ - $5 \text{ cm}^2$  (Morrison and Costanzo 1990). A commonly cited statistic suggests humans are capable of distinguishing 10,000 odors; however, there is no evidence for this commonly cited statistic (Touhara and Vosshall 2009). No one has catalogued the precise number of known odorants and no one has tested the number of odorants a human is capable of perceiving. Thus, the exact number of odorants an individual is capable of perceiving is still unknown. There is another form of odorant, the pheromone; these will not be discussed in this review. For information regarding the differences and similarities between the detection of pheromones and odorants please read: Touhara and Vosshall

(2009). A pheromone can be broadly defined as a substance used for intra-species communication, known to elicit both changes related to behavior and the endocrine system.

Stereo-specificity is the most widely accepted theory of olfaction, proposed by Amoore in 1963 (Amoore 1963). This theory suggests odor perception occurs through the binding of an odorant to a specific olfactory receptor. Odorants varying in different chemical structure can only bind to specific olfactory receptors. Strong support for this theory can be found in the discovery of the G-protein coupled receptors (GPCRs), the family of seven-transmembrane proteins, found to encode receptor proteins for odorants in rats (Buck and Axel 1991). G-proteins are also part of visual perception. Rhodopsin, also a GPCR, is critical to signal transduction in vision. The family of GPCRs identified in olfaction, is believed to be the largest subfamily of GPCRs and likely the largest single family of genes within the mammalian genome. Currently, it is not possible to predict which odorants will bind to which specific ORs. Furthermore it is not possible to predict odorant quality from odorant structure. Although the stereo-specific model of odorant binding is the most widely accepted explanation for how we detect an odorant, there are still several other theories: absorption, puncturing, radiational, and vibrational (Keller and Vosshall 2004, Burr 2002).

### *1.2.1 Olfactory Receptors*

In 1991, Axel and Buck (1991) identified a large gene family encoding olfactory receptors in rats, these were GPCRs. ORs are classified as GPCRs, due to their structural similarities to other GPCRs. In addition, OR activation occurs through coupling to heterotrimeric proteins. It is estimated there are ~800-1500 genes members in mammals, in contrast to fish who only have ~100 gene members (Mombaerts 2004, Niimura and Nei 2005). In homonides up to 50% of the genes are pseudogenes, in primates it is estimated between 25-50% of the genes are pseudogenes, and in whales and dolphins 70-80% of the olfactory genes are pseudogenes. Since audition is the primary form of sensory transmission in whales and dolphins it is speculated the more pseudogenes a species has the less dominant the sense (Touhara 2008). The difference in the number of genes found in mammals versus fish supports the theory of gene expansion as animals shifted from living in a primarily aqueous environment to a terrestrial environment. Each olfactory sensory neuron (OSN) represents a single OR member of the gene family.

### *1.2.2 Odorant Binding*

Olfactory transduction occurs when an odorant binds to an OR, converting the odorant into a neural (electrical signal). GPCRs are defined by their seven hydrophobic transmembrane domains, odorants bind to the hydrophobic binding pocket formed by transmembranes (TM), TM3, TM5, and TM6 (Katada et al. 2005). Figure 1 represents the structure and topology of the olfactory

receptor protein. The red dots represent variable amino acids in the OR family, while the blue dots represent the conserved amino acids in the OR family. The extracellular loops are characterized by conserved cysteines and there is a conserved glycosylation site in the N-terminal region (Katada et al 2004). Katada et al (2005), identified Ser113 located on TM3 as a critical for ligand hydrogen bonding to the receptor. They also identified Phe252 located in TM6 as crucial to leading to a conformational change within the OR, switching the OR from an inactive to an active state, and leading to the signal cascade. When these residues have been altered through single amino acid replacement studies, odorant binding does not occur. It is believed the large number of polymorphisms noted in humans is related to individual differences in olfactory perception.

### *1.2.3 Signal Transduction*

The signal cascade begins when the receptor activates a G-protein ( $G_{\text{Olf}}$ ), leading to the activation of adenylyl cyclase III (ACIII). For a visual representation of the G-protein cascade please see Figure 2. Signaling the conversion of intracellular adenosine triphosphate (ATP) into cyclic AMP (cAMP). The elevated levels of cAMP activate the opening of the cyclic nucleotide gated channel (CNG), releasing an influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ions, depolarizing the inside of the cell, thus altering the membrane potential. This action potential is propagated along the axon of the OSN, and into the

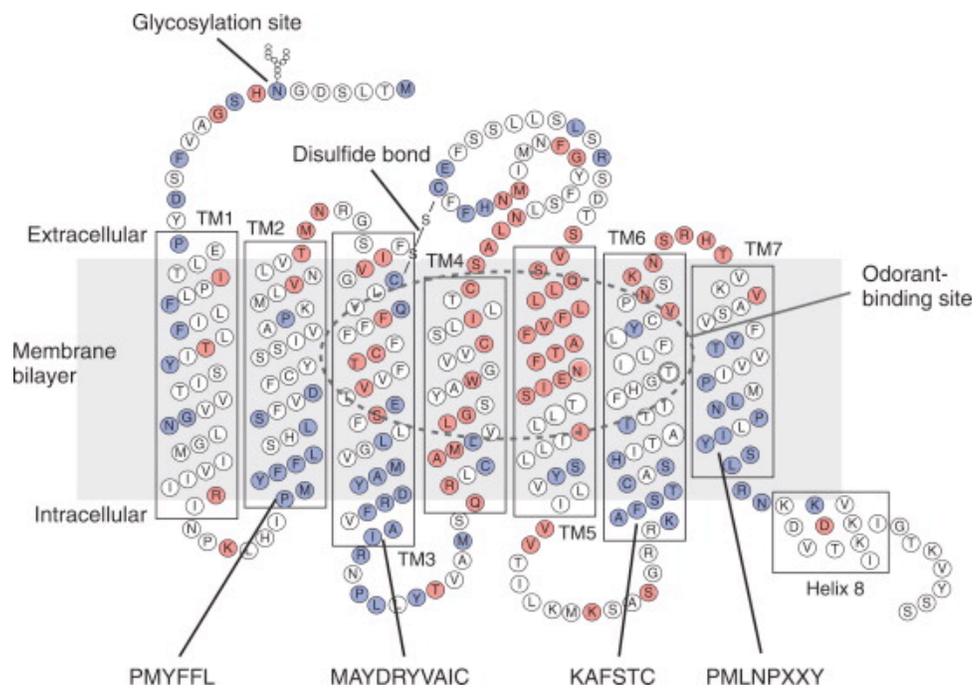


Figure 1: Olfactory Receptor Protein. This is a graphical representation of the 7TM OR protein. The conserved amino acids are represented in red. The variable amino acids are represented in blue. Reprinted by permission of Elsevier Inc. (Touhara 2008).

olfactory bulb (OB). The signal is amplified through the  $\text{Ca}^{2+}$  ions entering the CNG pathway; these ions activate a separate ion channel permeable to  $\text{Cl}^-$  ions. OSNs maintain a high negative potential, there is an efflux of  $\text{Cl}^-$  ions when the CNG pathway is activated (Firestein 2001). The net positive charge on the membrane results in a further depolarization, thus enhancing the signal response. Olfactory receptors became unresponsive in studies of gene knockout mice lacking  $G_{\text{olf}}$ , ACIII, or CNG (Belluscio et al. 1998, Brunet et al. 1996, Wong et al. 2000). The receptor depolarizes and returns to steady state through the binding of phosphorylated kinase A (PKA) and G protein-coupled receptor kinase (GRK). The elevated levels of  $\text{Ca}^{2+}$  from the opening of the CNG channel also mediate the negative feedback loop, which ensures the OR, returns to its steady state. For a more detailed explanation of the polarization and subsequent depolarization of the OR please see Touhara et al (2005), in addition this review suggests two other theories of odorant signal transmission.

The axons of OSNs are sent to the OB. Each OSN relays signals to specific glomeruli within the OB. A glomerulus is composed of a bundle of neurophil. Mombaerts (1999) identified that OSNs expressing a specific receptor type, converge onto specific glomeruli within the OB, regardless of their location within the epithelium. Glomeruli receive input from OSNs, there is cross-talk between glomeruli due to periglomerular cells, the signals from the glomeruli are transmitted down the lateral olfactory tract through mitral and

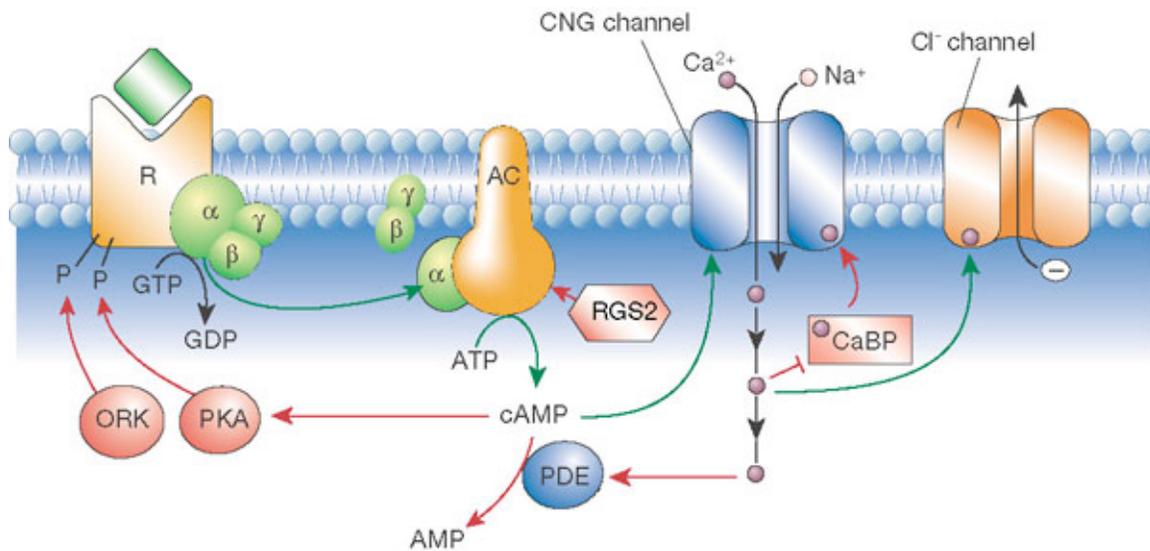
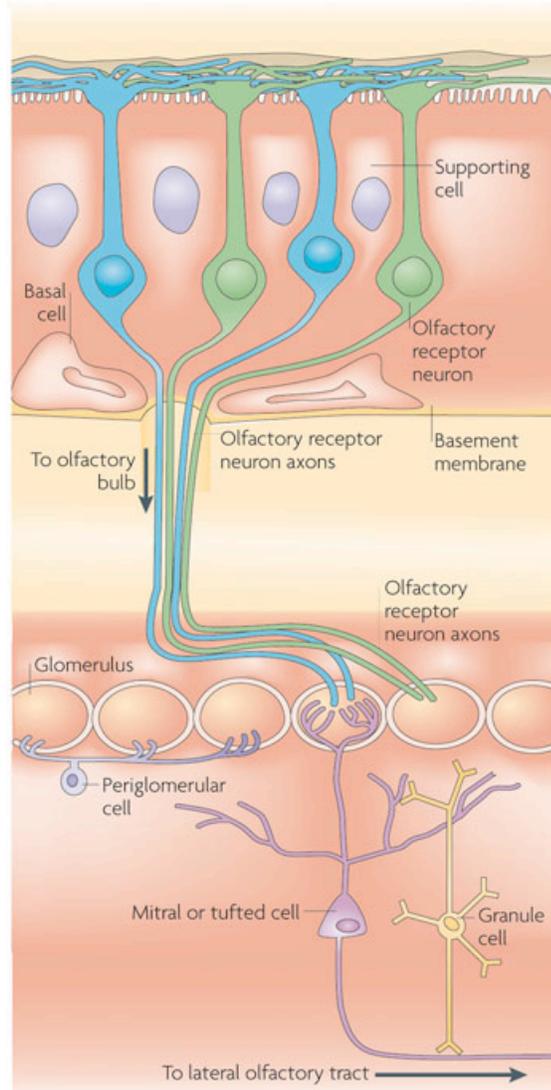


Figure 2. Olfactory Sensory Transduction. This is the sensory transduction cascade that occurs when an odorant binds to an OR. An odorant binds to R (receptor), G<sub>olf</sub>, which activates ACIII. The cyclase converts ATP to cAMP. cAMP then binds to the inner walls of the ion channel (CNG channel), allowing for the influx of Na<sup>+</sup> ions and Ca<sup>2+</sup> ions. The Ca<sup>2+</sup> ions are capable of activating the Cl<sup>-</sup> channel. Leading to an efflux of Cl<sup>-</sup> ions. The entering Ca<sup>2+</sup> ions are also involved in regulating the adaptation through the negative feedback pathway. Reprinted by permission of Nature Publishing Group (Firestein 2001).

tufted cells where the information is then projected into the olfactory cortex and higher brain regions Figure 3, illustrates Thus different odorants stimulate different glomerular activity patterns, it has even been found that glomerular activity patterns can be altered by the same odorant in different concentrations (Xu et al 2000, Leon and Johnson 2003, Mori et al. 1999, Oka et al. 2006). Each odor creates a unique odor image (Gottfried 2010). Imaging as well as classical neuroanatomy studies have identified the anterior olfactory nucleus, taenia teacta, olfactory tubercle, piriform cortex, amygdala, entorhinal cortex, and areas of the orbitofrontal cortex as regions of higher order cortical olfactory processing (Gottfried 2010, Zelano et al. 2005, Price 2008, Meredith and Stein 1986, de Araujo et al. 2005, Anderson et al. 2003).

#### *1.2.4 Receptor Tuning*

Beyond the level of the olfactory bulb there is little understanding of the specific of processing. Neuro-imaging studies have elucidated the areas in which higher order processing may occur, but little is understood regarding whether olfactory receptors are finely tuned or broadly tuned, meaning whether receptors are capable of binding several different types of odorant classes or are more specific to only certain odorant structures. (For two basic reviews discussing neuro-imaging and olfactory processing please see: Gottfried 2010, Lundström et al. 2011). A recent paper



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Figure 3 Wiring of the Early Olfactory System. The olfactory neuroepithelium is composed of OSNs, sustentacular cells (supporting cells), and stem cells (basal cells). Each OSN expresses only one of the ~1000 genes, and the axons from these cells converge on specific glomeruli located in the olfactory bulb. The mitral axons send signals from each glomeruli to areas of higher cortical processing. Periglomerular cells and granular cells provide for cross-talk. Reprinted by permission of Nature Publishing Group (Gottfried 2010)

revealed the variation in receptor tuning, most were finely tuned, while some were more broadly tuned (Nara et al. 2011). This explanation supports both the combinatorial and elemental theories of olfaction, suggesting that olfactory receptors more often bind specific types of odorants but other receptors are capable of binding a broader set.

#### *1.2.5 Theories of Olfactory Perception*

There are two theories of odorant perception: elemental and combinatorial processing. The elemental theory of processing suggests an individual is capable of identifying parts of a complex odor mixture (Nara et al. 2011, Laing 1994, Laing and Glemarec 1992, Laing and Willcox 1983). The combinatorial theory of olfaction proposes an odor mixture is perceived as a novel sum of its parts, representing no single aspect of the components of which it is composed (Le Berre et al. 2008). Likely our perceptions result from the way we attend to a stimulus. Human studies on olfaction suggest individuals are capable of detecting single parts of a mixture as well as experience blending qualities (Le Berre et al. 2008, Chiralertpong et al. 2008). The underlying arguments for both of these theories can be seen at the level of the olfactory bulb (Xu et al. 2000, Leon and Johnson 2003, Mori et al. 1999, Shepherd 2006, Cleland et al. 2002).

#### *1.2.6 Psychophysical Odor Evaluation Methods*

One of the most common methods for determination of the psychophysical detection threshold of an odorant is through n-alternative forced choice (AFC) testing. Pair-wise tests refer to when two samples are tested side-by-side. In this test the experimenter presents two samples simultaneously to the subject, one sample contains an odorant and the other does not. The subject is asked to indicate which sample contains the odorant (For a detailed explanation of pair-wise comparisons and methods for olfactory threshold evaluation please see: Lawless and Heymann 2010 and Gescheider 1997). In order to measure an olfactory threshold several factors must be controlled to ensure the experimenter is measuring the subject's sensitivity to the stimulus and not indirectly measuring something else. First to evaluate an olfactory threshold, the stimuli should be presented in ascending order of concentration, to control for adaptation. Several concentrations should be presented to the subject to collect a broad range evaluation points. Other factors that must be controlled when assessing an olfactory threshold are the method of odor delivery to control for uniformity across each sample tested, sniff size, sample purity, and temperature of the samples (Stevens et al. 1988). The overall goal of the researcher is to minimize testing variability, due to the wide range individual variability.

In a pair-wise test the statistical chance of choosing the correct sample (the odorant containing sample) is 0.5. When collecting an olfactory detection threshold it is advised to correct for the positive skew often created by the

panelist, this is done by calculating the threshold with a geometric mean. Furthermore, using Abbot's correction formula is also advised. Thus if the researcher wanted to calculate the 0.5 detection threshold for a panelist using a pair-wise comparison the panelist would have to get 0.75 correct (Lawless and Heymann 2010). Pair-wise comparisons may be one of the most common forms of collecting an olfactory detection threshold; however, a 3-AFC is a more sensitive test and therefore is preferred. In this test, two blanks are presented along with a sample containing an odorant. The subject must choose the sample containing the odorant.

Once all concentration levels have been evaluated, the researcher can create a psychophysical curve. The psychophysical curve is a plot of the log concentration vs. percent correct response. This will visually represent the response characteristics to a specific odorant. From a psychometric function it is possible to interpolate the point at which the odorant reaches the level of detection.

The following section describes four different threshold types, in which the same method of data collection can be used to identify the four different thresholds measurements. It is necessary to vary the question asked of the subject according to the type of threshold collected. The four different thresholds are: detection threshold, recognition threshold, difference threshold, and terminal threshold. This research is primarily concerned with detection thresholds and sometimes recognition thresholds. The level of detection is

defined as the minimum point at which point a subject is capable of detecting a sensation from a stimulus. In this example, a subject would choose the sample containing benzaldehyde odor 50% of the time, but would not be able to recognize the odor as benzaldehyde, the individual would be able to notice a difference between that sample and the other constant odorant. The difference between a detection threshold and a recognition threshold, is that at the level of a detection, the subject is often unable to identify i.e. name the odorant. At the point of recognition the individual is capable of naming the stimulus. Naming requires a higher level of cognition and accordingly a higher concentration of the stimulus. The subject must be able to both distinguish the stimulus from the blank, and name the nature of the stimulus (e.g. an individual can recognize and identify benzaldehyde as cherry odor in a solution). The difference threshold measures the minimum amount of concentration change necessary for a subject to identify a stimulus 50% of the time, when compared to a constant reference. An example of a difference threshold is being able to correctly identify the different concentration of benzaldehyde when presented with two solutions of benzaldehyde 50% of the time. A fourth type of threshold measurement is the terminal threshold, the point at which an increase in concentration yields no changes in response. There is a point, at which the maximum concentration for benzaldehyde has been reached, and the subject can no longer distinguish between samples,

there is no perceivable increase in stimulation with an increase in concentration.

### *1.2.7 Challenges in Measuring Olfaction:*

There are many challenges a researcher encounters when measuring olfactory perceptions. Individuals breathe at different rates, have different genes, as well as vary widely in sensitivity. Measuring the psychophysical perception of an odorant can be quite precarious if the researcher does not devise and follow a method that eliminates outside distractions, ensuring confident the subject is evaluating the odorant and not an unrelated cue. Training the panelist for a specific method can eliminate some variation. However, the experimenter must control for purity of sample, off-odors in the diluent, reliable blanks, a standardized method of measurement such as a forced choice method, understand the nature of the odorant he/she is using, and use instructions indicating when a subject should inhale and exhale (Stevens et al. 1988, Schmidt and Cain 2010).

Both Stevens et al (1988) and Schmidt et al (2010) stress the importance sample purity. Variability in threshold measurement can be the result of impurities within a sample. A lower than normal detection threshold can be an indicator of sample contamination (the contaminant is the odor the individual detects not the test odorant). Furthermore, if the blank or diluent is not close to odorless, the subject may evaluate the presence of a blank rather

than the presence of an odorant. It is important for the researcher to pre-screen all subjects to determine whether the subject is capable of detecting the odorant, a subject could exhibit a specific anosmia to an odorant.

The researcher should be aware of the timing of odorants in order to control for adaptation. Stevens et al (1988) suggest that much of the reported variability amongst individuals is related to experimental differences. In fact Schmidt et al (2010) further suggests the variability observed is usually a function of variation within the panelist testing from session-to-session. Therefore it is paramount the researcher be aware of changes in subject variation and reliability. Schmidt also stresses the importance of understanding the amount of odorant delivered during each evaluation and consistency. Overall, measuring thresholds requires a large amount of experimental control, the experimenter is capable of controlling stimulus purity, delivery, and measurement; however, large variability is often due to subject differences.

## **1.3 Vision**

### *1.3.1 Basic Structures in Phototransduction*

Most visual research has been conducted in primates and other mammals (cats, rabbits), with the advent of imaging techniques many of the prior findings in primates have been corroborated. Light first enters the eye and must pass through ganglion cells as well as horizontal, bipolar, and amacrine cells before reaching the photoreceptors. Phototransduction is the conversion

of wavelengths of light into electrical signals that result in visual perception. The process of phototransduction begins when a photon binds to a photoreceptor located on the retina. Light is defined by wavelength; humans are capable of detecting wavelengths in the range of 390-750 nm. The retina is capable of detecting a single photon of light, the smallest form of energy (Hecht et al. 1942).

