MULTISENSORY FLAVOR PERCEPTION IN YOUNG CHILDREN

BY

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## LIST OF ABBREVIATIONS

CEBQ: Children’s Eating Behavior Questionnaire

EMG: Electromyography

VDOR: Valence Dependent Odor Reactivity

VIOR: Valence Independent Odor Reactivity

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## ABSTRACT

Flavor perception is a key factor in dictating food choice, which directly relates to health outcomes. Between ages 2 and 6, children experience changes in food attitudes. This project examines the potential contribution of gustatory and retronasal olfactory perception to these behaviors. Although taste preferences are stable from birth, there is no knowledge of the development of retronasal smell perception, partly due to the difficulty in assessing sensory function in toddlers. Thus, the primary goal of this study is to implement a novel protocol for assessing flavor preference in toddlers to investigate changes in retronasal odor perception. Subjects were recruited and tested in the local community. Young children (n=22) and one of their parents (n=24) were asked to drink 8 solutions with either a taste or an odor compound, or plain water. Participants rated the solutions on a pictorial liking scale. Ratings from all solutions were analyzed to determine perceived intensity and valence. Taste intensity and valence were confirmed to be stable, but there were differences in individual odor valences and intensity of hibiscus odor in children compared to adults. This suggests that aspects of retronasal odor perception differ between children and adults and can potentially be modified by experience.

# CHAPTER 1: INTRODUCTION

Food preferences contribute to our risk for obesity, heart disease, and metabolic disease which may predispose us to Alzheimer's disease (Pugazhenthi, Qin, & Reddy, 2017). Food preferences are mediated through flavor perception. Commonly misinterpreted as taste, flavor is actually a multisensory construct. When you imagine your favorite ice cream, you might think it tastes like vanilla. You would probably think very little about the smell because we commonly think of flavor and taste as synonymous. Yet, the flavor of vanilla ice cream is actually produced by a combination of sweet taste (sugar binding sweet receptors on your tongue) and the vanilla odor of vanilla (vanillin released in the oral cavity traveling up the nasopharynx and binding olfactory receptors in the nasal cavity). Taste perception occurs when a chemical substance binds to the taste receptors in the tongue. The olfactory system consists of two pathways: orthonasal and retronasal (Bojanowski & Hummel, 2012). In the orthonasal pathway, odors enter the nose and bind to receptors in the olfactory epithelium; in the retronasal pathway, odors are ingested through the mouth bind to the same receptors in the olfactory epithelium. Processing of retronasal odors occurs in unique neural pathways and is specific to the context of eating and drinking. Taste preferences are generally stable from birth to adulthood where individuals tend to prefer sweet, salty, and umami tastes, and to dislike bitter and sour tastes (Mennella, 2014). While development of taste processing is well characterized, less is understood on when and how retronasal smell preferences develop. **I propose that development of retronasal smell perception affects eating behavior in early childhood.** The goals of this study are to characterize the developmental time course for sensitivity to retronasal odor in young children and to determine the relationship between retronasal odor sensitivity, eating behavior.

**Specific Aim 1: Determine the time course for development of retronasal smell perception.** I hypothesize that children experience changes in smell perception, but not taste perception, as they age which may impact eating behavior. To test this hypothesis, we will use a novel preference-testing protocol with a simple pictorial rating scale. Children and their parents will drink colorless solutions comprised of odors (mango, apple, hibiscus, broccoli, and pumpkin) or tastes (sweet, bitter) from cups with lids and straws. They will then rate their liking of each liquid on a 5 point face scale (1 = "really dislike," 2 = "really like"). To validate the scale, concentrations of sweet and bitter compounds were selected to elicit extreme ratings of 5 and 1, respectively. I will examine two measures of flavor preference: intensity (how much subjects care about the flavor compound in the solution; absolute value of the rating minus 3) and valence (whether the subject likes or dislikes the compound; rating minus 3). Because eating behavior changes dramatically during early childhood, I predict that the percentage of odors with negative valences will be lower in adults than in children, and that overall children will demonstrate more intense responses to odors than adults, suggesting heightened smell reactivity in childhood.

**Specific Aim 2: Examine how retronasal odor preference relates to eating behavior.** I hypothesize that retronasal odor perception will explain individual differences in eating behavior and changes in eating behavior that occur with age. I predict that higher reactivity to retronasal odor will correlate with food fussiness, satiety responsiveness, and slowness in eating. Alternatively, I hypothesize that lower reactivity to retronasal odor will correlate with food responsiveness and enjoyment of food.