The retina contains two basic photoreceptors: rods and cones. A third type of photoreceptor has been identified in mammals as responsible for regulating circadian rhythms (Berson et al. 2002). Rods regulate processing of achromatic vision, while cones process color vision. Rods are most sensitive to wavelengths of 510 nm and cones are most sensitive to wavelengths of 555, the difference known as Purkinje Shift, explains why night vision is undisturbed by the presence of red light. Rods are one of the best understood G protein mediated signaling processes (for full reviews please see (Luo et al. 2008, Palczewski and Saari 1997, Baylor 1996). Like in olfaction, visual transduction is also mediated by GPCRs 7-TM proteins, in vision they are referred to as opsins. Rhodopsin, found in rods absorbs primarily green-blue light. Light activates rhodopsin, converting it to the active form. This signals a G-protein cascade closing the CNG channel open during darkness where the signal is then propagated along the optic nerve. The cones are also mediated by opsin protein. There are three specific types of cones, enabling humans to see a full spectrum of colors (Krauskopf and Gegenfurtner 1992). Each cone-

type absorbs lights within a specific range: s-cones (~430 nm), m-cones (~530 nm), and l-cones (~560 nm). Human vision is trichromatic due to the presence of these three cones; however, some humans lack the s-cone thus resulting in dichromacy. For a review of color vision transduction please see: (Solomon and Lennie 2007).

Unlike in olfaction, vision is topographically mapped. Meaning, neighboring groups of cells in the retina relay neural information to neighboring groups of cells in the lateral geniculate nucleus (LGN), which send their information of to neighboring sets of cells in the visual cortex. Essentially, spatial arrangements of forms and objects are maintained through the neural encoding process, referred to as a retinotopic map (Kandel et al. 2000, Wolfe et al. 2006).

### *1.3.2 Visual Processing: Stream Specialization*

Visual information is processed in a hierarchical manner. This means the beginning stages of visual processing extract the localized and basic forms of vision, as the information reaches higher levels of processing the information is transformed into and abstract, holistic, and likely multimodal scene. At the neuronal level, as the flow of visual information progresses from the retina to the cortex the receptive fields of the neurons increases, thus suggesting more complex processes. In early vision processing, ganglion cells process the basic features of an object. From the earliest stages of visual processing in the

retina there are two types of retinal ganglion cells. Magnocellular ganglion cells (M-cells) and parvocellular ganglion cells (P-cells). Table 1 outlines the basic differences between these two cell-types. This is where object recognition begins. The subdivision of information that occurs due to the specialization of these cells is maintained up until the visual information reaches the visual cortex for higher processing, where it is believed to combine to form an overall gestalt. As visual information reaches higher levels of processing more complex cells have been identified such as facial recognition cells.

P-cells are slow, process information about color, have low luminance contrast sensitivity, high spatial frequency and low temporal frequency. P-cells are responsible for information about the shape of an object. In contrast, M-cells quickly process information, have high luminance contrast sensitivity, are color blind, have low spatial frequency, and high temporal frequency. For more information about specialization please see Livingstone and Hubel (1988). The divisions in specialization are maintained through the optic nerve, information from P-Cells and M-Cells go to specific parts of the LGN located in the thalamus. Figure 4 provides a map of the two pathways. Different layers within the LGN receive specific information from these cells. The specific layers then transfer this information onto specific areas for higher order processing. Information processed by P-cells is sent to the inferior occipitotemporal cortex along what is referred to as the 'what pathway' (ventral cortical pathway).

Table 1: Basic differences between the two visual processing streams

	Color Selectivity	Contrast Sensitivity	Temporal Resolution	Spatial Resolution
'Where Stream' Dorsal Pathway (Magno System)	No	High	Fast	Low
'What Stream' Ventral Pathway (Parvo System)	Yes	Low	Slow	High

Information from M-cells is sent to the dorsolateral parietotemporal cortex along the 'where pathway' (dorsal cortical pathway) (Milner and Goodale 2008, Goodale and Milner 2009).

The dorsal pathway processes information related to form and motion. Most motion processing occurs in the medial temporal area. The ventral pathway processes information about depth as well as color and form. These processes occur in area V4. The ventral pathway is primarily responsible for object recognition. The inferotemporal (IT) cortex is where facial recognition, cells have been found. Cells responsible for pattern recognition have also been found in the IT (Kandel et al. 2000, Milner and Goodale 2008, Mishkin et al. 1983).

### *1.3.3 The Binding Problem: Synthesis of Visual Information*

Although there are two distinct pathways involved in processing specific parts of visual information, this information is eventually combined to yield a perceptual experience. The two visual information streams have been well researched, but the underlying issue of how the information is bound together is still a mystery. How the information is combined to yield the experience of observing a basketball being shot into a hoop is not yet understood. The difficulty of understanding of how simple features are combined to create perceptions is known as the binding problem.

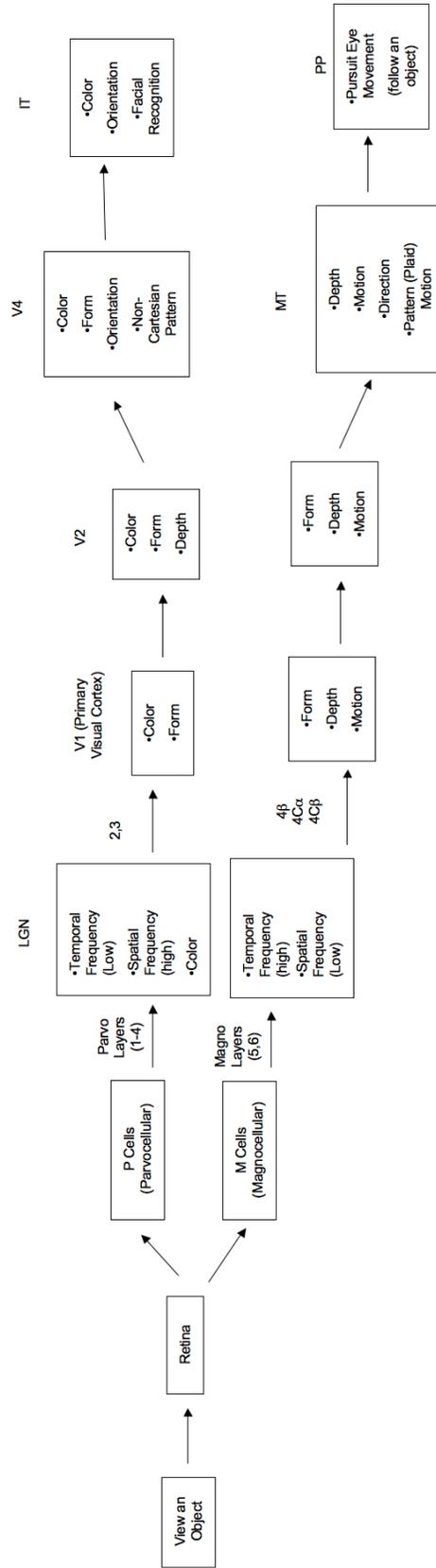


Figure 4 Flow Diagram of Ventral and Dorsal Processing Pathways.

Illusory conjunctions are strong evidence for the existence of the binding problem. An illusory conjunction occurs when visual information is improperly combined to create an image. For example, if there are two objects, one is round and red and the other square and green (Treisman and Schmidt 1982). If the subject is asked to recall the two objects, it is possible the subject might confuse the colors and shapes of the objects. The misattribution of object information is referred to as an illusory conjunction and presented as evidence for the binding problem. However, researchers argue whether illusory conjunctions are a function of memory rather than visual processing (Wolfe and Cave 1999).

Research has shown visual information may be bound together even earlier than the area V1 in the visual cortex (David 2009). Regardless of the exact location of binding, visual information is processed in separate areas and is most likely processed in a hierarchical manner, most importantly attention is essential to creating a perception. Object recognition utilizes both top-down and bottom-up processing. Bottom up processing is stimulus driven, and the subject is searching for differences in preattentive features. Top down processing requires a subject to search for an object with specific preattentive features.

#### *1.3.4 Visual Perception and the Role of Attention*

There are several different stages where visual information is attended to and processed, contributing to the visual experience. In order for a subject to see an object, the subject must attend to it (Wolfe 2001). Wolfe and Howard (2001) suggest there are several stages of processing: a preattentive phase, early vision processing, mid-level processing, and then an overall formation of a perception. Prior to the integration of attention, the visual system encodes dozens of features about an object in parallel which are represented as a bundle of features. In the preattentive stages of visual processing, the object is not well defined as the basic features are loosely attached to the object. Color, size, orientation and other basic features about an object are encoded at this stage; however, attention is essential in order for these features to be properly attributed to an object (Treisman and Schmidt 1982, Wolfe and Bennett 1997).

As shown through repeated search task studies, the binding or linking of features to an object can only occur in a single object at a time, thus attention is a bottleneck for visual information, and prevents observers from detecting change in objects if the change is either transient or hidden (Wolfe 2001, Wansink et al. 2000, Goldsmith 1998). Basic features such as luminance polarity, color, orientation, size, depth, line termination, curvature, target orientation and target polarity occur before more complex properties such as facial recognition, reading, object identification, or even perceiving the wetness of water. Attention is required for the second set of processes to occur.

The area or areas, where binding takes place and the theory of binding is still under debate. However, what is accepted is that attention is essential to object recognition. Objects that are not attended to, will not be recognized, and the preattentive information about the unattended object will be lost.

Furthermore, the binding problem researched by visual attention researchers is also a larger question. In real life we experience multiple sensations in a single perception, thus we bind many features at once from multiple sensory inputs. Crossmodal research examines how multiple signals are bound to create our sensations. Nowhere is the combining of the senses more apparent than when food is consumed.

#### *1.3.5 Visual Threshold Measurement*

Like in olfaction a forced choice methods are a commonly used approach for measuring the limitations of vision. In vision the 2-AFC is the most common method, often due to the limitations of a screen. Thresholds have been calculated for luminance, recognition, textures, motion, adaptation, and many different visual features (Solomon 2002, Donner 1992, Swanson et al. 1984, Foley, Legge 1981, Brown et al. 1953, Kohn 2007, Li et al. 2008, Enns and Di Lollo 2000, Tolhurst 2002). Masking has been used to study the limits of vision since 1925 (Piéron 1925). Many classical vision studies have used masking and paired comparison tests to evaluate the psychophysical perception of vision (Solomon 2002, Swanson, Wilson and Giese 1984, Foley and Legge

1981, Foley 1994, Legge and Foley 1980, Plant and Hess 1987, Turvey 1973, Weale 1955). Visual masking is a common psychophysical method used to evaluate the limits of vision. Two reviews outline the different uses and methods employed for visual masks: (Kahneman 1968) and (Enns and Di Lollo 2000) Through the various types of masking used, researchers have been able to determine the components necessary for object recognition. A visual mask can be presented in the form of a noise pattern superimposed over an image or as an object occluding another object. There are different levels of effectiveness of different types of masks (Enns and Di Lollo 2000, Legge and Foley 1980, Turvey 1973, Harmon and Julesz 1973, Kolers and Rosner 1960, Scheerer 1973, Schiller and Wiener 1963). The purpose of the mask is to increase the difficulty of a task, in doing so disrupts higher order processing (Enns and Di Lollo 2000). A mask is capable of reducing object recognition, such as obscuring the contours on an outline of two cherries making it more difficult to identify (Schiller and Wiener 1963, Kinsbourne 1962, Scharf 1966, Schiller 1965, Smith, Schiller 1966, Sperling 1963).

In a paired comparison using a visual noise mask, two visual stimuli patches are presented side-by-side. One patch, is the masking stimulus (MS), this is the noise mask presented alone overlaying a blank image. The other patch is the test stimulus (TS), containing the test stimulus, superimposed with a noise mask. The researcher can adjust the level with which the opacity of the noise mask to increase or decrease the difficulty of viewing the object

through the mask. The subject is required to choose the patch containing the TS. Unlike in olfaction, visual stimuli can be both presented increasing and decreasing in difficulty. Thus the stimuli are often delivered using a staircase method, stimuli are presented in a single session by increasing and decreasing the intensity (Cornsweet 1962). The staircase method is sometimes used to measure olfactory thresholds; however, it is modified to ensure stimuli are presented in an ascending order, to avoid the effects of adaptation.

When measuring a visual threshold, the researcher must control the head movements of the subject to ensure the subject views the images from a fixed visual angle. The lighting should be the constant during testing. A focal point should be used to direct the subject's attention on the task. The monitor used should be calibrated and it is advised the subject's become familiarized with the task prior to testing (Solomon 2002). Similar to olfaction, visual threshold determination needs to be properly controlled as well; however, the individual variation in vision is considered to be far less than in olfaction.

#### **1.4 Flavor Perception: a Multimodal Process**

Flavor perception occurs through the input of taste, smell, vision, audition, and touch. Two comprehensive reviews thoroughly describe the crossmodal interactions that have been researched as applied to food (Verhagen and Engelen 2006, Auvray and Spence 2008). Lawrence et al (2001) and Spence et al (2010) also investigated the influence of color on the consumer food

experience (Garber et al. 2001, Spence et al. 2010). All of our senses are used to assess the foods we are eating to create a flavor perception. The crossmodal integration of taste and smell has been widely studied (Small and Prescott 2005, Chiralertpong et al. 2008, Delwiche and Heffelfinger 2005, Prescott, Johnstone and Francis 2004, Small et al. 2004, Dalton et al. 2000, Stevenson, Boakes and Wilson 2000, Frank and Byram 1988, Gillan 1983). It is also clear vision is critical to informing our perceptions of flavor. Often the visual appearance of a food is assessed prior to even tasting the food and the aromas can offer further clues about the flavor profile of a food. However, how vision and smell combine to form our flavor perception is still not understood.

Consumers are easily persuaded by the visual appearance of a food, often basing flavor expectations on the presence of visual cues. Even professional wine tasters have been fooled by color-adulterated wine (Morrot, Brochet and Dubourdieu 2001). In 2001, Morrot et al (2001) used a professional panel of wine tasters to evaluate wine samples. These samples included a white wine as well as the same white wine that had been dyed red. Using descriptive analysis, the professional wine tasters used red wine attributes to describe the color-adulterated wine. Morrot suggested the evidence of visual cues overriding the other senses used to assess a wine's character is further evidence that higher order olfactory processing activates the primary visual cortex, area V1 as demonstrated by Royet et al (1999), suggesting vision may add to our olfactory perceptions.

#### *1.4.1 Odor/Color Associations*

The combined input of vision and olfaction powerfully influences our perceptions of foods. Several studies have demonstrated the strong associations of colors and specific odorants (Dematte, et al. 2006, Gilbert et al. 1996, Zellner et al. 2008). Although it is possible to argue these associations are based on learned pairings, Stevenson et al (2001) conducted a study in which participants learned to associate novel colors and odors (Stevenson 2001). Therefore, although color and odor associations may be strongly based on experience and expectation it is possible to learn new associations.

#### *1.4.2 Odor/Color Quality*

The following studies demonstrate the ability of visual stimuli to alter the perception of overall flavor quality. Several studies have examined the role of color to influence visual appearance to alter the odor quality of a product. In 1995 Francis conducted a review of the influence of color on the perceived quality of food products and found there are ideal color points that cue observers to associate color with flavor quality (Francis 1995). Schutz (1956) determined individuals perceive an ideal color for specific foods, he observed that these colors may not be the same as those found in nature (Schutz 1954).

Schutz (1956) showed through doctoring the color of orange juice, consumers found it to be more appealing. Christenson reported aromas paired with 'appropriate' colors were judged as having a higher quality aroma than those not paired with a color or paired with an 'inappropriate' color (Christensen 1983). DuBose determined the overall hedonic quality of a beverage could be significantly decreased or increased based on the color value of a beverage. Furthermore, a study using fMRI imaging confirmed the findings of others by demonstrating greater cortical excitation in pairs of congruent odors and smells judged as higher in hedonic quality (Österbauer et al. 2005).

#### *1.4.3 Odor/Color Pleasantness*

The combination of visual and olfactory stimuli can also alter judgments about the pleasantness of a food. This linkage is most likely related to theories of expectation, for a review please see Scharf and Volkmer (2000). Often alterations in the visual appearance of a stimulus can override the pleasantness of a flavor stimulus. Pleasantness is closely related to the perception of hedonic quality. Color and odor pairings can also influence the perceived pleasantness of a flavor in a product. Foster (1956) presented consumers with two types of chips, one speckled and one without speckles, consumers found the unspeckled chips more pleasant. However, when he informed the consumers the chips were 'charcoal grilled' the speckled chips

were preferred and were preferred overall (Foster 1956). Zellner et al (1991) presented subjects with several different odor color pairs. Those pairs judged as appropriate received higher pleasantness ratings than those odor-color pairs judged as inappropriate.

The linkage between pleasantness and acceptability in products due to discoloration is closely related to expectation and experience. The greater the discrepancy between the expectation and actual experience the consumer has, the greater the level of rejection and unpleasantness (Garber et al. 2001, Hutchings 2003, Cardello 2003).

#### *1.4.4 Odor/Color Identification*

Studies involving flavor identification, illustrate how a visual cue can drive our perceptions of flavor. One of the most common types of studies examining the role of vision and flavor involve flavor identification. Several studies have corroborated the reliance individuals have on visual cues in order to inform flavor judgments. As early as 1955, research dissecting the role of color and flavor perception in beverages found when colors were mismatched with beverages, individuals made large errors in flavor identification (Kanig 1955). A similar study devised by DuBose et al (1980) revealed how the color of a beverage influenced the identification of a beverage more than the flavor of the beverage. Another study where subjects were asked to identify the odor, odor

color pairs judged as 'appropriate' were identified correctly more often than odor-color pairs judged as 'inappropriate' (Zellner et al. 1991).

As previously mentioned color influenced wine tasting professionals to misattribute a white wine as a red wine based on the visual appearance alone (Morrot et al. 2001). Another study determined that subjects were likely to identify a cola flavored solution as cola when colored brown. When the experimenter changed the color of solution to orange, subjects misidentified the cola solutions as either orange flavored or tea flavored (Sakai et al. 2005). In a series of studies conducted by Shankar et al (2009, 2010) subjects were asked to identify the odor while the experimenter manipulated the timing of the color presentation. Shankar et al (2010) found the timing of the visual color stimulus influenced the identification of the odorant (Stanford et al. 2010). In an imaging study, Österbauer et al (2005) revealed greater cortical activation when congruent color-odor pairs were presented and identified correctly.

#### *1.4.5 Odor/Color Intensity*

The color intensity of a stimulus has been known to interfere with the ability of the subject to evaluate the flavor intensity of a stimulus. The other most common type of odor-color experiment asks subjects to evaluate flavor intensity. Overall these studies report an increase in perceived flavor intensity as the color intensity of the samples increased. Duncker (1939) conducted one of the earliest studies demonstrating the power of color to influence consumer

flavor judgment. In this study he first presented blindfolded subjects and presented them with two pieces of chocolate, both pieces were milk chocolate, one colored white and the other colored brown. Subjects were asked to describe the flavor of the chocolate samples. He then repeated the same set of questions but without the blindfold (Duncker 1939). The responses to the two chocolates were quite different when subjects were not blindfolded. In the evaluations where subjects were able to see the two samples, subjects reported a less intense chocolate flavor in the white chocolate sample (Duncker 1939).

Several studies have demonstrated the ability of color to enhance sweetness in solutions. Through a series of studies, Pangborn revealed the ability of color to influence the perceived sweetness of sucrose solutions; however, she did not find specific colors to influence the intensity, just the presence of a color in comparison to a colorless solution (Pangborn and Hansen 1963, Pangborn 1960). In another series of studies conducted by Johnson and colleagues, the concentration of color added to fruit flavored solutions yielded higher sweetness ratings and stronger flavor intensity ratings (Clydesdale et al 1992, Johnson and Clydesdale 1982). Similarly, Roth et al (1988) reported increases in perceived sweetness intensity in lemon-lime solutions as the yellow and green color within the samples were manipulated (Roth et al. 1988). It should be noted Frank et al (1989) demonstrated perceived increases in sweetness as a result of the presence of strawberry flavor rather than the intensity of the color in the solution (Frank et al. 1989).

Thus according to Frank, flavor is the driver of the change in perceived sweetness intensity not the visual cue. Furthermore, Lavin and Lawless (1998) showed depending on the level of experience of the consumer, color cues will not always influence the perceived sweetness of a product (Lavin and Lawless 1998).

DuBose et al (1980) reported changes in the redness of a cherry flavored beverage and changes in the concentration of orange color in an orange flavored beverage were both able to alter the perceived flavor of the beverage. However, DuBose did not find a direct relationship between the color of the solution and the perceived flavor intensity. Christensen (1983) reported subjects found appropriately colored foods perceivably more intense in both aroma and flavor than the inappropriately colored foods. Zellner and Kautz (1990) reported increases in odor intensity when odorants were presented with their expected color, they suggest the observed changes in intensity are the result of a perceptual change, and could be the consequence of conditioning. Engen (1972) found subjects were more likely to report an odor in colored solutions even in the absence of a color. Zellner suggests these associations are either learned or part of our multimodal processing.

#### *1.4.6 Color/Odor Influencing Detection*

Colors have also been found to alter psychophysical evaluations of odor stimuli. Two studies report psychophysical changes in perceptual detection

resulting from the presence of a visual stimulus. In 1972 Engen reported an increase in the number of reported false alarms when odorants were presented in colored solutions. When subjects performed the 2-AFC task, evaluating the odor of two solutions subjects were able to distinguish between the two colorless solutions. However, upon adding color to the solutions, the reported detection rates changed (Engen 1972). Engen attributed this finding to the role of expectations and cognition informing our perceptions.

In a separate imaging study, Gottfried and Dolan (2003) demonstrated the ability of a visual stimulus to increase detection rates of an odor. Gottfried and Dolan (2003) delivered puffs of olfactory stimuli through an olfactometer while displaying visual images on a monitor while analyzing the neural activity of subjects in an fMRI. They were able to find as the level of correspondence between the visual image and odorant presented increased, the detection of the odorants increased as well as the level of observed neural activity (Gottfried and Dolan 2003). The finding by Gottfried and Dolan (2003) suggests the combination of visual and olfactory stimuli can lead to an observed enhancement. Perhaps this observed increase in neural activity suggests signals from separate sensory pathways are enhanced when combined.