**Impact:** By determining how young children perceive flavor, we will be able to develop sensory interventions in early childhood to establish healthy eating practices and prevent disease. Characterizing relationships between flavor preference and eating behavior could be applied to select children at high risk of negative outcomes who would obtain the greatest benefits from interventions.

**Background**

Children undergo a major change in food attitudes between the ages of 2 and 6 years old (Dematte et. al., 2014; Wright et. al, 2007). Although picky eating behavior is considered a normal part of development, parents may give fussy eaters highly palatable, unhealthy foods to get them to eat, contributing to risk for obesity and diabetes (Brown et. al., 2016; Chao, 2018; Wright et al., 2007). There is also evidence that eating behavior in childhood may impact food choice in later life (Brown et al., 2016; Pesch et al., 2020). Young adults who were picky-eaters in childhood tend to consume less fruits, vegetables, and whole grains and opt for snacks, sugar-laden beverages, and fast food more frequently (Pesch et al., 2020). Excessive sugar consumption, particularly through consumption of sugar-sweetened beverages, is highly correlated with obesity and type II diabetes, which are key modifiable risk factors for Alzheimer's disease (Andres-Hernando et. al., 2020; Pugazhenthi, Qin, & Reddy, 2017; Zhou et. al., 2020). Notably, this provides an opportunity to alter risk for Alzheimer's disease by preventing obesity and type II diabetes through lifestyle interventions in early life. However, the causes of changes in eating behavior in toddlers are poorly understood, hindering the development of efficacious interventions. Given the major role of flavor perception in dictating food choices, changes in flavor perception may be implicated in altered eating behavior.

Developmental changes in flavor perception may contribute to changes in food attitudes in young children. Perception of flavor is multisensory and depends on input from gustatory and olfactory systems (Spence, 2015). Taste perception arises from molecules binding to receptors on the tongue and is constrained to sweet, salty, umami, sour, and bitter. The olfactory system can be divided into two pathways: orthonasal and retronasal (Bojanowski & Hummel, 2012). In the orthonasal pathway, odors enter the nose through sniffing and bind to receptors in the olfactory epithelium. In the retronasal pathway, odors are ingested through the mouth during eating or drinking and bind to the same receptors in the olfactory epithelium; however, retronasal odors are distinctly processed to contribute to perception of flavor. The taste and smell components of flavor are often confused (Auvray & Spence, 2008). People report loss of taste perception when their noses are closed. Additionally, taste descriptors such as "sweet" or "sour" are often used to describe odors despite the absence of taste receptors in the nasal cavity (Auvray & Spence, 2008). For example, strawberry odor would be classified as sweet, whereas spoiled milk odor would be described as sour. Although "flavor" and "taste" are often used interchangeably, 80-90% of flavors we experience arise from retronasal smell (Spence, 2015). If we want to understand the changes in eating behavior experienced by young children, we need to start by understanding flavor perception. Because both taste and smell contribute to flavor perception and food choice, it is important to consider the developmental trajectory of both systems.

Vigorous study been dedicated to the developmental course of taste perception. Taste preferences are innate and generally stable. From birth, children prefer sweet and salty tastes and dislike bitter tastes (Mennella, 2014). Infants are born with functional sweet receptors and can distinguish between different types and concentrations of sugar (Desor, Maller, & Turner, 1973). In a study examining consumption of various sugar solutions compared to water, infants consumed a higher volume of sugar solution (Desor, Maller, & Turner, 1973). Infants showed preference for sucrose compared to glucose and fructose, demonstrating that infants can discriminate between sugar types. Consumption also correlated with increasing concentration regardless of sugar type. In addition to relative consumption, facial responses have been used as an indicator for sweet preference in infants. Steiner originally described the phenomenon of the gustofacial response in adults and sought to determine if facial responses to taste stimuli were innate (1974). Infants show positive facial responses, such as smiling, facial relaxation, and increased sucking, in response to sweet stimuli. Sour stimuli induce pursing of lips, and bitter stimuli evoke aversive responses. Further, these responses were present in anencephalic and hydroanencephalic infants, suggesting that reflexive responses to tastes may be wired by the brainstem (Steiner, 1974). Although taste preferences seem to be evolutionarily hardwired, there are subtle changes in intensity over time. Compared to adults, children and adolescents prefer higher concentrations of sucrose (Mennella et al, 2011). Between ages 4 and 6, children may undergo some changes in taste preference and sensitivity (Vennerød et al., 2018). Preference for sweet taste appears to increase between ages 4 and 6. Sensitivity for sour and salty tastes increase, while sensitivity for sweet taste decreases. Thus, developmental changes in taste perception are subtle and restricted to minor variations in intensity, but valences for basic tastes remain constant. Overall, taste perception is stable.

Unlike taste perception, the development of odor perception is unknown. Little research has examined retronasal olfaction. One study found that adults demonstrated higher variability in responsiveness to retronasal odors than to tastes, but this variability decreased when the odors were paired with a congruent taste (Flagherty & Lim, 2017). Older adults showed greater variability in retronasal odor responsiveness than younger adults. There have been no studies for sensitivity, discrimination, identification, or hedonics of retronasal olfaction in children.

Studies of orthonasal smell perception in children have had mixed findings regarding odor sensitivity and identification abilities. Lehrner, Glück, and Laska investigated odor sensitivity and identification abilities across the lifespan (1999). Based on odor threshold, children (4-11 years old) and adults had equivalent sensitivity to odors, but children had lower odor identification and memory abilities. There was no difference in sensitivity between younger and older children. Contrarily, other studies have shown differences in sensitivity with age in children between ages 4 and 12 and between adults and 6-year-olds (Monnery-Patris et al., 2009; Stevenson, Mahmut, & Sundqvist, 2007). Another important dimension of smell perception is hedonic quality, which enables one to distinguish between foods that are suitable for consumption and foods that may be poisonous (Schmidt & Beauchamp, 1988). To investigate whether odor preferences are innate, Schmidt and Beauchamp used a forced-choice, like/dislike game procedure to determine if 3 year olds displayed adult-like odor preferences (1988). For most odors, children and adults showed similar preferences, indicating that many odor preferences are formed by early childhood. Adults may not be as sensitive to some odors, resulting in differential hedonic evaluation between children and adults for those odors. Contrarily, Rinck and colleagues found that changes in odor hedonics occurred between ages 3 and 5 (2011). In a longitudinal study, children were asked if they liked or disliked 12 odorants at ages 3, 4, and 5. At 3 years old, children liked and disliked equal proportions of the odor stimuli. However, the number of liked odors increased at 5 years old. It is proposed that the development of language skills enables children to organize odor perception. Because the odor preference test itself required children to respond verbally to indicate liking, it is possible that 3 year olds did not understand the task.

Although the development of smell preferences remains unclear, there has been an interesting link between eating behaviors and smell perception. Research in adults has suggested a link between food neophobia and odor identification (Dematte et al., 2013). Neophobic adults demonstrate diminished odor identification abilities (Dematte et al. 2013). This deficit may be explained by lack of exposure to novel odors. Differences in smell perception have also been observed in neophobic children (Monnery-Patris, 2015). Monnery-Patris and colleagues found that neophobic 20 month old children were more reactive to smell but not taste stimuli (2015). Differential smell reactivity may also explain why neophobic children primarily reject fruits and vegetables compared to other food groups (Demattè, Endrizzi, & Gasperi, 2014). This is often attributed to bitter taste compounds in vegetables, but sensitivity to bitter compounds is not related to willingness to try new foods in adults (Demattè, Endrizzi, & Gasperi, 2014; Lim & Padmanabhan, 2013). In a study on determinants of vegetable liking/disliking in adults, bitterness ratings did not differ between vegetable-likers and dislikers (Lim & Padmanabhan, 2013).

However, the intensity of vegetable liking/disliking was less when retronasal odor perception was compromised by closing the nose. This indicates that retronasal odor sensitivity may be a determinant of vegetable liking and eating behavior. It is also interesting to note that food neophobia peaks between ages 2 and 6, an age bracket that has been neglected in the literature due to challenges, such as those previously mentioned, in assessing sensory perception in young children (Demattè, Endrizzi, & Gasperi, 2014; Monnery-Patris et al., 2015).