#### *1.4.7 Studies where Olfaction Drives Visual Attention*

The following two studies suggest olfaction is capable of driving attention toward a visual stimulus. The other previously mentioned studies have

described how vision often overrides olfaction during the simultaneous presentation of olfactory and visual stimuli. The associations made between visual stimuli and olfactory stimuli often show visual stimuli dominating olfactory stimuli when the two are presented simultaneously. However, Zhou et al (2010) demonstrated an olfactory stimulus could influence vision. In this binocular rivalry study participants were presented two images, one in each eye, and an odorant. If the image and olfactory stimulant were congruent, the individual would spend more time looking at the image in the eye of the congruently presented image.

A study conducted by Seigneuric et al (2010) also demonstrated the ability to influence the visual modality through olfaction. In this study participants were presented with a visual scene, a still life of images associated with the odors that would be presented. Participants were connected to a visual tracking device. When subjects were presented with an olfactory stimuli congruent with a visual object within the visual still life tracking times were reduced, demonstrating the olfactory cue shortened the visual processing time and helped to direct visual attention (Seigneuric et al. 2010). A similar study, conducted by Seo et al (2010) also used eye tracking to assess the influence of olfaction on object recognition. Seo et al (2010) like, Seigneuric et al (2010) also found congruent odors assisted in object detection.

Overall the reported studies demonstrate strong links between olfaction and visual stimuli when the two images presented are considered to be congruent. The definition of congruency is often determined by the authors of the paper, and not through a test to determine whether the subjects within the study agree with the definition of congruency. Of the crossmodal olfactory-vision studies mentioned above only three screened to ensure the olfactory and visual stimuli were considered by the subject to be associated (Dematte et al. 2006, Gilbert, Martin and Kemp 1996, Österbauer et al. 2005). The methods in these three papers used to determine congruency varied.

#### *1.4.8 Observations about Prior Olfactory/Visual Studies*

Unlike, taste, texture, and touch, olfaction and vision are the first sensory inputs an individual experiences when interacting with a food. Even before the food is consumed, olfactory and visual cues contribute to the expectation of flavor.

In most of the reported studies, odorants were presented above the level recognition. It is necessary to mention, the method of visual presentation of stimulus is not standardized across these studies, nor is the type of olfactory stimulus or the method with which the olfactory stimulus is delivered and evaluated by the participant. Color vision is separately processed from black and white vision, the majority of the studies discussed above use color in the visual modality. Many of the studies use color alone i.e. the shape of the

objects is not changed, (Garber et al. 2001, Shankar et al. 2009, Stevenson et al. 2000, Dematte et al. 2006, Gilbert et al. 1996, Zellner et al. 2008, Christensen 1983, DuBose, Cardello and Maller 1980, Österbauer et al. 2005, Shankar et al. 2010, Clydesdale et al. 1992, Roth et al. 1988, Zellner and Kautz 1990) others use color photographs i.e. a scene and therefore a more sophisticated visual display (Gottfried and Dolan 2003, Seigneuric et al. 2010) only one evaluated crossmodal vision and olfaction by using black and white images (Zhou et al. 2010). Furthermore, presenting a block of color is different than presenting an image composed of lines, curves, shading, and depth. Thus depending on the visual image displayed to the subject different levels of visual processing and cognition are used to perceive the visual image. The more complex the image, a colored visual scene, the more parts of the visual system are engaged.

It is important to determine the parametric limits of multimodal interactions. Gaining insight into the features, which most strongly contribute to multimodal interactions, will yield more information about flavor processing and a greater understanding for how consumers interact with products. From the previous studies, it is unclear whether color is a necessary driver of these associations or whether the presentation of a curved line can signal the presence of a fruit. It may be that a curve is not enough to signal a higher order level of processing necessary to engage a multisensory enhancement.

Odorant delivery methods vary across these crossmodal experiments. Many of the experiments use flavored solutions, thus olfactory stimuli are evaluated through retronasal evaluation. Many of the studies actually examine the crossmodal interactions of odor and vision by using retronasal smell, due to the fact the researchers are examining combinations of taste, smell, and color (Morrot et al. 2001, Gilbert et al. 1996, DuBose et al. 1980, Pangborn, Hansen 1963, Pangborn 1960, Johnson and Clydesdale 1982, Roth et al. 1988). All of these studies require the subject to put the sample in one's mouth, thus odor is delivered retronasally. Koza et al (2005) reported differences in the influence of color on odor intensity when sampled orthonasally or retronasally. Other studies ask subject to smell the stimuli, thus orthonasal evaluation is used (Dematte et al. 2006, Christensen 1983, Österbauer et al. 2005, Zellner et al. 1991, Shankar et al. 2010, Zellner and Kautz 1990, Trygg 1972, Gottfried, Dolan 2003, Zhou et al. 2010, Seigneuric et al. 2010). In these studies the method of odor delivery varies, from the use of plastic bottles (Gilbert et al. 1996, Zellner et al. 1991, Zellner and Kautz 1990), glass jars and bottles (Engen 1972, Zhou et al. 2010, Seigneuric et al. 2010), to smelling a food or drink (Christensen 1983, Shankar et al. 2010), and olfactometers (Dematte et al. 2006, Österbauer et al. 2005, Gottfried, Dolan 2003). Diluents vary across studies; however, since odorants in these studies are presented above threshold the odorous nature of the diluent is not too disconcerting.

Österbauer et al (2005), Gottfried and Dolan (2003), and Seo et al (2010) delivered odorants through an olfactometer, precisely controlling olfactory delivery. The odorants used were complex smells (not pure single compounds). For instance, Österbauer et al (2005) presented subjects with blocks of color and odorants were spearmint, lemon, strawberry, and caramel. Gottfried and Dolan (2003) presented high resolution color photographs of everyday images. Both of these tests employed color stimuli and complex odor stimuli. The timing of odorants was controlled in both of these studies by the delivery method. Gottfried and Dolan (2003) used a detection task in order to evaluate responses, where subjects answered yes or no to the presence of an odorant. They also collected reaction times in order to determine whether congruency influenced response speed. Österbauer et al (2006) used a rating system, 1 = very good to 4 = very bad, to evaluate intensity, familiarity, and pleasantness for the odorants alone, they ultimately evaluated the 'fit' of odorants and colors using the same rating scale. All odorants were presented above the detection threshold.

Gottfried and Dolan measured whether the presence of an odorant alters the observed brain activity when an odorant is paired with a congruent or incongruent visual stimulus, or whether the stimulus is pleasant or unpleasant. However, this study does not examine the limitations necessary for the interaction to occur. The study reveals pleasant odors paired with its congruent visual stimulus yields more correct responses to the presence of an

odorant, as well as decreases reaction time, in comparison to incongruent bimodal presentation and unimodal presentation. Furthermore the congruent bimodal condition showed the strongest brain activation. However, it is not clear how much of an odorant must be presented in order to result in an enhanced response or how much of the visual stimulus needs to be present. Meaning, if the image has to be in color and whether the full photograph needs to be presented or it can be occluded or it can be an outline. There are many unanswered questions about the role of bimodal processing and its ability to enhance a response.

The research conducted by Österbauer et al (2005) leaves many unanswered questions about bimodal processing. The reader can extrapolate the importance of color odor congruency; however, it is unclear how intense the odorant must be in order to yield the observed super-additive brain activity response. Additionally it is unclear how long the visual stimulus must be presented to the subject. Österbauer et al (2005) suggests the observed increase in activity observed in the insular cortex due to crossmodal activity suggests color may influence certain aspects of olfactory processing. This supports the prior observations made by Öngür and Price (2000) and Rolls and Baylis (2004), in non-human mammalian species, that the OFC may receive input from both the primary olfactory cortex as well as the ventral visual pathway.

However, the observation that color is capable of influencing a perceived olfactory stimulus has been demonstrated in studies by others using varying methodologies (Shankar et al. 2009, Morrot et al. 2001, Christensen 1983, DuBose et al. 1980, Sakai et al. 2005, Shankar et al. 2010, Clydesdale et al. 1992, Roth et al. 1988). Thus it would be interesting to understand whether color is crucial to creating this enhancement, or if other information processed through the ventral pathway such as object recognition through form with the absence of color could also yield such an excitatory enhanced response. It is worth noting, Österbauer et al (2005) did not observe a suppressive brain activity response during the presentation of incongruent visual-olfactory stimuli, reported in other crossmodal studies with auditory and visual stimuli and taste and smell stimuli (Calvert 2001, Small 2004, Small et al. 1997).

The previously mentioned crossmodal studies examining olfaction and vision or even taste and vision have not isolated the necessary components of a visual stimulus to create an enhanced response, i.e. a psychophysical response greater than the response of a single sensory modality. Neither have they determined the types of olfactory stimuli that can elicit these responses. Although most individuals experience the world through a full spectrum of colors, it is worthwhile to determine whether color is critical for an enhanced response, if form alone is crucial for a response, or if is the pairing of form and color that can lead to a multisensory enhancement.

As mentioned previously both Österbauer et al (2005) and Gottfried and Dolan (2003) use computerized olfactometers to control the delivery of the olfactory stimulus. Dematte et al (2006) conducted a study using color and odors with the intention of improving on the experimental design of Gilbert et al (1996). Odorants were delivered through an olfactometer, they tested whether, semantic prompting could speed or change the accuracy of responses to the visual/olfactory stimuli. The results revealed the semantic prompting of key selection did not influence the subjects as much as congruency. Reaction times decreased for congruently paired stimuli, the researchers also observed an increase in accuracy for congruently paired stimuli. Thus this states a similar finding as Gilbert et al (1996), where individuals pair certain colors to certain colors. Dematte et al (2006) also report a semantic prompt is not as influential to changing accuracy of response as the congruency of the stimuli. However, from the design of the study it is clear the odorants are presented for a fairly long interval. The stimuli are all presented far above threshold, thus the reader does not gain a further understanding of how olfaction and vision are processed, whether the naming would have influenced the subject more had the odors been less recognizable or the visual stimuli presented for a shorter interval. It is possible the semantic element of naming, a higher level of processing, could facilitate in detection if the stimuli are not as easily detectable.

It should be noted the field of subliminal information processing reports on several examples where a subliminally presented signal can alter an individual's behavior. These have been demonstrated in both the olfactory and visual fields. However, the mechanism through which the subliminal information is processed in order to yield behavioral changes are not well understood, and many of these studies have had difficulty replicating prior results. Studies have shown the ability of a visual subliminal presentation to prime a subject's response, other studies have shown subliminal message presentation may not be as influential in helping a subject remember the message of a product or in persuading someone to purchase a product (Bar and Biederman 1998, Smith and Rogers 1994, Theus 1994, Williams 1938). The term 'priming' describes the ability of a stimulus presented prior to the test stimulus to influence the subject's response to the test stimulus, There are both negative and positive primes, priming is linked to implicit memory.

Subliminal stimulation has also be tested within the olfactory field, to determine if a subliminal olfactory cue can alter a subject's behavior. These studies like the visual studies suggest there may be underlying mechanisms which can influence a subject regardless of awareness; however, few studies have been conducted and their methodologies are not well tested (Hirsch 1995, Li et al. 2007, Stockhorst and Pietrowsky 2004). Theories related to subliminal olfactory influence often relate to hormonal influences. Based on the basic tenets of the role of attention in both olfactory and visual processing, it is

difficult to conclude how subliminal processing works if attention is required to form recognition.

From the above literature it is clear a study examining the limits necessary for a psychophysical crossmodal interaction between vision and smell has yet to be conducted. All of the preexisting studies use stimuli well above threshold and have not taken into consideration the complex nature of visual processing and olfactory delivery techniques.

## CHAPTER 2

### THESIS STATEMENT

Flavor perception involves the integration of sensory information from sight, sound, taste, and touch. All of the senses are involved in creating a flavor perception; however, the significance of each sensory contribution to the formation of the overall perception is not understood. The visual appearance of a food strongly informs the perceptual expectations of the food including its flavor. Research has shown the congruency of a food's color with its flavor has been found to increase performance in detection tasks, identification, and decrease reaction times in comparison to incongruent pairings. Researchers have attributed these observed increases in performance with the increased signal due to multimodal input as opposed to unimodal processing. Although, the integration of the senses undoubtedly influences our perceptions of a flavor how each sense contributes to the perception is not understood, nor are the specific parameters of each sense well defined. There are still many unanswered questions related to crossmodal processing of information. This research investigates crossmodal interactions between olfaction and vision in order to gain further insight into flavor perception.

There are many different variables that can be measured when testing a visual stimulus: length of stimulus presentation, contrast, luminance, color, occlusion, attention, peripheral or direct, shape of stimulus, movement of

stimulus. When assessing an olfactory stimulus it is possible to: intensity of the stimulus, length of presentation, purity of sample, timing of presentation (i.e. whether it is before, after, or during the visual stimulus), type of odor, retronasal, vs. orthonasal, and attention to the stimulus. For this study we seek to define the elements necessary to result in a measurable change in performance in visual task when presented with an olfactory stimulus or a measurable change in an olfactory task when presented with a visual stimulus. Figure 5, illustrates the project objectives and layout. In order to test the influence of a visual stimulus on olfactory perception, these experiments manipulate the visibility of a visual stimulus through a noise mask, color, and the shape of an object while measuring performance on an olfactory detection task. Furthermore the influence of an olfactory stimulus on a visual detection task is also measured in order to assess with an olfactory stimulus can result in measurable changes in performance of a visual detection task.

All experiments compare changes in detection performance resulting from crossmodal presentation in comparison to unimodal presentation. The first experiment will test whether the simultaneous presentation of perithreshold stimuli in olfaction and vision can influence performance in either a visual detection task or an olfactory detection task. These tasks will be conducted in the absence of color. During these tests, the visual fruit stimuli will either be congruent with the fruit odor or incongruent.

Figure 5: Experimental flow diagram of research.

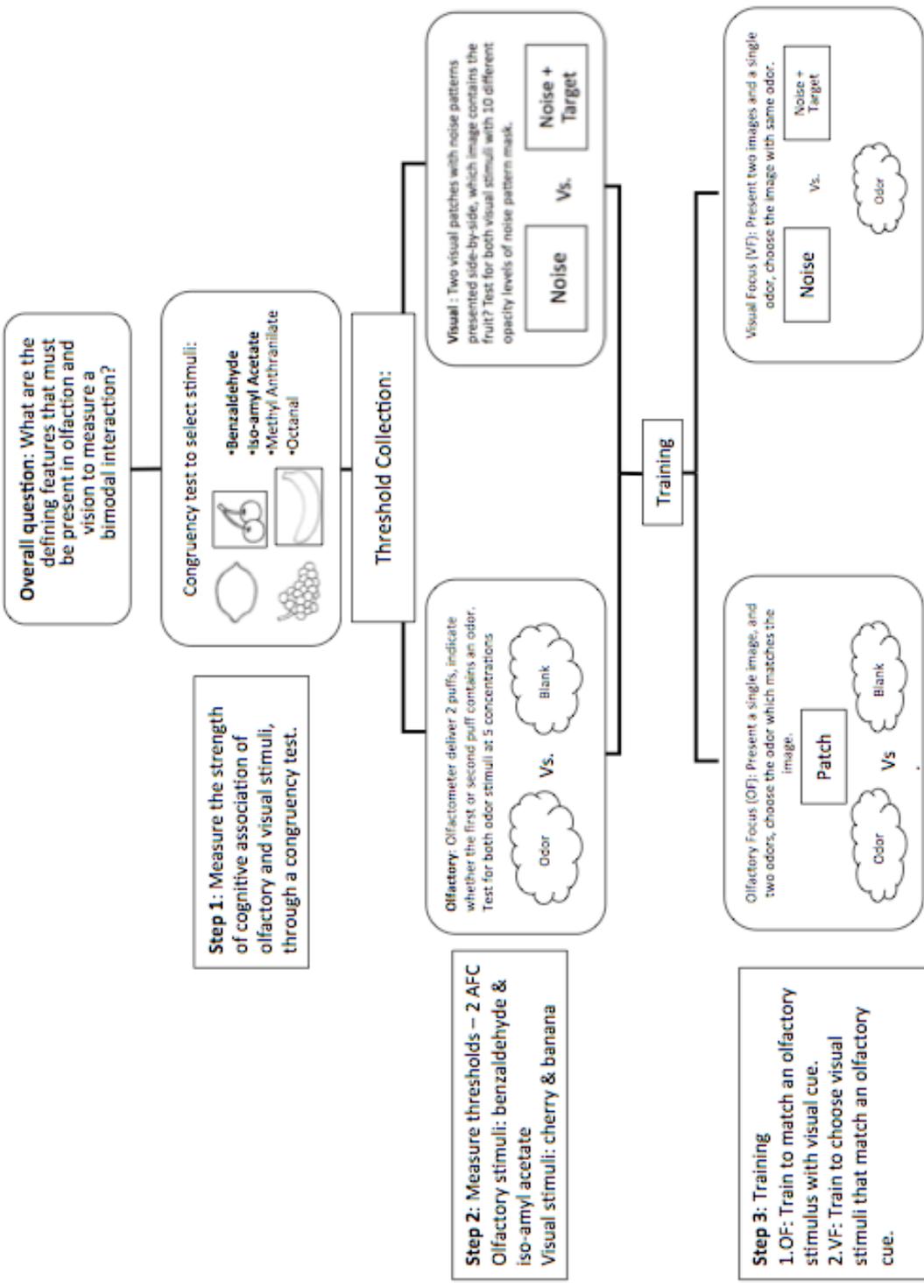
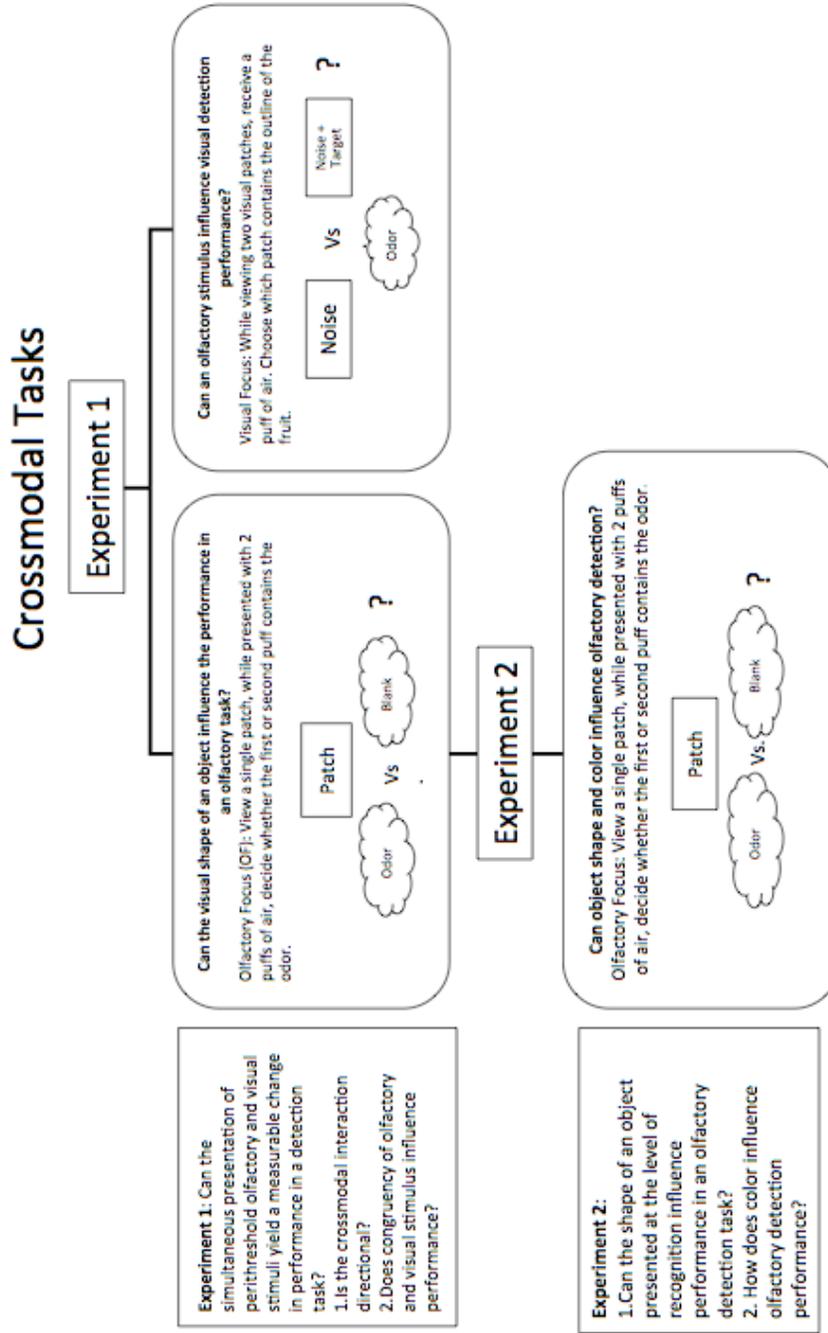


Figure 5 Continued



(I.e., benzaldehyde and cherry outline; iso-amyl acetate and a banana outline).

The effect will be measured by examining the influence of a congruent or incongruent fruit outline stimulus on the performance in an olfactory detection task. It will also be examined by measuring the effect of a congruent or incongruent presentation of an olfactory stimulus on the performance of a visual detection task. If perithreshold crossmodal enhancement were to occur in the congruent conditions, the performance in the crossmodal task would increase in comparison to its unimodal detection condition. Furthermore, if the simultaneous presentation of incongruent stimuli yield a decrease in performance in either the visual or olfactory detection tasks, it is then possible to conclude that the incongruent presentation of visual and olfactory information leads to a cognitive interference.

The next step in this research will assess if increasing the visibility of a visual stimulus to the level of recognition can influence the performance in an olfactory detection task. The role of color will also be investigated, whether the presentation of a congruent or incongruent color can influence the performance of an olfactory detection task. Finally visual stimuli at the level of recognition consisting of colored fruit shapes will be presented in both color congruent and color incongruent conditions while assessing the performance in an olfactory task. If color influences olfactory performance, it would be expected to see congruent colors yielding measurable increases in olfactory performance, and incongruent color presentation leading to decreases in olfactory detection

performance. If shape alone influences olfactory detection performance, the color of the fruit shape should not influence the olfactory detection performance.