As taste preferences are innate and stable, changes in taste preferences cannot account for the changes in eating behavior in childhood. Given the absence of knowledge regarding development of retronasal smell preferences and the links between smell perception and eating behavior, I propose that changes in retronasal smell preferences may explain changes in eating behavior in young children. Children between the ages of 2 and 6 years old are of particular interest due to prevalent changes in food attitudes within this age group. I hypothesize that retronasal smell reactivity and hedonics will differ between children and adults. Further, I predict that overall children will have greater smell reactivity than adults, and that the intensity of smell reactivity will peak between ages 2 and 6 years old.

A novel method is necessary to examine the development of flavor preferences because typical methods used for assessing preferences in adults are not appropriate for young children. The reliability of typical methods of flavor preference testing is often constrained by age due to limited verbal ability (Monnery-Patris et al., 2009). When asked to rate odors as “like” or “dislike,” children ages 3 and 4 rate banana, feces, and sweat as positive stimuli (Stein, Ottenberg, & Roulet, 1958). This illustrates the need for different procedures to assess liking in toddlers. Facial responses have been used to assess taste and orthonasal odor preferences. In infants, facial reflexes are often used as an alternative measurement of taste liking (Menella, Forestell, Morgan, and Beauchamp, 2009). A weakness of approaches that rely solely on facial responses is that responses to pleasant stimuli are not as well characterized as responses to negative stimuli. Furthermore, there is little evidence of facial responses to unpleasant and pleasant odor stimuli (Armstrong, Hutchinson, Laing, and Jinks, 2007). Armstrong and colleagues examined the feasibility of using facial electromyography to assess taste and smell preference in children (2007). In this experiment, taste and orthonasal smell stimuli were presented to 6-9 year olds. The facial muscle responses to unpleasant and pleasant stimuli were consistent with typical adult responses, but the participants were older than the target population for my study. Facial EMG may not be well suited for younger children because it requires placement of electrodes on the face which might be upsetting or uncomfortable for toddlers and would require children to sit still and remain attentive for a longer session.

Other creative approaches to assess orthonasal smell perception in young children have presented tests in the context of a game (Mennella and Garcia, 2000; Schmidt and Beauchamp, 1988). Schmidt and Beauchamp tested orthonasal odor preference in 3 year olds using Sesame Street character puppets (1988). Children were instructed to give good things to one puppet and bad things to another puppet. Using this method, 3 year olds demonstrated general odor preferences that were similar to preferences of older children and adults. Mennella and Garcia applied the smell game to study orthonasal odor preference in 4 to 6 year olds (2000). This validated the method in 4 to 6 year olds based on responses to positive odors (bubblegum) and negative odors (pyridine); however, developmental changes were not examined in the study. A limitation of this procedure is that it requires a pretest with known likes and dislikes to ensure that the child understands the instructions.

A simpler procedure might be to use a pictorial rating scale. Yuan and colleagues assessed food preferences in 5 year olds using a 3-point pictorial face scale (2016). Children were presented with pictures of foods and asked to rate liking by pointing to a positive face, neutral face, or frowny face. They established the external validity and test-retest reliability of using this method in children as young as 5. Similarly, Liem, Zandra, and Thomas used a face scale to assess ice cream flavor preference in 3 to 10 year olds and compared children's ratings to questionnaires completed by their mothers (2010). They found that the mothers' ratings were not reliable; mothers were only 40% accurate at predicting children's flavor preferences. Overall children reliably reported flavor preferences over two consecutive days using the simple rank task. The reliability of ratings was higher among children ages 5-10, suggesting that younger children may not fully understand the instructions, lack attention, or still be forming flavor preferences (Liem, Zandra, & Thomas, 2010). However, they noted that children as young as 3 are generally able to perform simple rank tasks.  This type of task might be extended to assess taste and retronasal smell preference.

# CHAPTER 2: METHODS

## Subjects

Healthy children ages 3 to 6-year-old (n = 24) and one of their parents (n = 24) were recruited from locations in the community including the Kaleideum North and Downtown. Children were excluded from the study if they did not speak English or had a known food allergy, nasal congestion, impaired sense of taste or smell, motor or speech delay/difficulties, other developmental delays, or an autism spectrum disorder. Two children were excluded from the final data analysis. One child asked to stop after tasting the bitter solution, and another child was excluded due to rating bias (rating all solutions as "really liked" without consuming any solution). Parents were asked if they wanted to participate in an experiment in which they and their children would taste different clear liquids containing flavors found in everyday foods and rate how much they like them. Children were asked if they wanted to help a scientist with an experiment and were told that they could say no or stop at any time. Parents completed a child eligibility form and provided informed consent, and children provided verbal assent prior to testing.