Finally, if the intensity of the visual stimulus influences olfactory detection, there may be observable differences in performance from the first experiment to the second experiment.

Overall these experiments will reveal how each sense contributes to an overall flavor perception by investigating the influence of stimulus intensity, stimulus shape, stimulus color, and stimulus congruency.

## CHAPTER 3

### MATERIALS AND METHODS

#### **3.1 Congruency Test**

In order to select the test stimuli, a set of four olfactory and four visual stimuli were chosen to be included within a congruency test. The congruency test assessed how strongly the participants associated the olfactory and visual stimuli. Based on the results of this test the two odorants and visual stimuli with the strongest associations were chosen to be the test stimuli.

##### *3.1.1 Panelists in Congruency Test*

Eleven female and four male healthy individuals with no reported olfactory or visual impairment participated in the congruency test, whose mean age was  $33 \pm 11$  years. Of those nine female and one male partook in all parts of the study. The University Committee of Human Subjects of Cornell University approved and reviewed all research protocol. Testing took place in a smell isolation room, ensuring a constant flow of pure air.

##### *3.1.2 Materials for Congruency Test*

Visual stimuli were presented on a sheet of paper, the visual stimulus sheet.

The visual stimulus sheet, illustrated in Figure 6, consisted of a single sheet of

paper containing black and white outlines of all four visual stimuli: a banana, cherries, a bushel of grapes, and a lemon located on the four corners on the sheet of paper. Panelists used five black bottle caps to respond to evaluations of olfactory stimuli. Olfactory stimuli consisted of four Teflon squeeze bottles each retrofitted with a Teflon ball for nasal comfort. Each bottle contained a single odorant. All odorants were diluted in Polyethylene Glycol 400 Lot J33647 (J.T. Baker, Mallinckrodt Baker Inc, Phillipsburg NJ). Odorants were: 2.94 mM benzaldehyde ( $\geq 99.5\%$ , Sigma Aldrich, St. Louis MO), 26.91  $\mu\text{M}$  iso-amyl acetate ( $\geq 97\%$ , SAFC (Sigma Aldrich), St. Louis MO), 772.6  $\mu\text{M}$  methyl anthranilate ( $\geq 99\%$ , SAFC (Sigma Aldrich), St. Louis MO), and 263.5  $\mu\text{M}$  octanal ( $\geq 99\%$ , Sigma Aldrich, St. Louis MO).

### *3.1.3 Procedure for Congruency Test*

The congruency test method was adapted from Gilbert et al (1996). Subjects were presented with a sheet of paper consisting of four black and white images of fruit outlines. The four black and white fruit outlines were: a banana, two cherries, a cluster of grapes, and a lemon. While explaining the procedures, the experimenter demonstrated for the panelist, the proper method of sniffing from the squeeze bottles. The subjects received five black bottle tops. Subjects were instructed to sniff the odorant from the squeeze bottle and then place the five game pieces on the image they felt best fit the odorant. All five bottle tops could all be placed on a

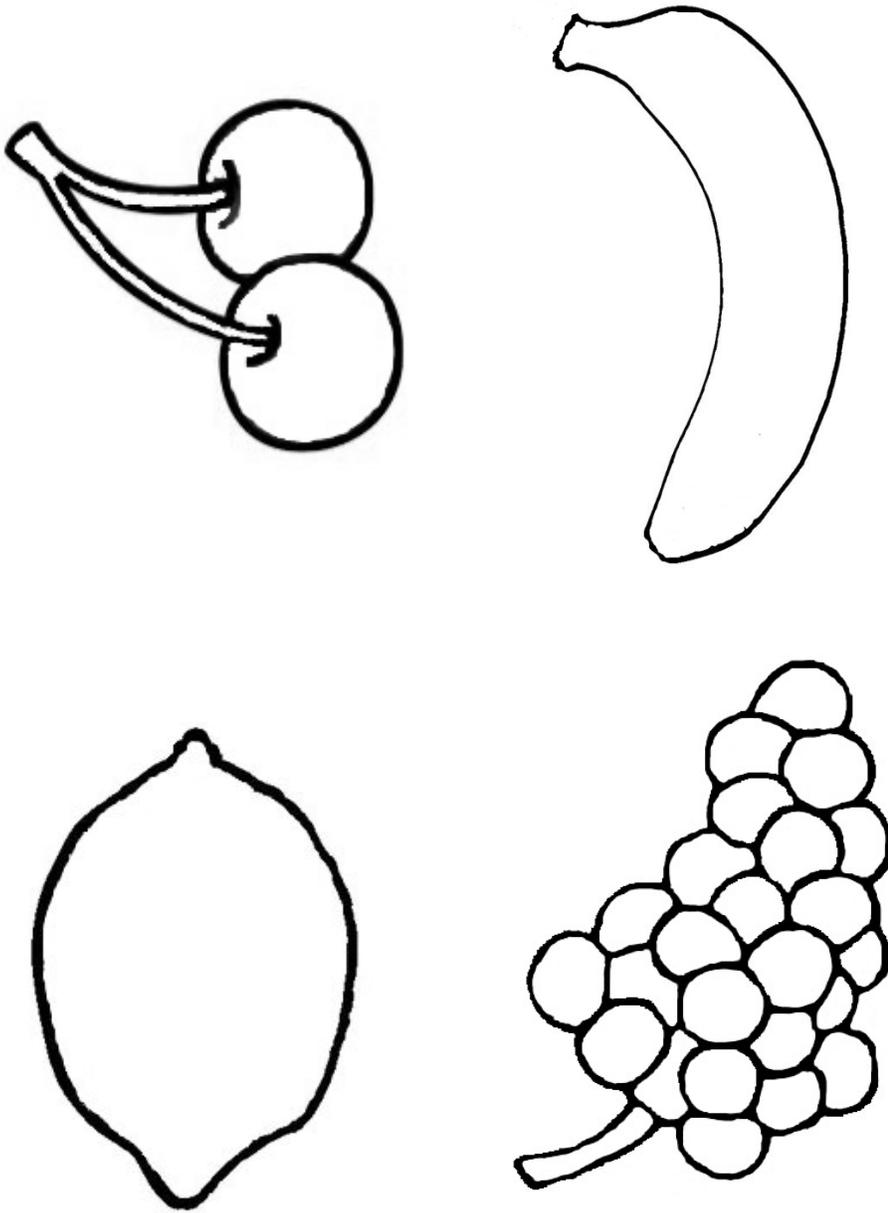


Figure 6: Visual stimuli presented in the congruency task. Four fruit outlines were presented: a lemon, cherries, a bunch of grapes, and a banana. The banana and cherry stimuli were used in both experiment 1 and experiment 2 as the visual stimuli.

single fruit outline or distributed amongst the four outlines. Following each trial, the experimenter recorded the placement of the five game pieces. After a minimum of a timed 45-second break the subjects evaluated a different odor repeating the same process as before. Participants evaluated each odorant three times. Testing took approximately 15 minutes. The order of odorant presentation was randomized.

### *3.1.5 Data Analysis for Congruency Test*

The number of times a game piece was placed on each image was tabulated. The distributions of the five game pieces across the four fruit outlines were tabulated for each trial. The total number of game pieces placed on each outline for each fruit presented was totaled. Percentages for the number of game pieces placed on cherry when presented with benzaldehyde were calculated, number of game pieces placed on banana when presented with iso-amyl acetate, the number of game pieces placed on grape when presented with methyl anthranilate, and the number of game pieces placed on lemon when presented with octanal.

## **3.2 Visual Threshold Determination**

Ten participants (nine female and one male) who partook in the congruency study partook in crossmodal study. All protocols were approved by the University Committee of Human Subjects of Cornell University. Testing took

place in a smell isolation room, to ensure a constant flow of pure air. Visual thresholds were determined for each participant through a forced choice visual noise mask task.

### *3.2.1 Materials for Visual Threshold Determination*

All visual stimuli were black and white outlines of fruit created in GIMP, the same black and white fruit outlines used to determine the visual stimuli in the congruency test were used in the visual threshold task. Two visual thresholds were calculated: a threshold of the outline of a banana, the other threshold of the outline of two cherries. All visual stimuli were presented on a screen with a resolution of 2560 X 1440. Images were prepared in GIMP. A black and white noise pattern was created in GIMP. The transparency of the noise pattern was manipulated in GIMP, from 85% transparency to 99% transparency (nearly opaque). The noise pattern was layered on top of the images of the fruit, the transparency for the visibility of the fruit outline through the noise pattern varied from 85%-100%. During testing, all except for one panelist were tested with a transparency range of 90% to 99% transparency (not visible), transparency increased in single step increments. One panelist, due to demonstrated poor visual performance, was tested with a range from 85%-94%. Subjects were seated at a fixed distance of 60 cm from the screen; their heads were stabilized with a chin and forehead rest. Images were viewed at a visual angle of 18.21° X 12.12°. The fixed distance of 60 cm was chosen

based on prior visual testing protocol (Seigneuric et al. 2010, Zhang et al. 2011). All stimuli were presented using a program written in PsychoPy v 1.64.00 where timing of the instructions, stimulus presentation, interstimulus intervals (ISI), randomization, and data collection could be effectively controlled (Peirce 2007, Peirce 2009)

### *3.2.2 Method for Visual Threshold Determination*

Visual thresholds for banana stimulus and cherry stimulus were determined in two separate sessions. Testing took place in a smell isolation room. Panelists were seated in front of a computer monitor, with a chin and forehead rest located at a fixed distance of 60 cm from the screen, as illustrated in Figure 7a and b. Upon entering the testing area, the experimenter adjusted the chair height for each panelist ensuring uniform eye level. All stimuli were presented using a two alternative forced-choice method, similar to the classic two the visual masking methods using in (Foley and Legge 1981, Foley 1994, Legge and Foley 1980, Garcia-Perez 1998). The computer program used to evaluate visual thresholds, written using PsychoPy (Peirce 2007, 2009) instructed the subject to evaluate two images side-by-side one image located on the left side of the screen and one image located on the left side of the screen.

Figure 8 is a schematic of the experimental. Following the first set of instructions were examples of the two reference images: a patch of noise followed by a patch of noise+target. Six practice trials with feedback followed

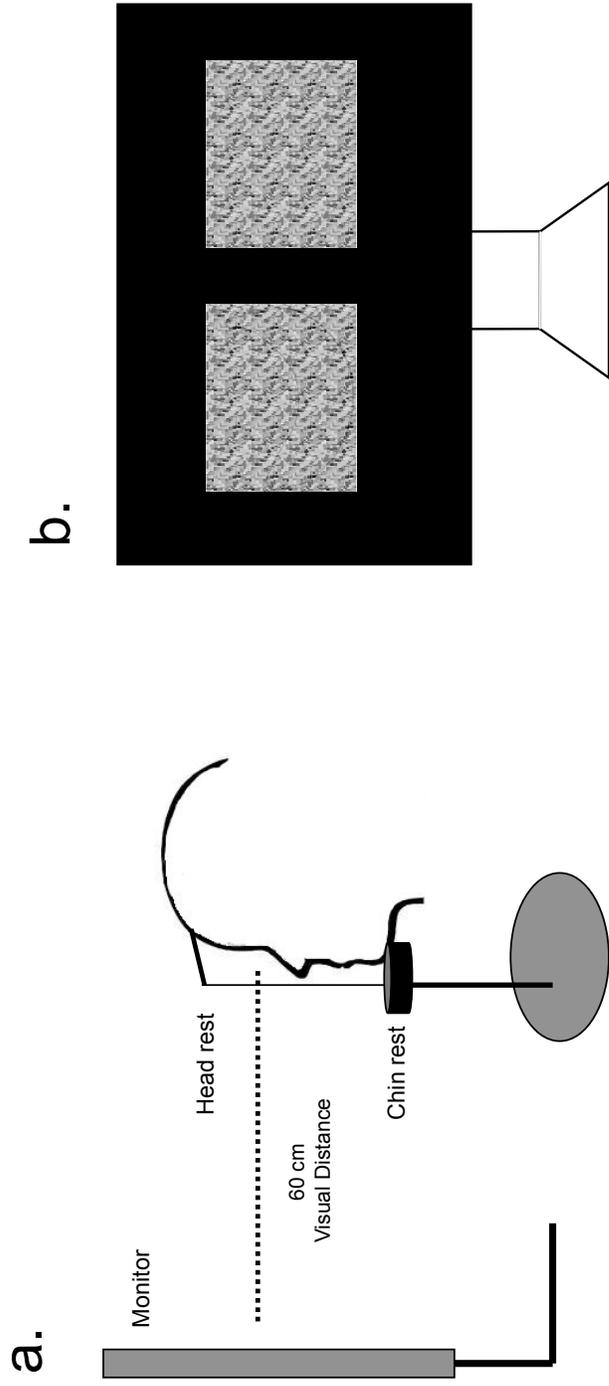


Figure 7a and b: Set-up for the visual threshold detection task. a) A cartoon depiction of the set-up, where the panelist rests her chin in the chin-rest at a fixed distance of 60 cm from the computer screen. b) Depicts how the visual-task would look like to the panelist.

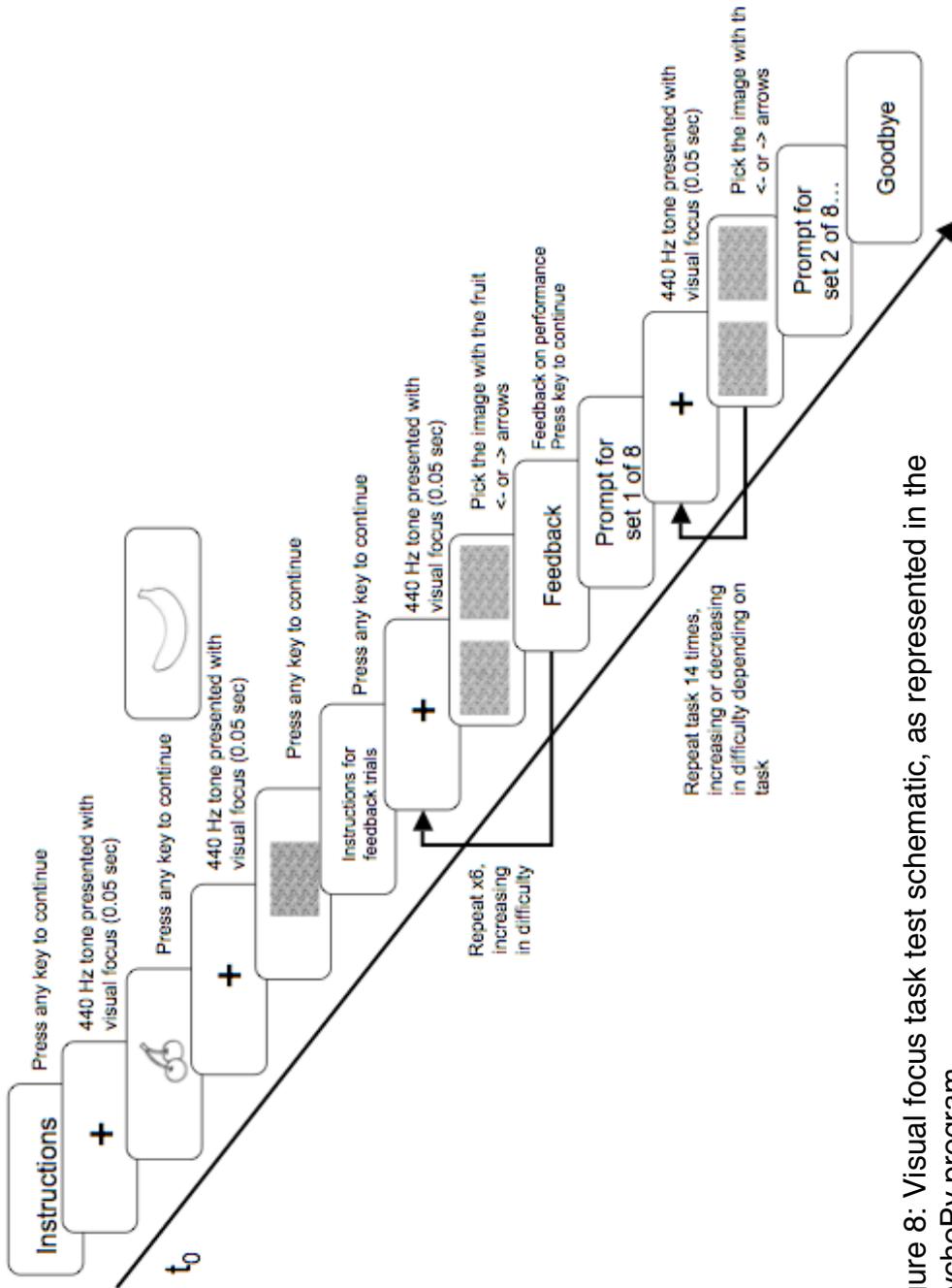


Figure 8: Visual focus task test schematic, as represented in the PsychoPy program.

the presentation of the references. Practice trials, like the test trial presented the subject with two images (noise and noise + target) presented side-by-side. All images were evaluated using a forced-choice method, where noise and noise+target were always presented side-by-side on the screen. Subjects were instructed to select the noise+target image. Presentation location of noise+target was randomized to appear on either left or right side of the screen. Subjects responded by pressing either the left or right arrow keys on the keyboard, indicating the location of the noise+target stimulus. Subjects were instructed to respond as quickly and accurately as possible. A 440 Hz tone simultaneous with the presentation of a focal cross-hair centered in the middle of the screen, alerted the subject to the beginning of each trial.

Subjects were told to focus on the cross-hair at the start of each trial. Subjects were instructed to press any key to continue. After the practice trials, subjects were alerted to the start of testing. Each session consisted of 8 test sets, each containing 14 trials. The test sets alternated between increasing and decreasing in visual detection difficulty. Each transparency level was presented two times during each test set. A test set increasing in difficulty would always be followed by a test decreasing in visual detection difficulty. Whether a subject began testing with a test set that increased or decreased in difficulty was randomized across subjects. Subjects rested for 1-minute between each test set. There were a total of eight sets of trials.

### *3.2.3 Data Analysis for Visual Threshold Determination*

The number of correct responses was tabulated for each mask level presented to each panelist. The 0.75 correct level was calculated for each participant using the geometric mean.

### **3.3 Odor Threshold Determination**

Odor Thresholds were first collected without the use of an olfactometer, using a 3-AFC method Kurtz et al (2010). In order to control for timing and uniform puff delivery across all panelists it became increasingly necessary to engineer an olfactometer. The design of the olfactometer integrated the Teflon puff bottles into the design. By pressing in the sidewall of the squeeze bottle a fixed distance, the same size puff could be delivered to each panelist.

Additionally the olfactometer could receive commands from the computer indicating when to deliver a puff of air to the panelist. This allowed the panelist to focus on the required task rather than be distracted by stimulus delivery.

Attention could be effectively directed toward the visual stimulus or olfactory stimulus. Two thresholds were determined, one for benzaldehyde (cherry) and the other for iso-amyl acetate (banana).

#### **3.3.1 Materials for Odor Threshold Test**

Testing for two odorant thresholds occurred over separate sessions. All dilutions were prepared in Polyethylene Glycol 400 (J.T. Baker, Mallinckrodt

Baker Inc, Phillipsburg NJ) (PEG). All solutions of benzaldehyde were made from  $\geq 99.5\%$ , (Sigma Aldrich, St. Louis MO) and all solutions of iso-amyl acetate were made from  $\geq 97\%$ , SAFC (Sigma Aldrich), St. Louis MO) Five concentrations were prepared for benzaldehyde:  $46 \mu\text{M}$ ,  $92 \mu\text{M}$ ,  $184 \mu\text{M}$ ,  $368 \mu\text{M}$ , and  $736 \mu\text{M}$ . Five concentrations of iso-amyl acetate were also prepared:  $0.13 \mu\text{M}$ ,  $0.41 \mu\text{M}$ ,  $1.24 \mu\text{M}$ ,  $3.73 \mu\text{M}$ ,  $11.21 \mu\text{M}$ . All odorants were presented in 250 mL Teflon bottles manufactured by Nalgene. A Teflon ball was affixed to the top of each bottle to avoid panelists from accidentally sticking the bottle up their noses. Each bottle contained 10 mL of solution. All bottles were labeled with random three-digit codes except for the reference bottles, which were marked 'Ref'. All solutions appeared clear and colorless. Solutions were made 24 hours in advance of testing, and pipetted into bottles approximately 1-hour prior to testing.

### *3.3.2 Method for Odor Threshold Determination*

The protocol followed a 3-AFC threshold method described in Wise et al (2008) (Wise et al. 2008) and used by Kurtz et al 2009. Panelists evaluated three references at the beginning of each session a reference for the blank (a bottle containing only PEG) and two odor references, labeled 'low reference' and 'high reference' containing the lowest and highest concentrations of the test odorant. The panelists evaluated three bottles per trial, references were available throughout the testing session. Two of the three bottles were blank

and one of the bottles contained the odorant. The researcher randomized the order of bottle presentation. After the panelist selected the bottle containing the odorant, the panelist recorded the bottle number and the location of the bottle on the ballot. Once the subject completed her assessment, the subject rested for a timed 45-second break. After the break, the individual repeated the same procedure for the next set of three-bottles. There were a total of three trials per concentration level, odorant concentration increased throughout the test. Each testing session began with the lowest concentration and ended with the highest concentration. Subjects evaluated a total of 15 trials during a single training session, lasting approximately 20-minutes. Every panelist completed a total of two testing sessions per odorant, for a total of four odorant-threshold testing sessions. The level of detection is defined as 66.7% correct; however, we were interested in a slightly higher level of detection, in between that of detection and recognition, and thus chose the level of 75% detection.