## Test Stimuli

Taste and odor stimuli were presented individually in plastic cups (2 oz) with lids and straws (5 mm diameter) to prevent orthonasal detection. Each solution consisted of a tastant or odor dissolved in purified water. Tastants (Fischer Scientific) included sucrose (0.3 M, sweet), quinine-sulfate (0.1 mM, bitter). Concentrations were selected to elicit maximum responses in young children. Odors (Givaudan Flavors and Fragrances Corporation) consisted of mango flavorant (0.2%), honey crisp apple flavorant (0.2%), pumpkin flavorant (0.1%), broccoli flavorant (0.1%), and hibiscus flavorant (0.2%).

### Child Eating Behavior Questionnaire (CEBQ)

The CEBQ is a 35-item questionnaire that assesses eating behavior based on 8 subscales: Food Responsiveness, Enjoyment of Food, Desire to Drink, Slowness in Eating, Satiety Responsiveness, Food Fussiness, Emotional Over-Eating, and Emotional Under-Eating (Wardle et. al., 2001). A version of the CEBQ with 27 items was administered, excluding the emotional over- and under-eating subscales for brevity. Each item evaluates frequency of a behavior on a 5 point Likert-scale, where 1 indicates "never" and 5 indicates "always."

### Testing Procedure

Testing took place in a closed room with minimal distractions. Each parent completed a demographic questionnaire and the CEBQ while the child completed the task. The child participant sat at a small table, across from the experimenter. The experimenter provided the following instructions: "You will have to be a very good listener, can you be a good listener? I will give you cups with different drinks. You need to drink from the cup and tell me if you like it or not. If you like it, you will point to the YUM face; if you don't like it, you will point to the YUCK face. If you don't know, you will point in the middle. You can drink as much or as little as you like." They were asked to indicate their liking on a 5-point pictorial face rating scale (where 1 meant "really disliked, 2 meant "disliked", 3 meant "I don't know", 4 meant "liked" and 5 meant "really liked;" see Figure 1). Before the stimuli were presented, the child participant was instructed to repeat back the instructions to ensure that they comprehended the task. The child received 25 ml of each solution and was instructed to drink from the cup. The order of stimulus presentation was randomized. They were allowed 30 seconds to sample each solution. Facial and verbal responses were recorded using a video camera positioned across from the child participant. Ratings were recorded manually. Cups were weighed before and after testing to determine amount consumed. Once the child had completed the task for all 8 stimuli, the parent was tested separately to prevent the responses of the parent from influencing the child. As compensation, parents each received a $25 Target gift card, and children received stickers. The study was approved by the Wake Forest School of Medicine Institutional Review Board.

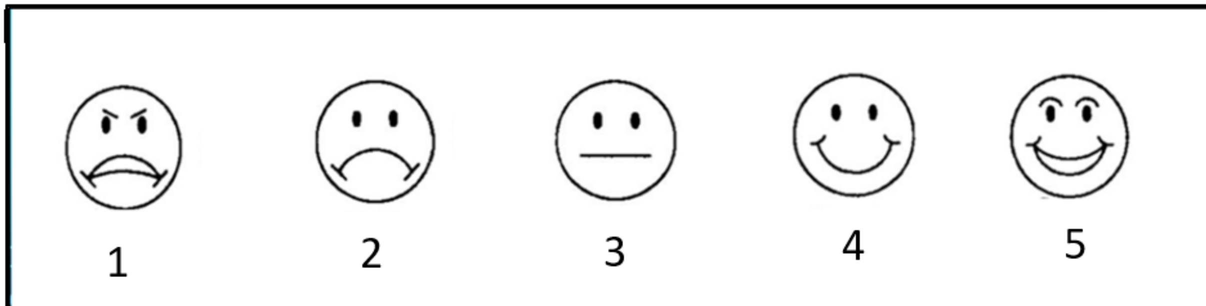


Figure 1. 5-Point Liking Scale. 1 indicates "really disliked", 2 indicates "disliked", 3 indicates "I don't know", indicates "liked," and 5 indicates "really liked."