### *3.3.3 Olfactometer Design*

In order to ensure accurate and measurable delivery of olfactory stimuli this experiment utilized an olfactometer, capable of communicating with the PsychoPy computer program. The olfactometer used throughout these experiments was engineered specifically for this research. From the outside the olfactometer appeared as a white rectangular box, with two side doors, a

chin rest, a forehead rest, and a single piece of Teflon tubing located during in front of the chin rest, pointing up toward the participant's nostrils. The subject rested her chin on a chin rest affixed to the top of the olfactometer and rested her forehead against a forehead rest also affixed to the olfactometer. Odors were emitted through a Teflon tube centered directly beneath the participant's nostrils and located directly in front of the chin rest. Figure 9 a and b is a diagram of the puff olfactometer (Kurtz et al, *in preparation*). Each side-door accessed the Teflon squeeze bottles. These bottles could be easily switched in and out of the machine during testing. Two actuators affixed with circular discs, one for each bottle were used to press in the side of each squeeze bottle to deliver a puff of approximately 11 mL in volume as confirmed through a soap bubble test. The olfactometer was controlled through a program written in Python code from ActiveState Komodo IDE version 7.0.0 (ActiveState, Vancouver, BC) connected to an Arduino board (<http://arduino.cc>, Italy). The program PsychoPy regulated timing of odorant delivery from the olfactometer.

#### *3.3.4 Olfactory Threshold Method with Olfactometer*

Threshold determination for benzaldehyde and iso-amyl acetate with the olfactometer used a 2-AFC forced choice method (Delwiche and Heffelfinger 2005). Thresholds for the two odorants were collected in separate testing

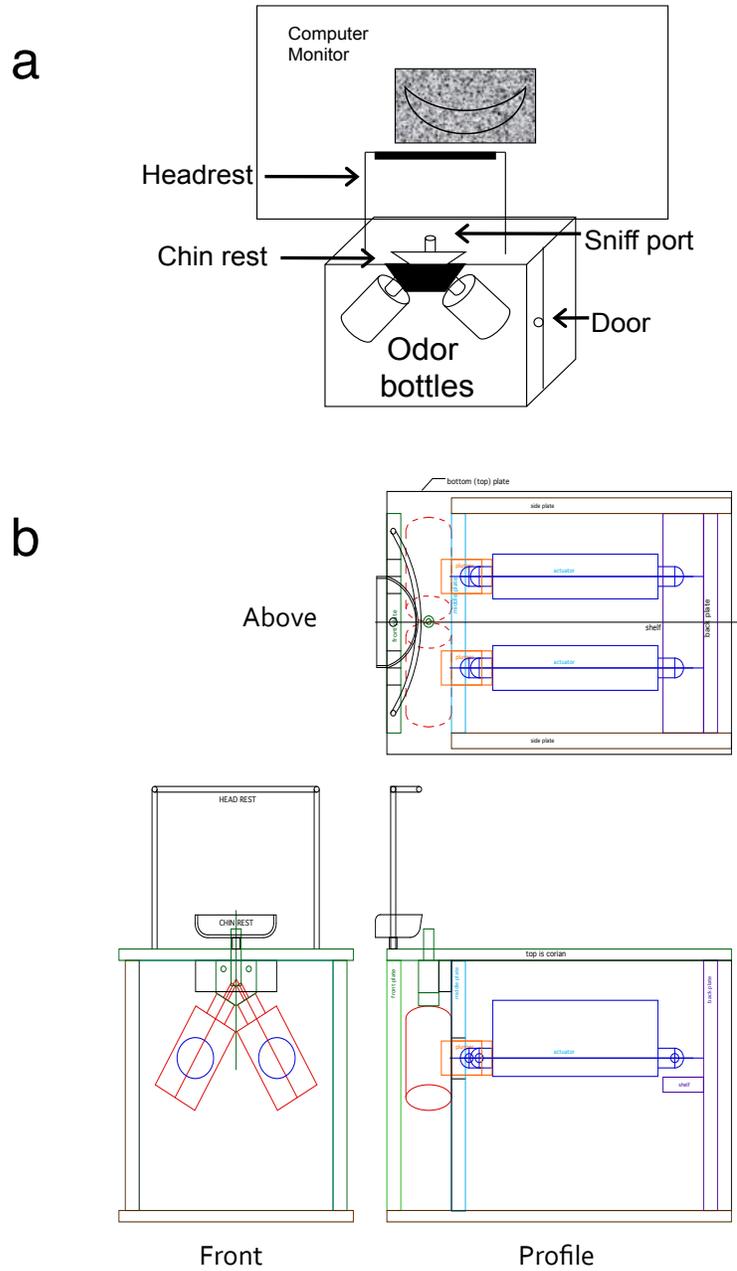


Figure 9 a and b: Puff-olfactometer design. a is a cartoon drawing of the olfactometer and computer set-up. b) is a schematic of the olfactometer design, from above, from front and profile views. From above the two horizontal objects are the actuators. From the front, the two squeeze bottles, chin rest, and forehead rest are visible.

sessions to reduce olfactory fatigue. Five concentrations of iso-amyl acetate were prepared 1.24  $\mu\text{M}$  - 11.24  $\mu\text{M}$  and five concentrations of benzaldehyde 92  $\mu\text{M}$  - 368  $\mu\text{M}$ . All solutions were diluted in PEG. 10 mL of solution were pipetted into a 250 mL Teflon Nalgene squeeze bottle. These concentrations were determined to be within the detection range from the 3-AFC test set. Two bottles were inside of the olfactometer throughout testing. One bottle contained only PEG (blank) and the other bottle contained the odorant (target). In order to avoid bias due to unknown differences between left and right actuators, bottle location was randomized throughout the trials. Upon the subject's arrival, the height of the chair was adjusted in order to ensure a the subject's chin rested comfortably in the chin rest located on top of the olfactometer, this also ensured all panelists viewed the visual stimuli from approximately the same angle.

To measure the olfactory threshold a program written in PsychoPy, delivered a set of instructions on a computer screen stating two puffs of air would be delivered in sequence for each trial. Panelists wore Bose noise canceling headphones throughout testing to block external noise. Headphones were connected to the computer where white noise played throughout testing, subjects prompted with tones throughout testing. In order to alert panelists to the beginning of a trial panelists were alerted with a 440 Hz tone along with a focal crosshair, located in the center of the monitor. Panelists were instructed when to inhale and exhale in order to regulate breathing patterns across

panelists. During testing one puff always contained the odor while the other contained no odor, panelists were told regardless of whether they detected an odor to make a decision. The program alerted the panelist to the onset of the first and second puffs; panelists were instructed to use the left and right arrow keys to record their responses (left arrow = 1<sup>st</sup> puff; right arrow = 2<sup>nd</sup> puff). At the beginning of each testing sessions panelists completed five practice trials with feedback in order to ensure that he or she understood the task as well as to serve as a warm-up. A schematic of the testing protocol can be seen in Figure 10.

In order to test the threshold, testing always began with the comparison of the lowest concentration and increased up to the highest concentration. Panelists evaluated each odorant at five concentrations. Concentration levels were repeated four times in a row, separated by 15 second breaks. After repeating the same concentration step four times, a timed 1-minute break took place, followed by the presentation of the next concentration step. Thus each panelist completed 20 total trials, five for each concentration step for each odorant tested. Thresholds were calculated through the presentation of five different odorant concentrations.

### *3.3.5 Data Analysis Olfactory Threshold Determination Method*

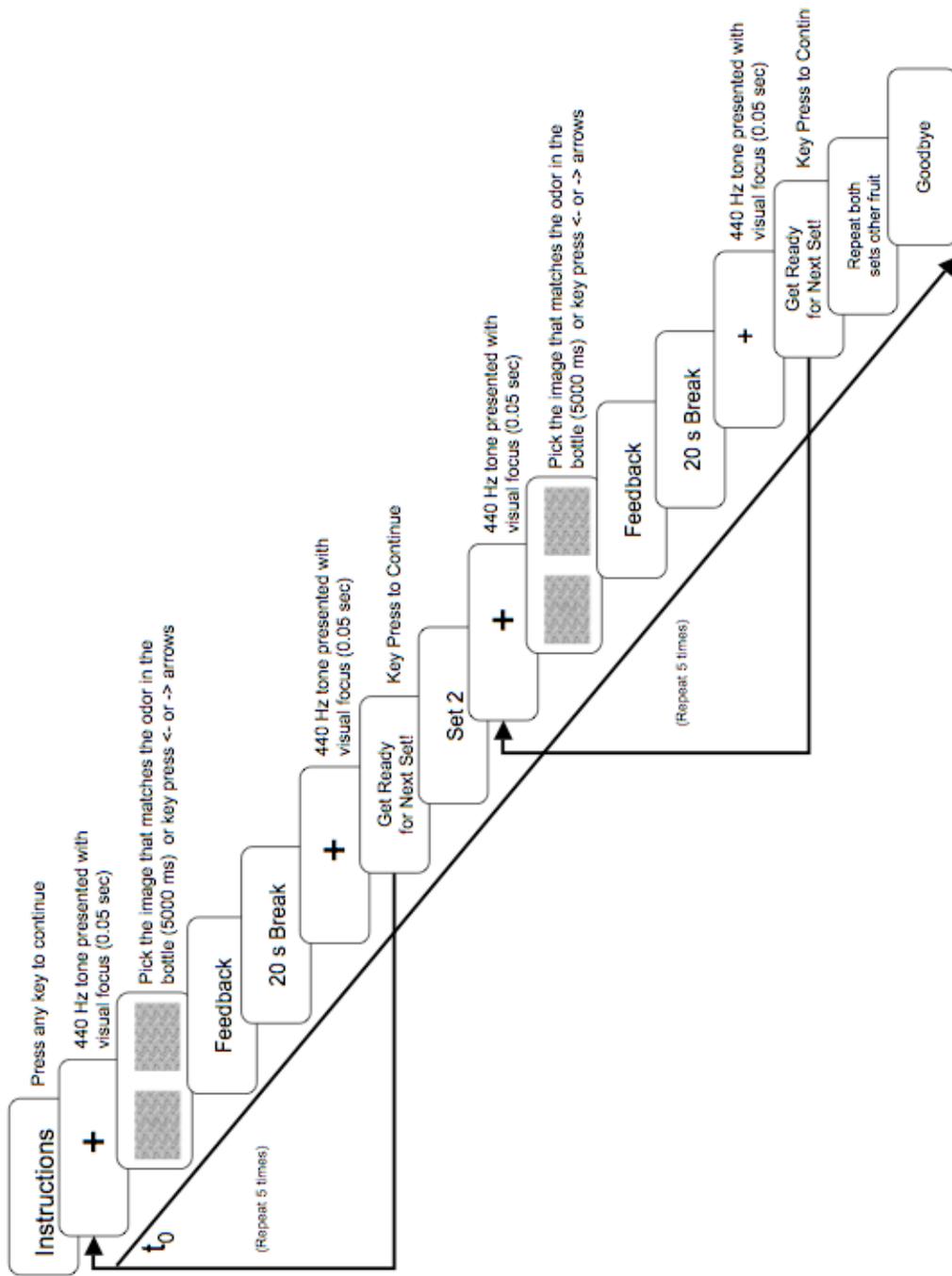


Figure 10: Schematic for the olfactory detection task used in conjunction with the puff olfactometer.

The number of correct responses was tabulated for each concentration level presented to each panelist. The 0.75 correct level was calculated for each participant using the geometric mean.

### **3.4 Training Method**

A set of two training sessions took place prior to the engineering of the olfactometer, to determine whether individuals were capable of associating visual images with olfactory cues.

#### *3.4.1 Materials for Training*

All visual images were presented using a program written in PsychoPy on a screen with a resolution of 2560 X 1440. Panelists rested their head on a chin and forehead rest, located at a fixed distance of 60 cm from the screen. All visual images were viewed at a visual angle of  $18.21^\circ \times 12.12^\circ$ . Images were either of a noise pattern or of a fruit outline (either banana or cherry) occluded by noise. All images were created in GIMP. Images were the same as those described in the methods for determining the visual threshold. Images were presented above threshold at the 85% transparency point. All stimuli presented were above the calculated thresholds of detection for all panelists. Odorant concentrations during training were above threshold: benzaldehyde  $736\mu\text{M}$  and iso-amyl acetate  $11.21\mu\text{M}$ . Blank bottles, those containing only PEG, were also presented to panelists. All bottles were labeled with 3-digit

codes, except for the three reference bottles. Reference bottles were labeled 'Ref 1,' 'Ref 2,' and 'Blank'. Ref 1 contained iso-amyl acetate, Ref 2 contained benzaldehyde, and Blank contained PEG. As in the other tests, all bottles were 250 mL Teflon Nalgene squeeze bottles, retro-fitted with a Teflon ball on top for nasal comfort. Each bottle contained 10 mL of clear colorless solution.

#### *3.4.2 Method for Training Session*

Panelists completed a training session to gain experience associating images and odorants. Each training session took approximately 30 minutes. Training took place in two separate sessions. For the first training session taught the panelist to use associate a visual stimulus with an odorant. Figure 11 illustrates the testing scheme used during the olfactory discrimination task used to train subjects. Subjects were presented with a single image, centered on the screen either target + noise or noise alone. The subjects were presented with either a visual image of noise or an image of noise + target. The researcher presented the participant with two bottles, one odorant bottle and one blank bottle. The subject then would choose the blank bottle if she perceived the visual patch to be noise or select the odorant bottle if she detected a fruit outline in the visual patch. The participant indicated to the experimenter which bottle best fit the image on the screen. The experimenter recorded the participant's answer and provided feedback. Testing began with

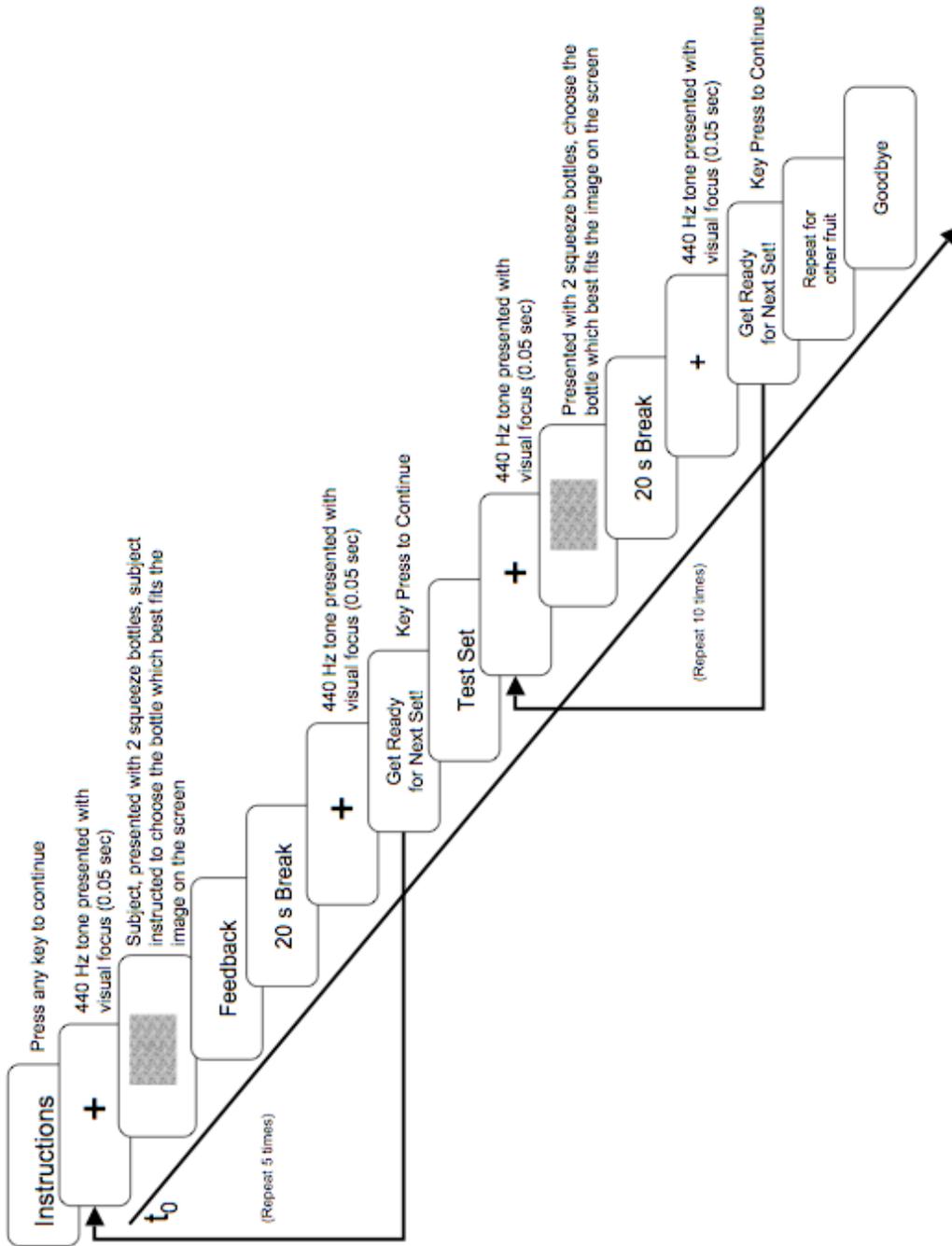


Figure 11: The test schematic for olfactory task training.

two example trials for the odorant/visual stimulus to acquaint the participant with the task. Participants completed five practice trials with feedback prior to beginning the test. Each participant repeated the test for a total of 10 test trials per odor. All participants were required to receive a score of 90% correct by the conclusion of the second test trial. After a timed 20 second break, the panelist repeated the procedure for the second odor and image. A 440 Hz tone signaled the beginning of each trial, along with a message prompting the participant. The participant pressed any key to continue, prompting another 440 Hz tone and a focal crosshair to appear in the center of the screen. A visual patch appeared in the center of the screen until the participant made his or her decision, at which point the participant was instructed to press any key to continue which prompted a 20-second timed break. After completing the second set of trials, the same procedure was repeated for the second odor/visual pairing, beginning with the two example trials.

During the second stage of training, two images were presented side-by-side the same as in the visual threshold test, one an image of a fruit + noise (either banana or cherry at 85% transparency), and the other of noise alone. Subjects were presented with a single squeeze bottle, to be sniffed as they examined the two visual images. Subjects used the left and right arrow keys to choose the image best fitting the bottle presented with a single bottle, either containing an odorant (matching that of the fruit image on the screen) or a blank (PEG). Subjects were instructed to choose the image that best

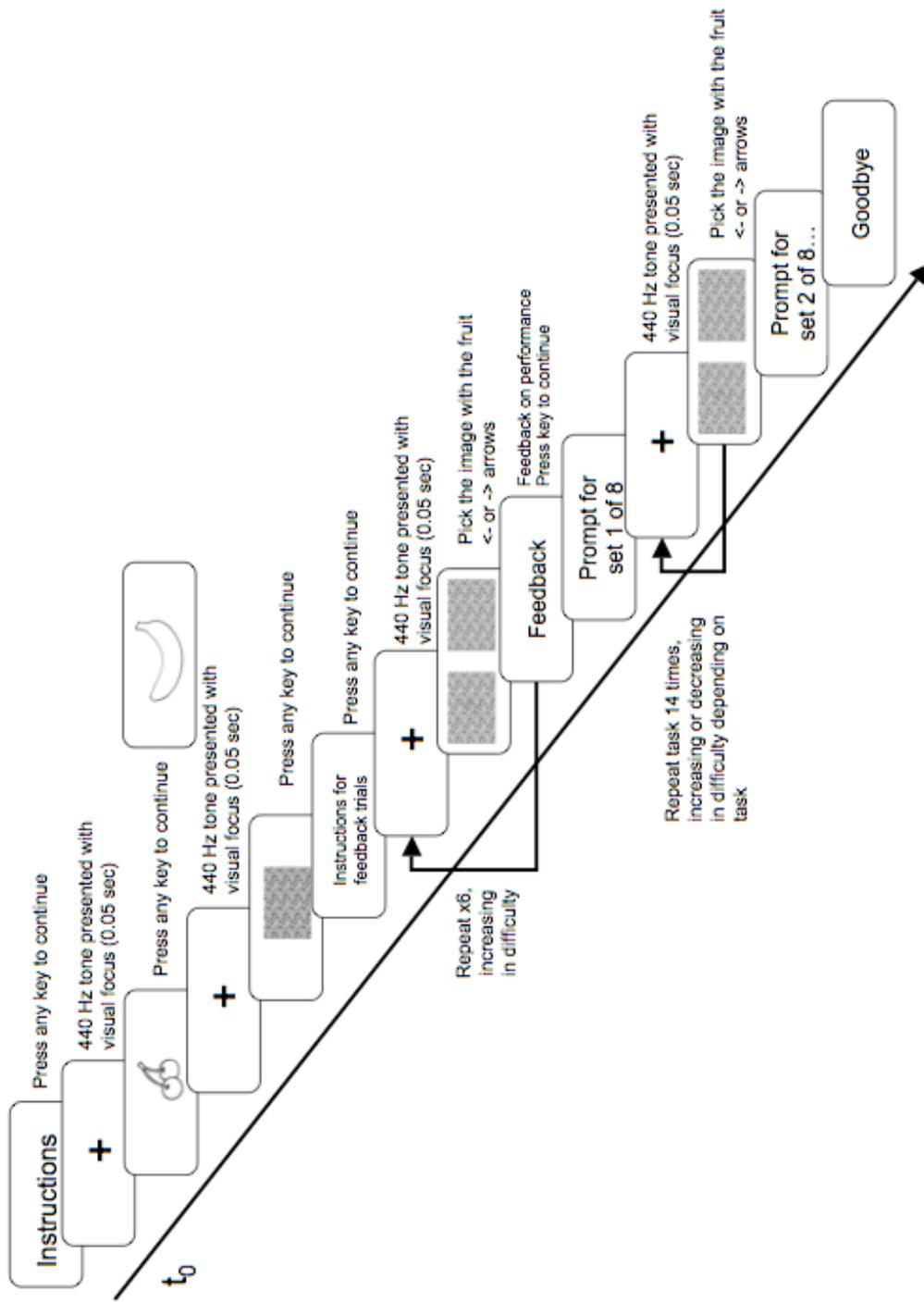


Figure 12: Test schematic for visual focus task training.

matched the odorant in the bottle. If the subject were presented with a blank bottle, the subject should choose the image of noise. Training for cherry and banana occurred separately. Training proceeded as follows. The test schematic for the visual discrimination crossmodal training is illustrated in Figure 12. The participants were presented with two example trials, two images were presented on a screen, panelists were also presented with a single squeeze bottle. The test session began with a 440 Hz tone accompanied by a screen informing the participant to 'Get Ready', and to press any key to continue. The next screen presented a focal cross-hair along with a 440 Hz tone followed by the presentation of two images side-by-side. The individual was instructed to evaluate the visual images while simultaneously sniffing from the squeeze bottle, and make her selection with the left or right arrow keys. A timed 20-second break immediately followed the subject's response. A 440 Hz tone along with a screen to 'Get Ready' appeared after 20-seconds and this procedure was repeated five times and then again for second test, for another five trials. After which, the subject was introduced to the second odor and the entire procedure was repeated for the second odor and image.