**Data Analysis**

For each of the stimuli, the intensity was determined by calculating the absolute value of the rating minus three, and the valence was indicated by the sign of the rating minus three. All statistical tests were conducted using R statistical software. For children and adults, average intensity ratings for each of the taste and odor stimuli were calculated. Wilcoxon Signed Rank Tests were used to evaluate differences between intensity for sucrose and quinine within children or within adults. Rank Sum Tests were used to examine differences in average intensities for sucrose and quinine between children and adults. Rank Sum Tests were also implemented to evaluate differences in average intensities for each of the odor compounds between children and adults.

All intensity scores were averaged across tastes and across odors for each age group to determine the average intensity for taste and the average intensity for odor in children and adults. Wilcoxon Signed Rank Tests were used to compare average intensity for odor and for taste within each age group. Rank Sum Tests were used to evaluate differences in average odor intensity and average taste intensity between age groups.

Valences for each of the taste and odor stimuli were represented by the percentage of negative ratings out of total nonzero ratings within each age group. Differences in the valences for sucrose, quinine, and each of the odor stimuli between children and adults were assessed using chi-squared tests for contingency. After observing the trend of odor valences to become less variable, and therefore, more extreme in adults, I transformed each of the valences to provide a measure that was relative to 50%. For the individual odors within each age group, the absolute value of the percentage negative ratings minus 0.5 was calculated. An average for all odors was then determined for children and adults, and a rank sum test was used to assess the difference. Kolmogorov–Smirnov tests were used to assess differences between the distributions of valence ratings for children and adults for each of the stimuli.

Finally, linear regression models were used to investigate relationships between retronasal odor reactivity and each of the subscales of the child eating behavior questionnaire in order to examine the potential effects of retronasal odor reactivity on individual variability in eating behavior. One child was excluded from the analysis of retronasal odor reactivity and eating behavior due to incomplete questionnaire data. For each child (n=21), the items corresponding to each of the subscales of the CEBQ were averaged to obtain a score for each measure of eating behavior: slowness in eating, food fussiness, satiety responsiveness, desire to drink, food responsiveness, and enjoyment of eating. Higher scores represented higher frequency of the behavior. Two measures of retronasal odor reactivity were calculated for each child subject: valence independent odor reactivity and valence dependent odor reactivity. Valence independent odor reactivity was determined by averaging the odor intensity scores (absolute values of each odor rating minus 3) for each child. Valence dependent odor reactivity was obtained by calculating the percentage of negative odor ratings out of the total number of odors (5) for each child. Linear regression models were used to analyze the relationships between VIOR and VDOR and each of the subscales of the CEBQ. R-squared values were determined for each relationship, and F-tests were conducted to assess significance.

$$VIOR = AVERAGE(Abs(Apple - 3), Abs(Mango - 3), Abs(Hibiscus - 3), Abs(Broccoli), Abs(Pumpkin - 3))$$

$$VDOR = \frac{Total\ Negative\ Odor\ Ratings}{5}$$

# CHAPTER 3: RESULTS

## Taste ratings do not change with age

Because previous research has suggested that taste preferences are innate and stable, I first examined ratings for taste compounds in children and adults ($n = 22$ children, 55% female, mean age = 4.62 years old, 86.4% white and 13.6% other ethnic group; $n = 24$ adults). I expected sucrose and quinine to elicit ratings of 5 and 1, respectively, because concentrations were selected to elicit optimal liking or disliking. Within children and adults, both sucrose and quinine were overall rated as 1 and 5 (see Figure 2). There were no statistically significant differences in intensity for sucrose compared to quinine in either age group. Statistical analyses revealed that children exhibited a higher intensity for sucrose than adults do ($p < 0.05$). However, intensity of quinine was comparable between children and adults. In order to analyze the differences in valences for tastes between children and adults, I calculated the percentage of negative ratings out of all nonzero ratings for sucrose and quinine in each group. Valences of sucrose and quinine did not differ between children and adults. Both age groups consistently rated quinine as negative and sucrose as positive. Additionally, when the distributions of valence ratings were examined for each taste, there were no differences in the distributions of ratings between children and adults (Figure 3). I confirm that ratings of taste are stable with age, although children care more about sweet taste than adults.