Completion of this training task enabled the researcher to ensure individuals were able to distinguish the target odor from the blank. All testing took place above every individual's calculated threshold, in order to observe

whether the individuals were capable of completing the task with a 95% success rate.

### **3.5 Method for Crossmodal Tasks**

#### *3.5.1 Experiment 1: Participants in Crossmodal Task*

Nine female and one male participant who had partook in all other parts of training and testing, completed the eight testing sessions. The same stimuli that had been used in the previous training and detection determination sessions were used in the testing sessions. Stimulus preparation followed the same protocol as described above.

#### *3.5.2 Overview of Method for Experiment 1*

Two tasks were devised for evaluating crossmodal interactions between the olfactory and visual systems. The visual focus task (VF) examined whether an olfactory stimulus could alter visual detection sensitivity, the olfactory focus task (OF) examined whether a visual stimulus could influence the olfactory detection sensitivity. Figure 13 a and b illustrate the VF and OF tasks. The OF task required the panelist to complete an olfactory 2-AFC with the simultaneous presentation of a single visual stimulus, the VF task required panelists to evaluate a visual 2-AFC during the simultaneous presentation of an olfactory stimulus. All stimuli used during these tests were delivered at the

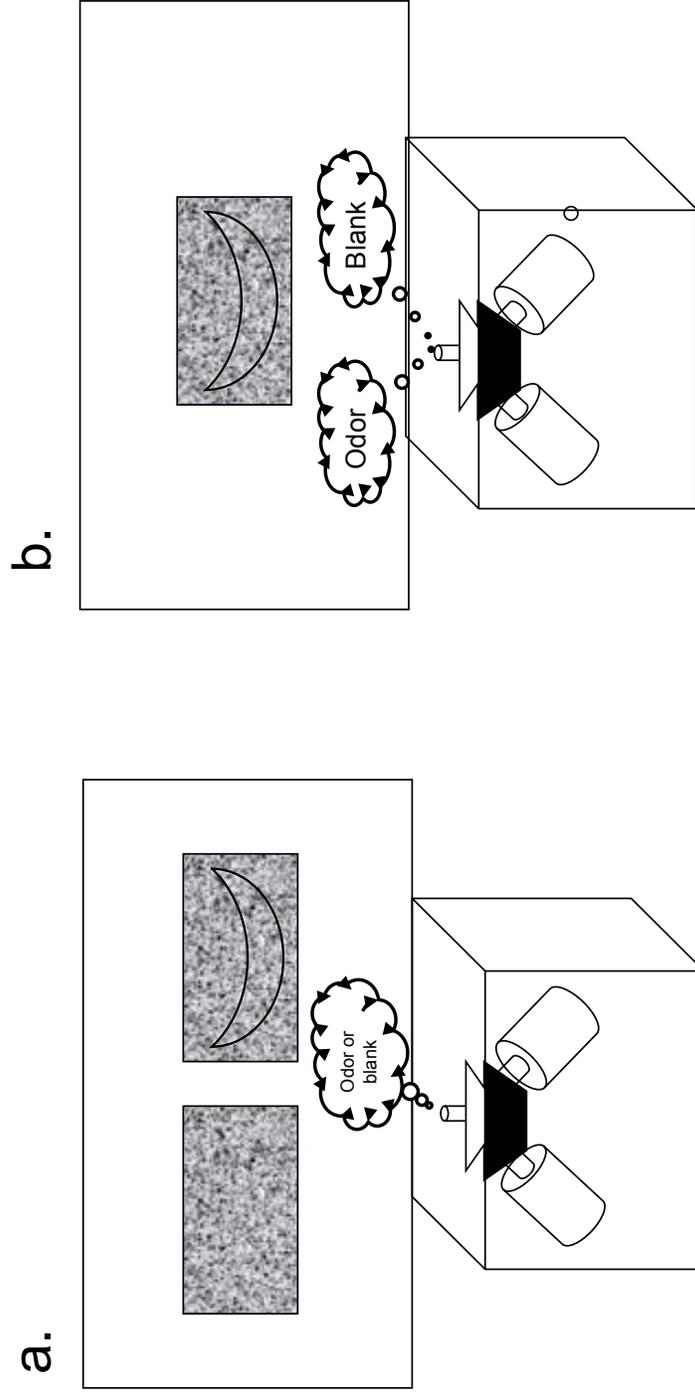


Figure 13 a and b: Set up of the two focus tasks conducted in experiment 1. a) Set-up of the visual focus (VF) task. The subject performs a visual detection task, in the presence of a single odor puff. b) the olfactory focus task (OF), the subject is presented with two simultaneous odor puffs, and must decide which puff contains the odor, while a single visual patch is presented.

calculated 0.75 detection threshold for each panelist based on their performance on the olfactory and visual threshold tests. Olfactory and visual stimuli were presented in both congruent (c) (cherry image and benzaldehyde; banana image and iso-amyl acetate) and incongruent (i) tests (cherry image and iso-amyl acetate; banana image and benzaldehyde). Congruent and incongruent manipulations were tested separately. The different test conditions are illustrated in Tables 2 and 3. Table 2 outlines the different test conditions in the VF task, while Table 3 outlines the conditions presented in the OF task. There were two basic tests conducted, OF and VF, both of these tests were conducted for the both the congruent (c) and incongruent conditions (i). Thus there were a total of eight test conditions, four of these focused on banana (B) and four focused on cherry (Ch): cBOF, cBVF, iBOF, iBVF, cChOF, cChVF, iChOF, iChVF. Thus each panelist completed a total of 8 test sessions.

All programs for testing the VF and OF tasks were written in PsychoPy the schemes for the VF and OF tasks are illustrated in Figure 14 illustrates the testing schemes written in PsychoPy for the VF task, and Figure 15 illustrates the testing scheme for the OF task written in PsychoPy. To begin each test session, adjustments to chair height were made ensuring panelists viewed the visual images at the proper visual angle and received a direct puff of air from the olfactometer. All testing occurred in the smell isolation room, panelists wore Bose noise-cancelling headphones during all testing to isolate noise.

Table 2: Stimuli presented in the visual focus condition. The top row defines the stimuli in the visual detection task, the odorants presented were either congruent, incongruent, or no odor (PEG) for a control.

<b>Visual Task</b>		
	Banana + Noise vs. Noise	Cherry + Noise vs. Noise
Congruent	iso-amyl acetate	benzaldehyde
Incongruent	benzaldehyde	Iso-amyl acetate
Control	PEG	PEG

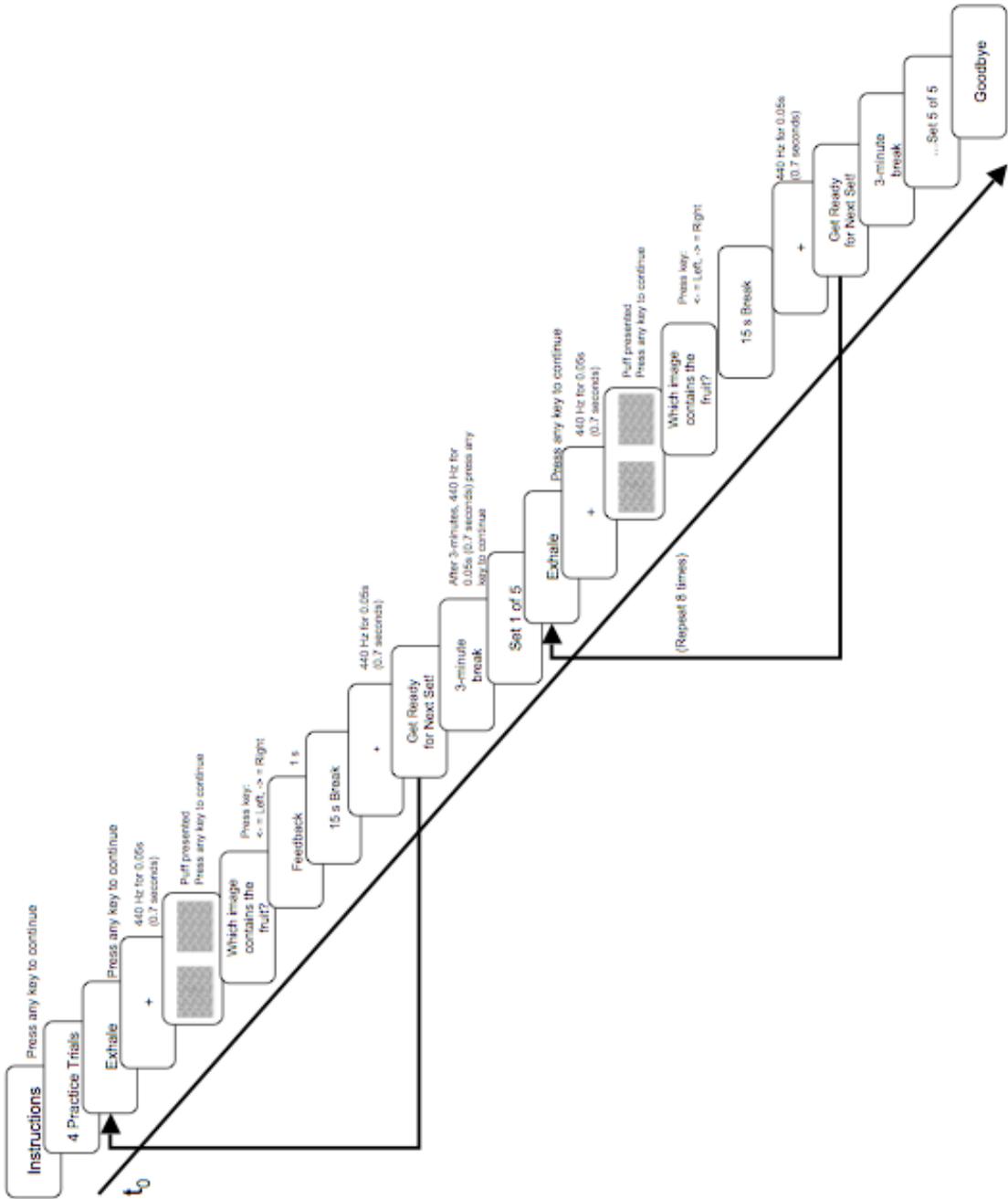


Figure 14: Visual focus task testing scheme.

Panelists listened to white noise during testing and received 440 Hz tone alerting them when to sniff. Each testing session began with a set of instructions alerting the panelist to the type of task he or she would complete. The PsychoPy programs delivered instructions to panelists, instructing them when to inhale and exhale, to ensure breathing be as controlled across panelists as possible. For both OF and VF tasks each test began with a presentation of the reference stimuli as well as a set of five practice trials with direct performance feedback, serving as both a warm-up trial as well as to gauge performance, if further adjustments were necessary.

As in the prior tests a 440 Hz tone along with a focal crosshair indicated the delivery of each stimulus. Across all testing timed 15-second timed breaks separated each trial presentation, and 3-minute timed breaks after the presentation of a set (each set consisted of eight trials, four test trials and four control trials). For both the OF and VF tasks a total of five test sets were evaluated during a single testing session excluding the practice trials. Thus a total of 24 test trials and 24 controls trials were tested per session.

### *3.5.3 Procedure for VF Task*

Panelists conducted a two-alternative forced choice visual masking procedure using the mask of the panelist at their calculated level of the 0.75 detection threshold. Simultaneous with the presentation of the visual two-alternative forced choice procedure were timed presentations of single puffs of odor.

Panelists were instructed when to inhale and exhale through instructions on the screen as well as 440Hz tones along with the focal crosshair signal. During the VF task, panelists were told to evaluate the two images on the screen, choosing the noise+target image while sniffing from the olfactometer. The test condition consisted of an odorant presented above the panelist's calculated level of detection, at the 0.75 level, the control condition presented the panelist with a puff of PEG (blank). For each trial the panelist was told to choose the visual image containing the fruit outline, by pressing the right and left arrow keys. The control condition served to measure whether the level of detection for the visual threshold changed during the test condition. Testing lasted approximately 20 minutes.

#### *3.5.4 Procedure for OF Task*

The OF task required panelists to complete a similar task to the olfactory threshold task; however, simultaneous with the presentation of the two puffs of air, panelists were presented with a single visual image in the center of the screen. All visual and olfactory stimuli were presented at the panelist's calculated 0.75 detection threshold. Instructions and 440 Hz tones along with a cross-hair visual stimulus alerted the panelist to the delivery of a puff of air. Instructions were presented on the screen indicating when the panelist should inhale and exhale in order to regulate breathing across panelists. Panelists were asked to respond to whether the first or second puff of air contained an

Table 3: Stimuli in the olfactory focus task. The top row indicates the olfactory task performed, the visual stimuli are presented in the rows, congruent, incongruent, and control. Visual noise was presented alone in the control condition.

<b>Olfactory Task</b>		
	Benzaldehyde vs. PEG	Iso-amyl Acetate vs. PEG
Congruent	Cherry + Noise	Banana + Noise
Incongruent	Banana + Noise	Cherry + Noise
Control	Noise	Noise

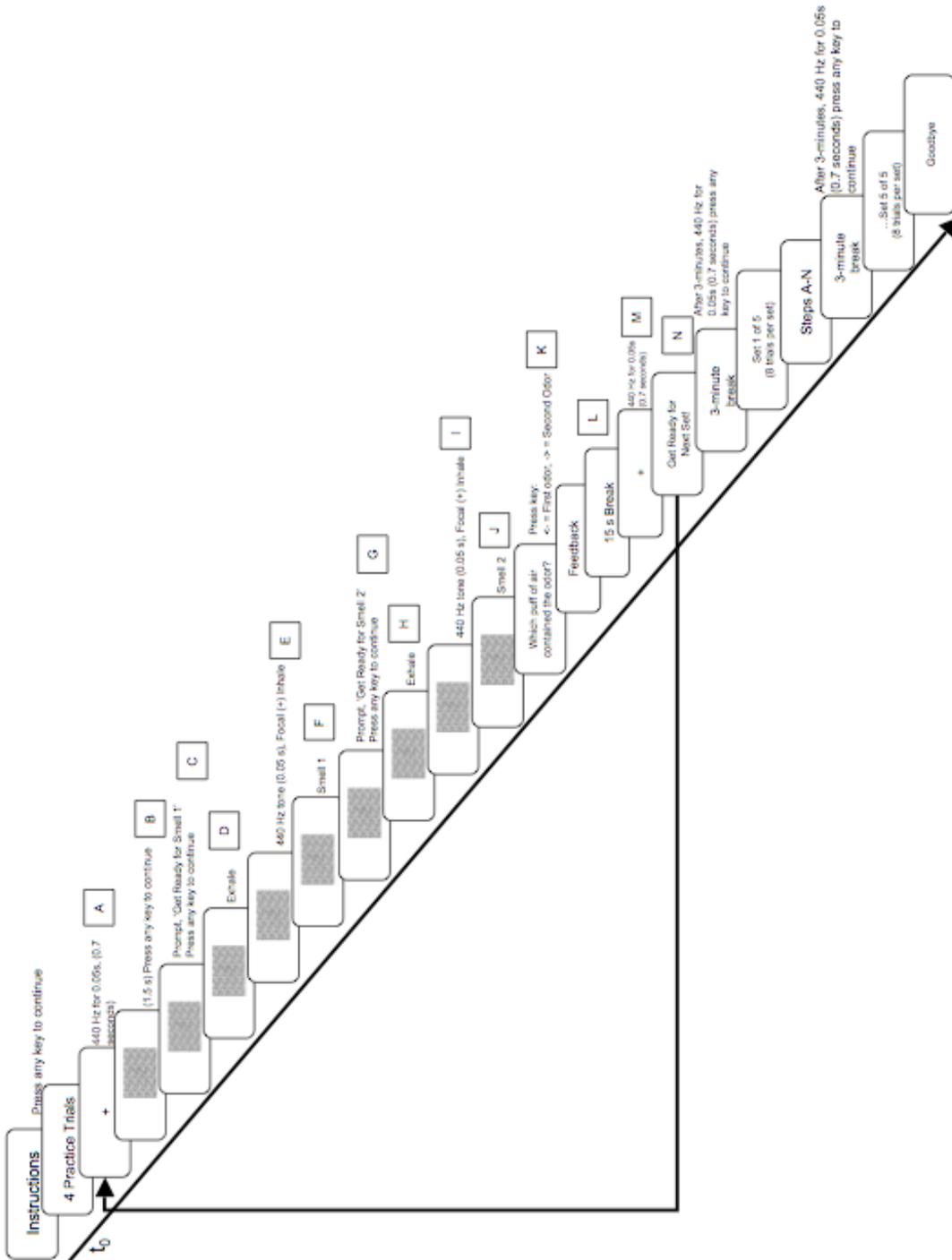


Figure 15: The test scheme for the olfactory focus task.

odorant. As in the VF task, each test sessions contained five test sets and one practice set, with each set containing eight randomized trials, four test trials and four control trials. During each trial an odorant was always presented; however, the order of presentation was randomized across each trial. During the control condition, noise alone was presented in the visual image, rather than noise + image. The tested whether the olfactory threshold remained at the 0.75 calculated detection threshold level. Between each trial the panelist rested for a timed 15-second break, with a 3-minute timed break between each test set. Testing lasted approximately 20 minutes. Like in the VF task, 20 test trials and 20 control trials were collected.

### *3.5.5 Data Analysis for Experiment 1: VF and OF Tasks*

Data for the OF and VF tasks were analyzed separately. Mean percent correct values and associated uncertainty intervals were calculated for each condition type. Figures 17-20 incorporate significance tests of differences between means using the uncertainty intervals of Andrews et al. (1980)  $\alpha=5\%$  least significant intervals (LSI) were calculated as

$$L = \bar{x} \pm \frac{t_{\alpha/2,v} \sqrt{2s^2/n}}{2}$$

Where  $\bar{x}$  is a mean  $t_{\alpha/2,v}$  is the upper  $\alpha/2$  point of Student's  $t$  distribution on  $v$  degrees of freedom,  $s^2$  is the error mean square from the analysis of

variance, and  $n$  is the number of observations contributing to each mean (Andrews et al. 1980). The least significant intervals include the error mean square from analysis of variance, thus they are more conservative than confidence intervals. Two means significantly differ if their least significant intervals do not overlap. One way ANOVAs were performed for the both the OF and VF data.

## **3.6 Experiment 2: Methodology**

### *3.6.1 Panelists*

Six men and four women completed the crossmodal task for this study, with an average age of  $30.5 \pm 10$  years. Testing consisted of six training sessions and four test sessions. All protocols were approved by the IRB. The testing protocol remained the same as in the OF task, except some visual stimuli were in color.

### *3.6.2 Materials*

Visual stimuli included black and white noise, used in the Part 1 of the experiment, as well as the two black and white fruit outlines. Color transforms of the noise patterns were made, these color masks were also applied to the two fruit outlines. A transform was performed on the black and white noise pattern to create a red and yellow noise pattern. The red noise mask had a RGB value of  $R=255, G=0, B=0$  and  $H=0, S=100, V=100$ . The yellow noise

mask had a RGB value of R=255, G=255, B=0, H=60, S=100, V=100. These same color transforms were applied to the black and white fruit outlines. All visual stimuli were presented at fixed levels of opacity. Noise stimuli were presented as follows: black and white noise 85%, red noise 88%, yellow noise 93%. Black and white fruit outlines were presented as: banana 85% and cherry 85%. Color noise masks were created of the fruit outlines: red banana 88%, yellow banana 93%, red cherry 88%, yellow cherry 93%. These levels were chosen based on the ability to recognize the visual stimulus. The same concentration levels and preparation used to determine the olfactory thresholds in part 1 were used in part 2.

### *3.6.3 Experiment 2: Method*

Panelists completed an OF task as described above as well as the VF task. The range of visibility for the stimuli presented in the VF task ranged from 88%-97% occlusion by the mask. A short test in order to assess whether visual stimuli were at the level of recognition was devised. Cherry and banana stimuli with visual masks ranging from 92-94 were presented in a randomized order, where each image was presented a total of 6 times. Panelists had to press 'b' on the keyboard if he or she saw a banana and 'c' if he or she saw a cherry. The accuracy of response indicated the ability to recognize as well as distinguish the two visual stimuli.

Table 4: Stimuli tested in experiment 2. Benz= benzaldehyde, IA= iso-amyl acetate. The + = when the condition was presented. The Top rows indicate the visual stimuli presented during the olfactory detection performance task.

		<b>Olfactory Task</b>										
		Visual Stimulus										
		Banana + Noise			Cherry + Noise			Noise				
		B/W	Red	Yellow	B/W	Red	Yellow	B/W	Red	Yellow	Red	Yellow
Benz					+	+	+	+	+	+	+	+
I-A	+	+	+	+								

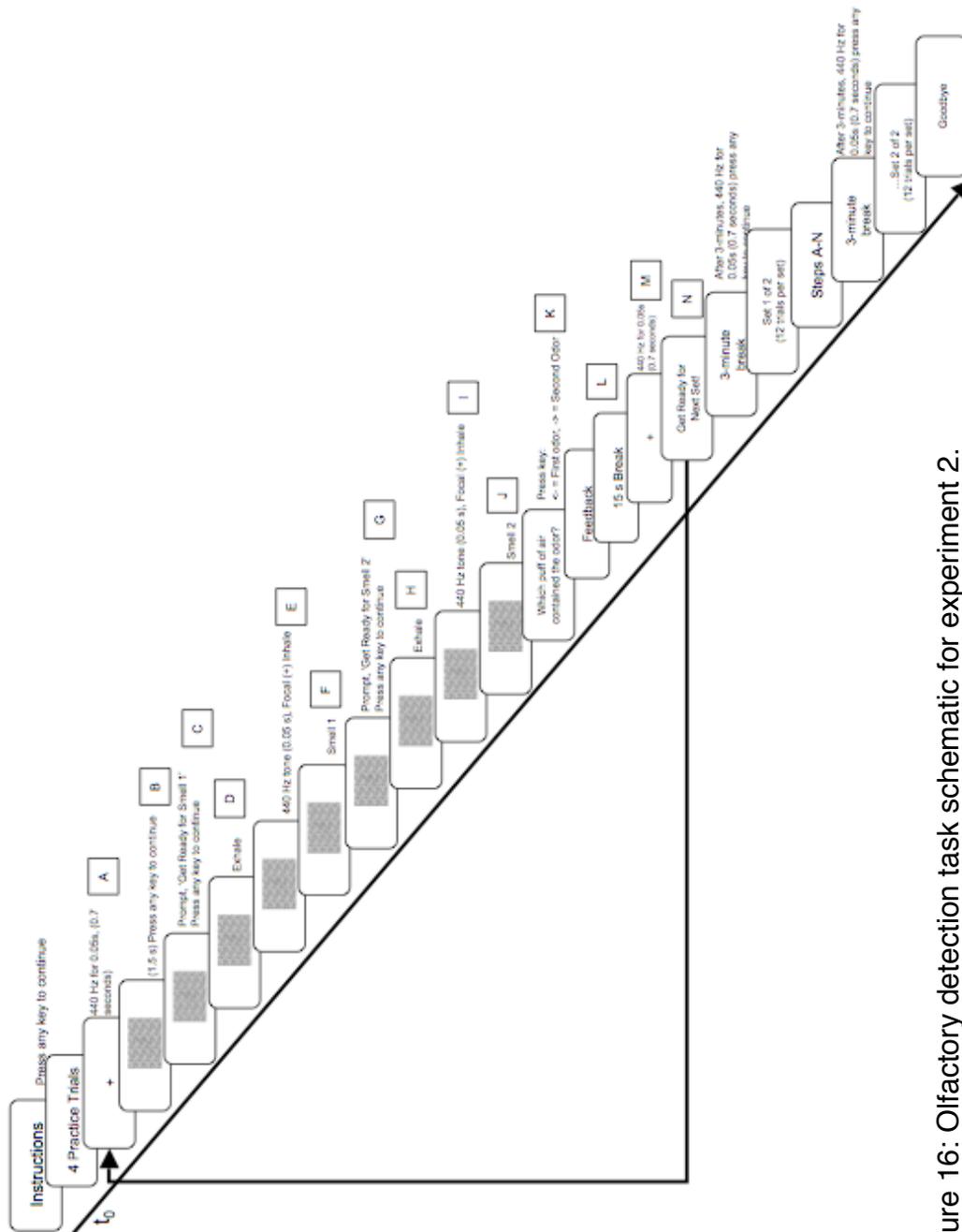


Figure 16: Olfactory detection task schematic for experiment 2.

For the OF task, stimuli presentation were congruent i.e. benzaldehyde odor was presented with cherry images and iso-amyl acetate was presented with banana images. All visual images were created in GIMP. Visual stimuli were noise (black and white), noise (yellow), noise (red), banana + noise (black and white), banana + noise (yellow), banana + noise (red), cherry + noise (black and white), cherry + noise (yellow), and cherry + noise (red). The testing conditions are outlined in Table 4. The OF task proceeded as described above. The testing scheme is illustrated in Figure 16. There were four practice trials, with direct feedback after each response, followed by two test sets. Each test set contained 12 trials, six trials control trials and six test trials, a 15-second timed break occurred between each trial presentation, and a 3-minute timed break separated the set presentations. Testing lasted approximately 20 minutes.

#### *3.6.4 Data Analysis for Experiment 2*

As in section 3.5.5 data were analyzed using LSIs. Data were analyzed by the effect of color on olfactory detection and the influence of shape and color on the olfactory detection task (Figures 19 and 20). Mean percent correct values and associated uncertainty intervals were calculated for each condition type. One-way ANOVAs were performed for the both the color and color and shape conditions.

## CHAPTER 4

### RESULTS

#### **4.1 Congruency Test: Results**

Results of the congruency test were analyzed to determine the stimuli to be used for the crossmodal tasks. Panelists placed the game pieces on the cherry outline when presented with benzaldehyde 88% of the time, followed by banana and iso-amyl acetate 79.5%, grape and methyl anthranilate 78.2%, and lastly lemon and octanal (72%). Table 5 reports the frequency counts of game piece distribution as well as the upper and lower 95% confidence limits (Goodman, 1965). Based on these results the two test stimuli chosen were the outline of the banana and the outline of the two cherries. The two olfactory stimuli were benzaldehyde and iso-amyl acetate.

#### **4.2 Experiment 1: Results**

Figures 17 and 18 represent the findings from the VF and OF tasks. In fig.17, plots the mean percent correct performance in the visual task, while presented with either a congruent or incongruent olfactory stimulus. The mean percent correct for visual detection performance in the congruent condition remained around 60% performance, in both the control and test conditions. The visual stimuli presented to each subject were at the calculated 75% level of detection, thus at the perithreshold, performance did not vary greatly from this

Table 5: Results of Congruency Test. Bolded frequency counts represent when the odorant and shape are significantly different from the other pairings as a result of the 95% confidence limits (B=banana, C=Cherry, L=Lemon, G=Grape). The strongest odorant and shape association is for iso-amyl acetate and banana, followed by benzaldehyde and cherry, the two pairings chosen for testing in the crossmodal tasks.

Odorant	Benzaldehyde				Iso-amyl acetate				Methyl anthranilate				Octanal			
	B	C	L	G	B	C	L	G	B	C	L	G	B	C	L	G
Shape	16	<b>179</b>	17	13	<b>198</b>	7	15	5	8	33	8	<b>176</b>	25	24	<b>162</b>	14
Count	28.45	192.2	26.68	24.72	207.9	16.87	27.22	14.07	18.22	48.39	18.22	189.6	39.21	38.04	177.3	25.97
Upper Limit	8.75	162.19	9.47	6.65	183.5	2.83	8.04	1.73	3.42	21.89	3.42	158.9	15.51	14.73	144.0	7.34
Lower Limit																

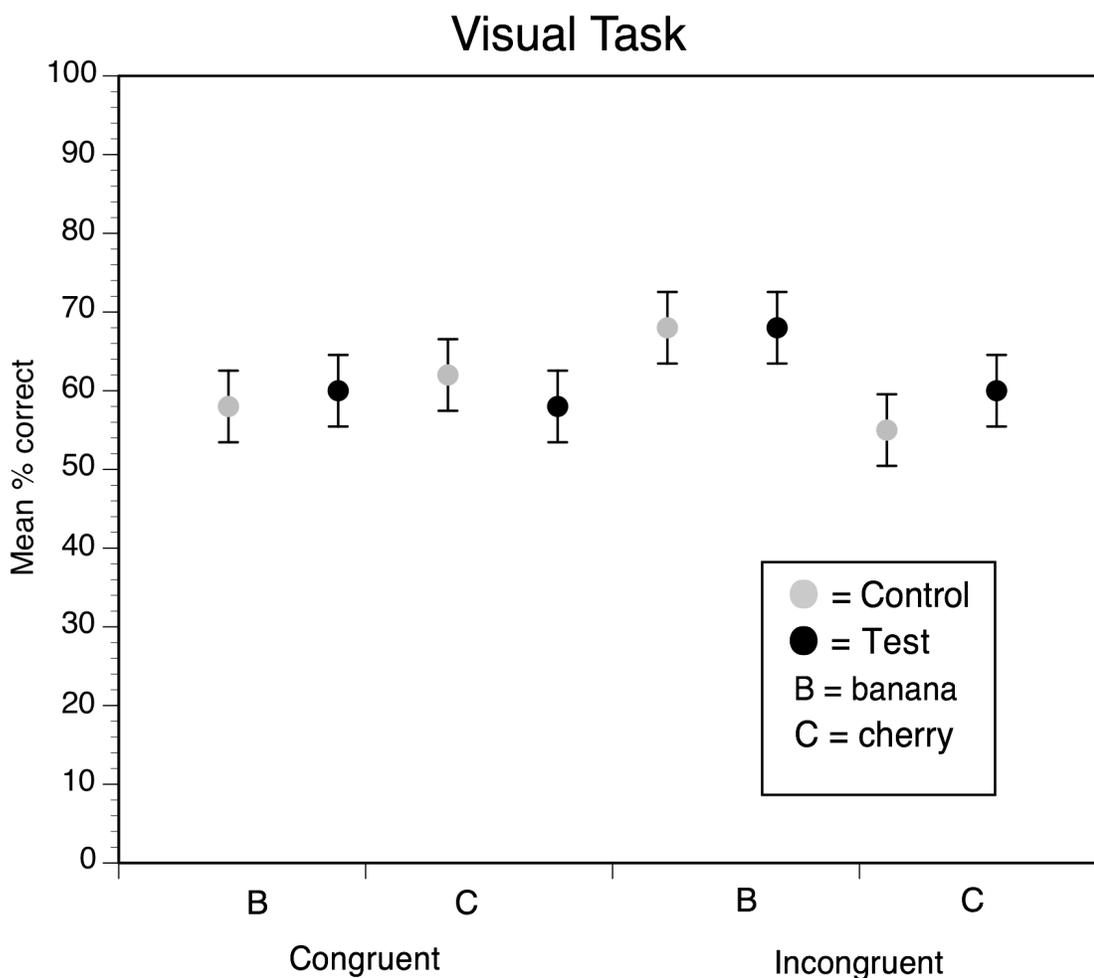


Figure 17: Results from VF Task. Influence of the presence of an olfactory stimulus, while performing a visual detection task. Mean percent correct for performance in the visual task is plotted against the congruency of the olfactory stimulus. Grey circles represent the control condition and black circles the test condition. The whiskers indicate the 95% LSIs based on pooled error from the ANOVA. In the control conditions, PEG is presented. In the congruent test conditions iso-amyl acetate is presented while evaluating a banana, benzaldehyde while evaluating the cherry image. In the incongruent condition, benzaldehyde is presented while evaluating the banana visual stimulus and iso-amyl acetate is presented while evaluating the cherry visual stimulus.

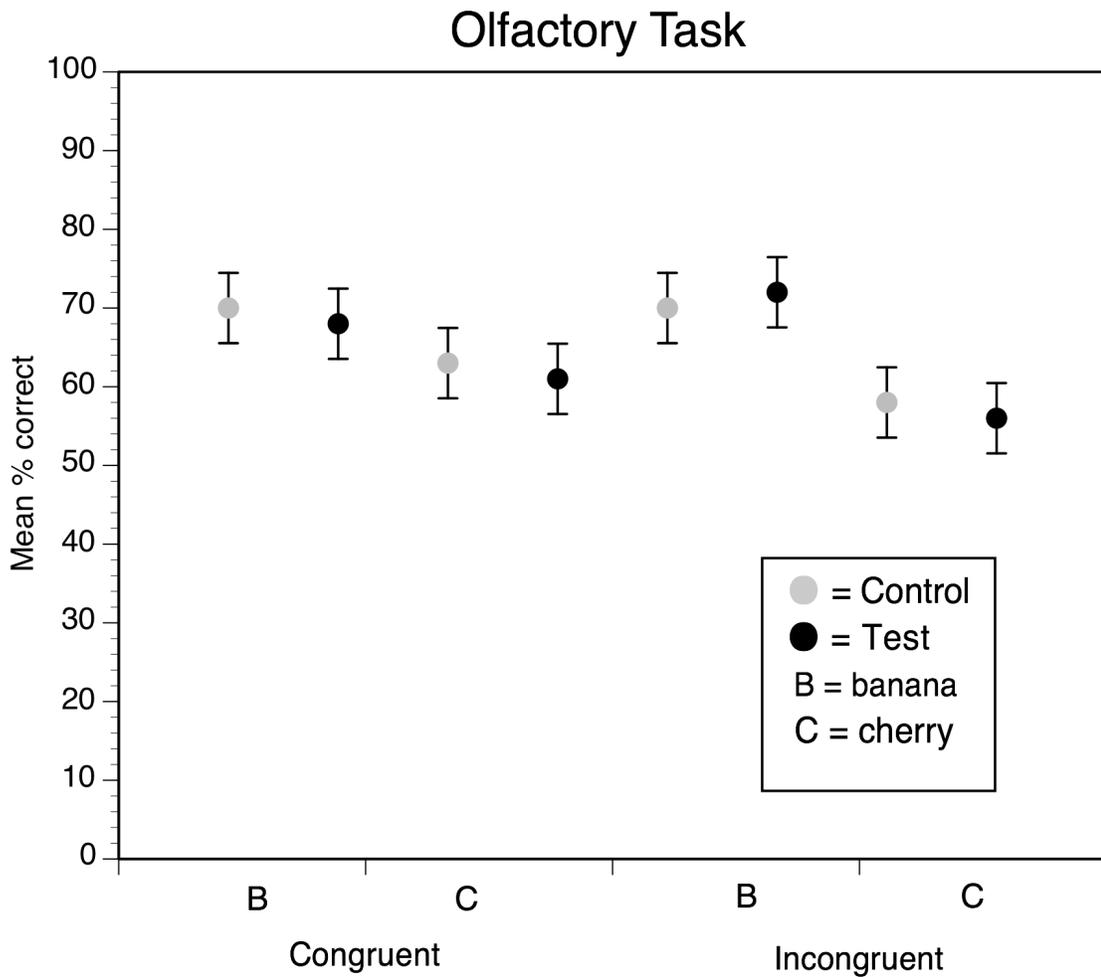


Figure 18: Results from OF Task. The influence of perithreshold shape on olfactory detection performance. Percent correct performance in the OF task is plotted against the congruency of the condition. Grey dots represent the control condition, and black dots represent the test condition. The whiskers indicate the 95% LSIs based on pooled error from the ANOVA. In the control condition, a noise patch is presented. In the congruent task, a banana figure is displayed in the presence of iso-amyl acetate, a cherry figure in the presence of benzaldehyde. In the incongruent conditions, iso-amyl acetate is evaluated while looking at cherries, and benzaldehyde is evaluated while looking at a banana.

level. The consistency of performance between the control and test conditions, indicates the drop in percent detection is likely due to the difficulty of the visual task. When presented with an incongruent olfactory stimulus, the performance in the visual task is slightly lower for overall in the cherry incongruent condition for both the control and test condition (in the presence of iso-amyl acetate),  $F(7,1752) = 2.05, p < 0.05$ .

Figure 18 represents the performance in the OF task. The mean percent correct values, lie around 70%, very close to the perithreshold levels calculated for each subject. The olfactory detection performance for benzaldehyde was lower overall; however, in the congruent condition, as indicated by the overlap in the LSIs, this difference is not statistically different from the performance in the iso-amyl acetate detection task. Performance in the incongruent olfactory task condition, where iso-amyl acetate was evaluated in the presence of a cherry and benzaldehyde in the presence of a banana, performance is significantly different in both the control and test conditions of the iso-amyl acetate odor condition from all other evaluations. However, since the control (evaluation of iso-amyl acetate in the presence of black and white noise) does not significantly differ from the test condition.

### **4.3 Experiment 2: Results**

Figures 19 and 20 represent the findings from experiment 2. Figure 19 plots the mean percent correct performance in the olfactory task in the presence of

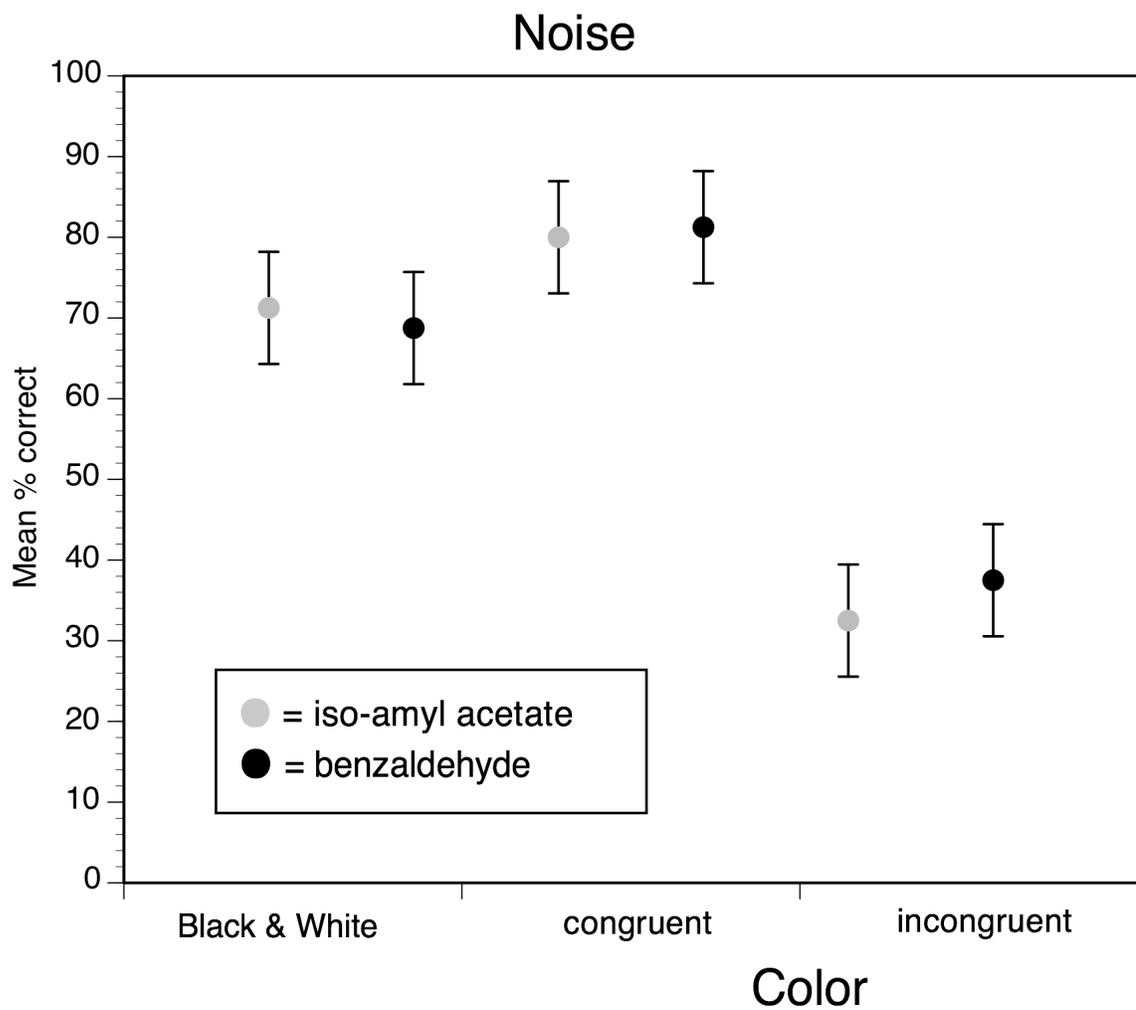


Figure 19: The effect of color on olfactory performance. Mean percent correct in the OF task is plotted against the color of the noise patch. The grey dots represent iso-amyl acetate evaluation and the black dots represent benzaldehyde odor evaluation. The whiskers indicate the 95% LSIs based on pooled error from the ANOVA. Congruent color is yellow while evaluating iso-amyl acetate and red while evaluating benzaldehyde. Incongruent color is red while evaluating iso-amyl acetate and yellow while evaluating benzaldehyde. Color strongly influences the ability to perform in the olfactory detection task.

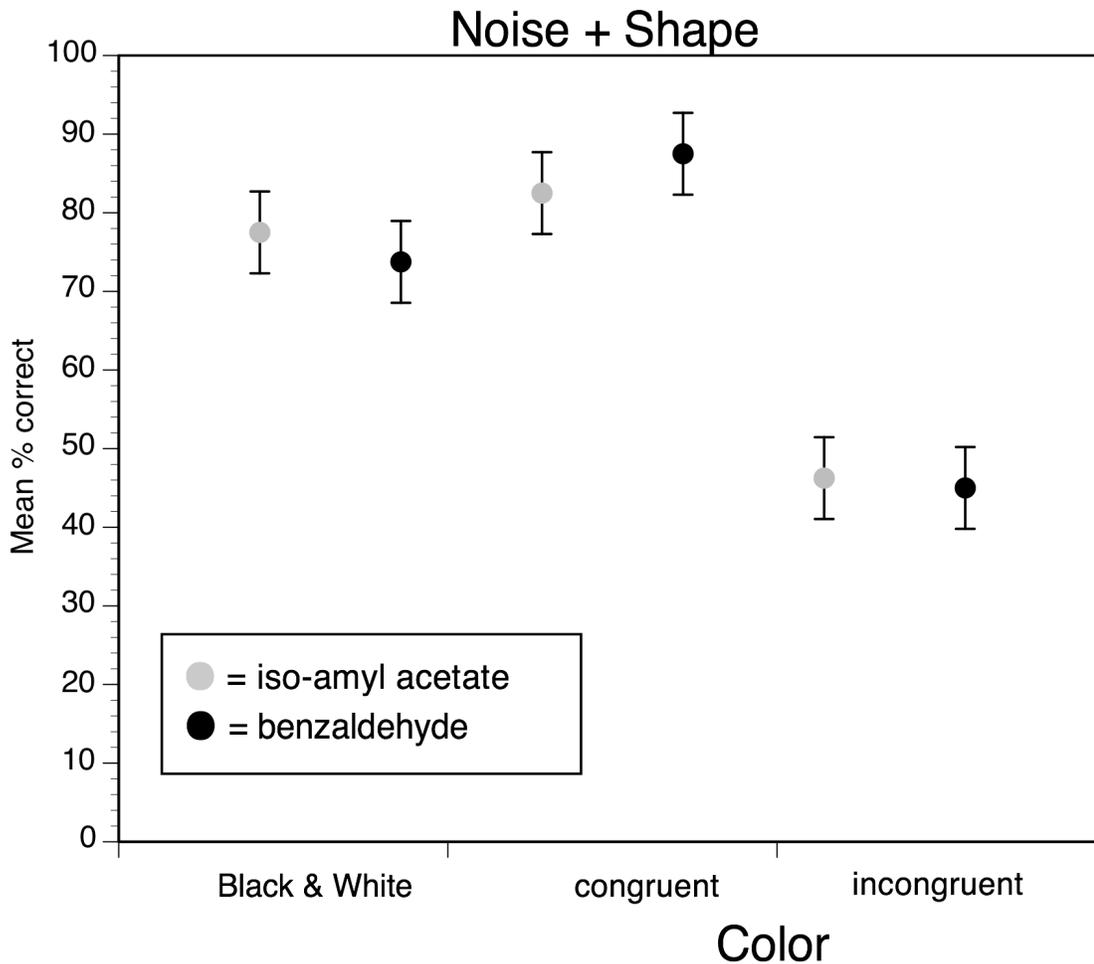


Figure 20: The influence of color and congruent shape. Mean percent correct in the olfactory task is plotted against the effect of color. Grey dots represent iso-amyl acetate evaluation and black dots represent benzaldehyde evaluation. The whiskers indicate the 95% LSIs based on pooled error from the ANOVA. Congruent color is yellow while evaluating iso-amyl acetate and red while evaluating benzaldehyde. Incongruent color is red while evaluating iso-amyl acetate and yellow while evaluating benzaldehyde. The effect of congruent shape is not as strong as the impact of color.

visual noise, presented in black and white, yellow and red. Performance In the black and white visual condition, is around 70% detection, there is no significant difference in the detection performance of iso-amyl acetate and benzaldehyde. When congruent color is presented during the olfactory detection task, yellow in the presence of iso-amyl acetate evaluation and red in the presence of benzaldehyde odor evaluation, performance rises to 80% detection. However, these results are not significantly different than in the presence of a black and white noise pattern. Olfactory detection performance decreases to approximately 35% detection for both iso-amyl acetate and benzaldehyde when in the presence of an incongruent noise stimulus. Thus a yellow patch decreases performance in the benzaldehyde detection condition and a red patch decreases olfactory detection performance of iso-amyl acetate,  $F(5, 474) = 18.35, p < 0.001$ .

Figure 20 plots the influence of color and shape on olfactory detection performance, when the visual stimulus is presented at the level of recognition. Olfactory detection performance when presented with a black and white noise pattern over a congruent object shape, yielded performance close to 80% detection. The addition of a congruent color mask to the congruent fruit shape, increased olfactory detection in both the iso-amyl acetate and benzaldehyde conditions to approximately 85% detection; however, this is not a significant change. Olfactory detection performance dropped significantly when an

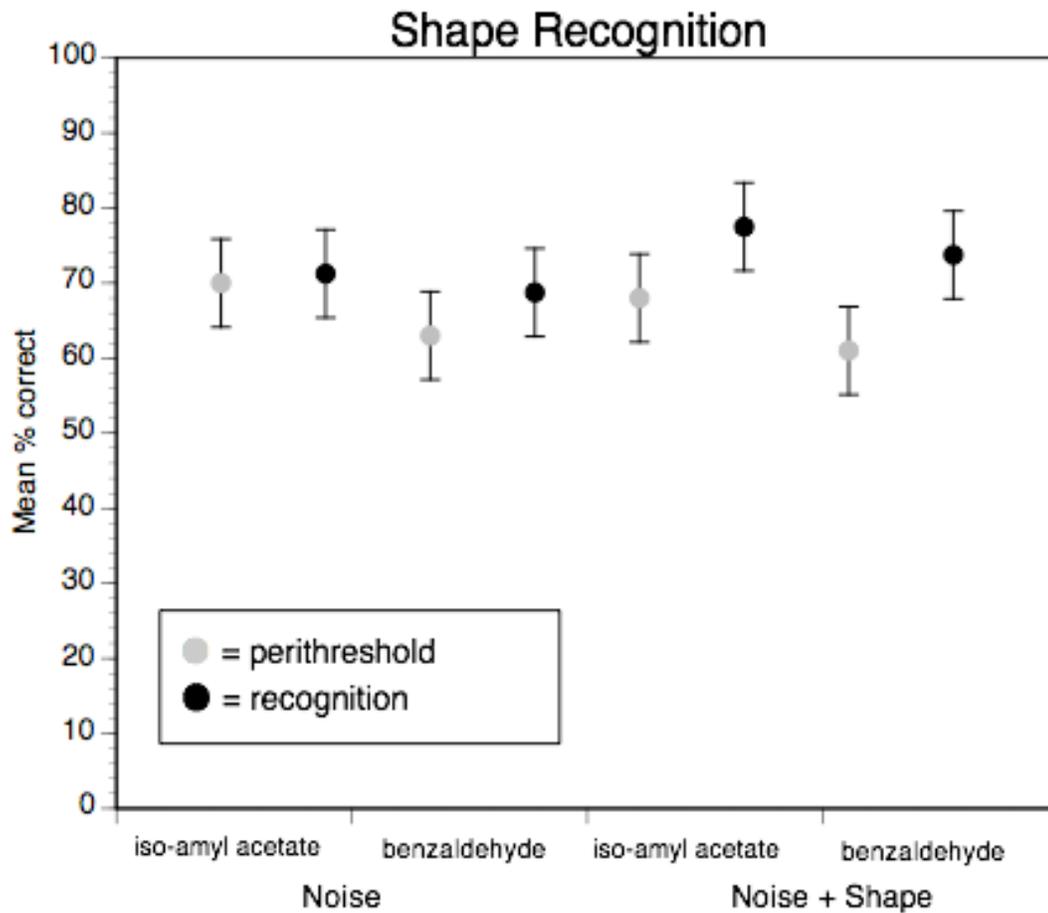


Figure 21: The Impact of a Recognizable Shape. The influence of visual object recognition on olfactory detection performance. The whiskers indicate the 95% LSIs based on pooled error from the ANOVA. The grey dots represent the evaluation while the visual image is presented at the perithreshold level, and the black dots indicate when the visual stimulus is presented at the level of recognition. There is a difference in olfactory task performance when presented with the congruent fruit outline at the level of recognition; however, this is not a significant change.

incongruent color mask occluded the fruit object, for both iso-amyl acetate odor detection and benzaldehyde odor detection,  $F(5, 474) = 24.41, p < 0.001$ .

#### **4.4 Combined Results for Experiment 1 and Experiment 2**

Figure 21, plots data from both experiment 1 and experiment 2, it compares olfactory detection task performance when presented with black and white noise from both experiment 1 and experiment 2 as well as olfactory task performance when presented with a congruent shape at both perithreshold (experiment 1) and at the level of recognition (experiment 2). Olfactory task performance remains around 70% detection for all black and white noise conditions as well as for perithreshold noise + shape conditions; however the noise+shape condition presented above the perithreshold, at the level of recognition in experiment 2, yields a significant difference in olfactory detection performance in comparison to the noise+shape presentation in experiment 1. Olfactory detection in the noise+shape recognition condition rose from  $68\% \pm 3.16$  (standard error) to  $77.5\% \pm 1.97$  in the banana outline condition, and  $61\% \pm 3.33$  to  $73.75\% \pm 4.95$  in the cherry outline condition. However, the calculated 95% LSI values indicate this is not a significant difference, thus likely due to differences in error and number of individuals tested the difference between performance due to visibility of the fruit outline did not yield a significant difference in olfactory performance.

## CHAPTER 5

### DISCUSSION

Flavor perception involves the integration of input from sight, smell, sound, taste, and texture. Both smell and vision are crucial to forming first impressions of a food. This research sought to investigate crossmodal interactions between olfaction and vision through a psychophysical approach. Prior research examining olfactory and visual interactions has investigated identification and hedonic ratings; however, this research sought to determine whether detection sensitivity could be altered through bimodal presentation in order to reveal new insight into flavor perception. From prior crossmodal olfactory visual experiments there is evidence that a visual stimulus can influence olfactory perception (Morrot, et al. 2001, Österbauer et al. 2005, Zellner, et al. 1991, Zellner and Kautz 1990, Engen 1972, Blackwell 1995, Davis 1981, Koza et al. 2005).

This research investigated the necessary parameters to be presented in one sense to result in a measurable change in detection performance in a different sense. Two different olfactory stimuli were tested and several different visual manipulations were assessed including: object shape, color, and visibility. Two different attention tasks were used to measure crossmodal interactions: The olfactory attention task (OF) measured whether a visual

stimulus could influence olfactory detection performance, while the visual focus task (VF) measured whether an olfactory stimulus could influence visual detection performance. All tasks in this first set of experiments were conducted at slightly above the calculated level of detection, at the 0,75 response level. Performance in a crossmodal tasks were compared to the performance in a unimodal task. Neither the results in the OF nor the VF task show measurable change in performance from the crossmodal condition to the unimodal condition. Figure 18, illustrates the findings from the OF task. The performance of both benzaldehyde and iso-amyl acetate detection remained constant, regardless of whether the subject viewed black and white noise (control), the congruent visual stimulus, or incongruent visual stimulus. This finding suggests that the simultaneous presentation of perithreshold black and white shape objects does not alter the performance in the olfactory detection task.

### **5.1 Experiment 1 Findings Explained**

Due to the consistency of performance, across both the unimodal and crossmodal conditions simultaneous presentation of a visual cue while performing an olfactory performance task did not disrupt nor enhance visual processing. There is also the possibility that the nature of the visual task does not allow for a crossmodal disruption or enhancement to occur. It is also likely that the strength of the visual stimuli must be stronger to result in a

measurable change. Due to the attention-related resources being allocated to the olfactory detection task a question requiring greater overall processing integration might have yielded a different response. If the task required the individual to evaluate a hedonic measure as well or choose from a list of fruits that best fit the odorant smelled rather than just performing a detection task there would have been a more measurable change due to the higher level of processing integration needed when performing these other types of tasks.

Figure 17 illustrates the results of the VF task. Like the findings in the OF task, there were no significant differences between in the visual detection task in either crossmodal (test) condition or unimodal (control) condition. Performance in both the detection of the banana stimulus and cherry stimulus remained around 60% performance. Performance in the visual detection task did not change from the control condition where the panelist was presented with a blank puff or in the test conditions. Within the test conditions, neither the simultaneous presentation of a congruent odor nor the presentation of an incongruent odorant resulted in a measurable change in the visual detection task.

Like the results of the OF task, it is possible the nature of the task does not enable for sensory disruption. If the task involved greater integration across the senses, rather than full attention of visual resources, there could be a measurable change. The task asks the subject to choose the visual patch with the fruit, if the task required the subject to perform a visual search

amongst several other visual fruit outlines, perhaps it would result in a different type of interaction (Seigneuric et al. 2010).

Taken together the results of Experiment 1, both the olfactory task condition and visual task condition, show that crossmodal stimulation at the perithreshold does not influence the performance of the task being attended to. Thus the addition of sensory information did not improve or decrease performance. One theory is the signal strength from the crossmodal stimuli did not interfere with the performance in the detection task. This would suggest that if a signal presented above the perithreshold level were presented simultaneously with the detection task, performance might change. It could also be possible that these two processing paths do not cross in a way in which cognitive interference occurs.

## **5.2 Cognitive Interference in Crossmodal Processing**

Cognitive interference, a term commonly found in cognitive psychology, refers to the measurable change in response due which occurs when the processing of a stimulus is interrupted by the processing of another stimulus. This can result in a decrease in performance as well as measurable changes in reaction time due to the disruption in processing (Stroop 1992, MacLeod 1991, White and Prescott 2007, Djordjevic et al. 2004, Kane and Engle 2003). One of the most well known examples of cognitive interference is the Stroop Task. In this task an individual is presented with a word, spelling the name of a

color; however, the color of the letters are different than the word, e.g. the word 'RED' in blue type-face. The subject is instructed to name the color of the typeface, not the word. When the typeface color is the same as the word (congruent) subjects perform with better accuracy and at faster speeds than when presented with the incongruent condition. This is observed effect is known as Stroop interference (Stroop 1992, MacLeod 1991). There have been many different variants on the Stroop task (for a review please see: (MacLeod 1991)); two tests even used Stroop like tasks to evaluate the influence of odor and taste on word evaluations (White and Prescott 2007, Pauli et al. 1999).

### **5.3 Experiment 2: Increasing Signal Strength**

One possibility for the lack of measurable change in experiment 1 could have been due to the strength of stimuli used or the type of task not creating interference. In order to test whether signal strength could alter performance, experiment 2 visual stimuli were presented at the level of recognition.

Experiment 2 only evaluated the influence of a visual stimulus on the olfactory detection task. In addition to increases the strength of the visual stimulus to the level of recognition, red and yellow noise patterns were used to test the role of color. The olfactory stimuli remained the same; all olfactory tasks were again performed at the calculated level of detection. By presenting the olfactory detection task at the level of detection and the visual stimulus at the level of recognition the change in olfactory performance due to a stronger

visual signal could be measured. Black and white stimuli were presented in addition to, yellow noise, red noise, and fruit outlines occluded by red and yellow noise patterns. In this experiment, object shape was always congruent with the olfactory task; the cherry outline was presented in the presence of benzaldehyde and the banana outline in the presence of iso-amyl acetate. Figure 19 plots the robust effect of color on olfactory perception. When the black and white noise condition (unimodal) is compared to the olfactory detection performance of bimodal congruent color presentation and incongruent color presentation, it is clear that presence of incongruent color while performing the olfactory detection task resulted in a significant decrease in performance. This dramatic decrease in performance is best explained by both cognitive interference as well as literature on the profound role of expectation in forming our perceptions.

Figure 20 illustrates the effect of noise + shape. Figure 20 displays the same pattern of results as Figure 19; however, this plot shows how congruent object shape, paired with color influences olfactory detection. It is clear color is driving the performance in the olfactory task, because if shape were driving the task, the change would not be as dramatic since the object shape is congruent with the olfactory task. There is a slight overall increase in the performance with the presence of the shape; however, color appears to have a greater impact.

## **5.4 Findings from Experiment 1 and 2 Compared**

In order to determine the role of signal strength of an object's shape to influence performance in an olfactory detection task, results from the perithreshold crossmodal OF experiment conducted in experiment 1 were compared with the results of the findings in experiment 2, collected in the presence of a visual cue at the level of recognition. This comparison enabled us to determine whether a shape presented at the level of recognition is capable of altering olfactory detection performance, these findings are plotted in Figure 21. The results in this Fig. 21 illustrate the impact of increasing the visibility of the visual stimulus from perithreshold to detectable yielded a measurable and significant change in the performance in the olfactory detection task. Thus object shape does contribute to the crossmodal processing of olfaction; however, color is still a more powerful factor. However, due to differences in the number of times the stimuli were tested the pooled error between experiment 1 and experiment 2 yield a non-significant difference between olfactory performance when presented with a visual stimulus at the level of recognition when compared to olfactory detection performance when presented with a visual stimulus at the perithreshold level. Further testing with a larger number of sample repetitions and greater number of subjects could yield a significant finding

### *5.4.1 Differences in Congruency Reflected in Error*

It should be noted that in experiment 1 and experiment 2, there is a difference between detection performance error in benzaldehyde and iso-amyl acetate. Likely, this observation can be traced to the congruency test, where banana and iso-amyl acetate had the strongest association, while cherry and benzaldehyde followed. The difference in their associations is expressed in the variation in performance.

### *5.5 Experiment 2 Findings Explained*

Congruencies and expectations are learned associations. The effect of color to alter performance in crossmodal tasks is well documented, for a thorough review, please read (Spence et al. 2010). Through experience individuals learn color and food associations. Due to the element of experience, some associations may not be as strongly engrained as others (Lavin, Lawless 1998). When presented with a colored solution, there is an automatic association of the color with a flavor as well as a taste profile (Shankar, Levitan and Spence 2010). These associations vary across cultures. However, these associations profoundly inform the way in which consumers interact with foods. Many of these associations are gathered through implicit learning, where the simultaneous presentation of color and odor is learned through an intentional process (Dematte et al. 2006, Degel, et al. 2001, Degel, and Köster 1999, Stevenson and Boakes 1998).

The more often sensory stimuli are commonly presented together, the consumer expects when he or she sees a specific food it will taste and smell a certain way. When these expectations are broken there are changes in performance (Schifferstein and Spence 2008). Research in the area of expectation has focused on food liking and acceptability (Garber et al. 2001, Hutchings 2003, Cardello et al. 1985, Cardello 2003, Blackwell 1995, Schifferstein and Spence 2008, Zellner et al. 2004, Armand V. 1995, Frewer et al. 2001, Wilson et al. 1989). The findings have shown that when visual appearance and expected flavor differ there is a strong dislike and even rejection of the product. Researchers have used a combination of theories to explain this finding. All of these theories rely on the concept of expectation versus actual experience. These theories suggest, the greater the distance between the expectation of the experience and the actual experience, the greater the consumer disappointment (Cardello et al. 1985, Festinger 1962, Carlsmith and Aronson 1963, Scharf and Volkmer 2000, Anderson 1973, Hovland et al. 1957).

A well-cited explanation is the assimilated-contrast model, which a consumer based range of acceptability and rejection (Scharf and Volkmer 2000, Anderson 1973, Hovland et al. 1957). Essentially if expectation and acceptance vary slightly, the consumer will excuse the discrepancy and accept the product. However, if the discrepancy between expected and actual is too large, the product will be rejected. Perhaps, the dramatic decrease in olfactory

detection performance could have been due to the expectation of a different odor, prompted by the incongruent visual cue, leading to an interference reaction. The decrease in performance due to the presence of an incongruent color might be explained by the combination of the expectation model as well as the allocation of attention-related resources. The presentation of a red visual stimulus cues the attention-related resources of the subject to cherry odor, thus when iso-amyl acetate is presented, olfactory performance may decrease due to the unexpected nature of the odorant. Thus to due to the cognitive interference of expectation and resource allocation, there is a dip in olfactory performance in the presence of an incongruent visual cue.

The lack of measurable crossmodal interaction in experiment 1, suggests the need for one signal to be above the perithreshold level in order to yield a crossmodal interaction. Perhaps when both signals are presented at the perithreshold level attention-related resources from the focused attention task are not distracted from the task. This is contrary to mixed reports within the subliminal stimulation literature of reports of behavioral responses due to signals presented at the subliminal level (Bar and Biederman 1998, Smith and Rogers 1994, Theus 1994, Williams 1938, Li et al. 2007). In order to attend to a task, attention-related resources must be allocated, according to Wolfe and Bennet (1997) in order to actually see a stimulus, regardless of subliminal message or not, the individual must attend to it, thus if the visual stimulus cannot be seen or the individual does not know where the stimulus will be

projected, it would then be extremely unlikely that the visual stimulus would be processed and thus lead to a behavioral or measurable psychophysical response. Thus if the individual cannot see the stimulus, not only would the individual not process the visual information, there would logically not be a crossmodal or behavioral response.

It is well understood that consumers use visual information in order to assess foods before consumption. These visual assessments enable the consumer to create an image of the expected product experience. Color as well as label information strongly inform these perceptions (Shankar et al. 2009, Yeomans et al. 2008, Wansink et al. 2005, Herz, von Clef 2001 and Wansink et al. 2000, Cardello et al. 1985, Zellner et al. 2008, Cardello 2003, Zellner et al. 2004, Cardello 1995, Vazquez et al. 2009, Zampini et al. 2007). Thus understanding the roles of color, object shape, and strength of the visual signal and its ability to influence olfactory perception is important in order to better understand consumer preference formation. Understanding that olfactory sensitivity might be influenced by visual information should be understood by the product developer when creating packaging and labeling information due to its implicit influence on overall hedonic acceptance as well as evidence for influence of the sensitivity of sensory information processing.

## **5.6 Further Areas for Research**

This research only begins to examine the many possible psychophysical interactions, which can be observed between olfaction and vision. This research investigated the effect on detection performance when simultaneously presented with a bimodal stimulus. However, it would be worthwhile further researching the influence of timing, such as cue on olfactory detection. As well as further testing the limits on the necessary strength of a crossmodal stimulus to yield a measurable interaction. Further investigation into the role of shape is crucial to understanding how visual appearance can alter the consumer's experience. Additionally, understanding how labeling, an already burgeoning area of research, can influence the olfactory experience is also crucial to understanding how the consumer experience can be changed even before consuming the product.

## CHAPTER 6

### CONCLUSIONS

This research sought to determine a consistent method for measuring crossmodal olfactory and visual interactions using psychophysical methods. The experiments were designed in order to better understand olfactory and visual interactions to further investigate flavor perception. The first experiment tested whether olfactory and visual crossmodal interactions could be observed when bimodal stimulus stimulation occurred at the calculated perithreshold level. These experiments assessed whether a black and white object shape outline could influence the perception of an olfactory stimulus or whether an olfactory stimulus could influence the detection of an object shape. No measurable changes were observed for bimodal versus unimodal presentation. This suggests regardless of the attention task or stimulus congruency that crossmodal olfactory / visual interactions do not occur at the perithreshold.

Experiment 2 measured the influence of visual stimulus presentation at the level of recognition; shapes were presented as well as color. In this experiment measurable changes between the presentation of bimodal versus unimodal stimulation occurred. Presentation of incongruent color most strongly influenced olfactory detection performance. The change in olfactory performance due to the presentation of color, suggests attention-related

resources are strongly allocated to the visual information resulting in interference in olfactory processing. Furthermore the presence of a recognizable congruent object shape resulted in a change in olfactory sensory performance; however, not to the extent of color.

Overall these findings highlight the importance of visual information in forming expectations and cognitive associations. The visual information a consumer uses in order to evaluate novel products is based on expectations and associations from experience. Although new color and odor associations are made through implicit memory, working memory is associated with confirming the familiarity and thus acceptability and pleasantness of the experience. When introducing novel products into a marketplace it is paramount to understand the cultural implications of colors and odors.

If both the color and the odor are novel, a label may offset the preconceived association by creating a context for the flavor of a new product. The label can inform consumer expectation and perhaps avoid a large discrepancy between expectation and experience, leading to a pleasant and acceptable product experience. Marketers and product developers must work together to create both visual and sensorial experiences that enable the consumer to use prior experience to inform the expectations of a new experience this could help to ensure product acceptance and success in the marketplace.

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