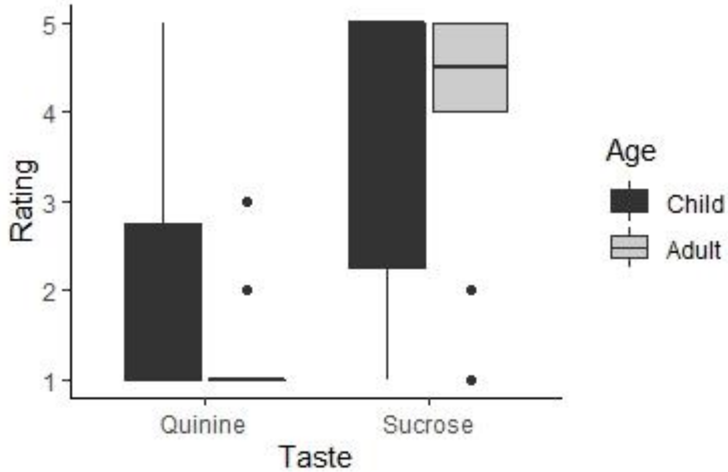


**Figure 2. Ratings for sweet and bitter tastes in children and adults.** The average rating of 0.1 mM quinine (black) and 0.3 M sucrose (gray) for children (n=22) and adults (n=24).

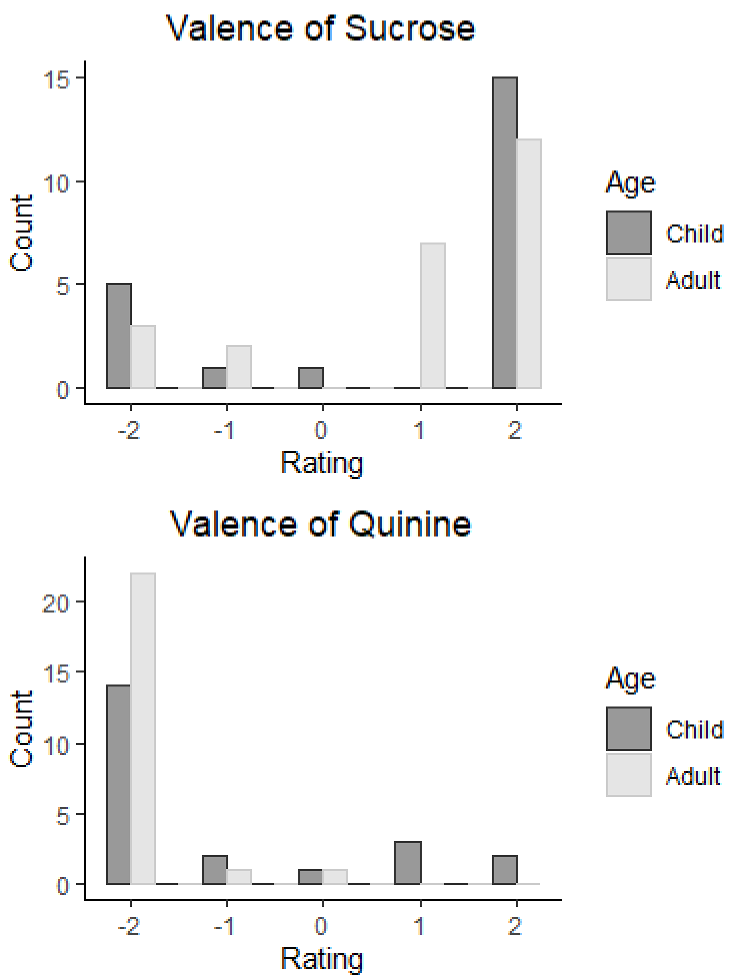


**Figure 3. Distribution of valences of tastes.** Histograms of valence ratings for 0.3 M sucrose (top) and 0.1 mM quinine (bottom) for children (n=22; dark gray) and adults (n=24; light gray). KS Test.

**Intensity is stable for food odors, but changes for hibiscus**

After validating the rating scale based on the stability of taste ratings with age, I next examined how odor intensities vary between children and adults (Figure 4). Concentrations of taste compounds were selected to elicit maximum intensities, but intensities of odor compounds were undefined. There were no significant differences in average intensities for mango, apple, broccoli, and pumpkin. Interestingly, intensity of hibiscus was higher in children than adults ($p < 0.001$). Intensities of food odors did not change with age. However, intensity of the nonfood odor, hibiscus, did differ between children and adults.

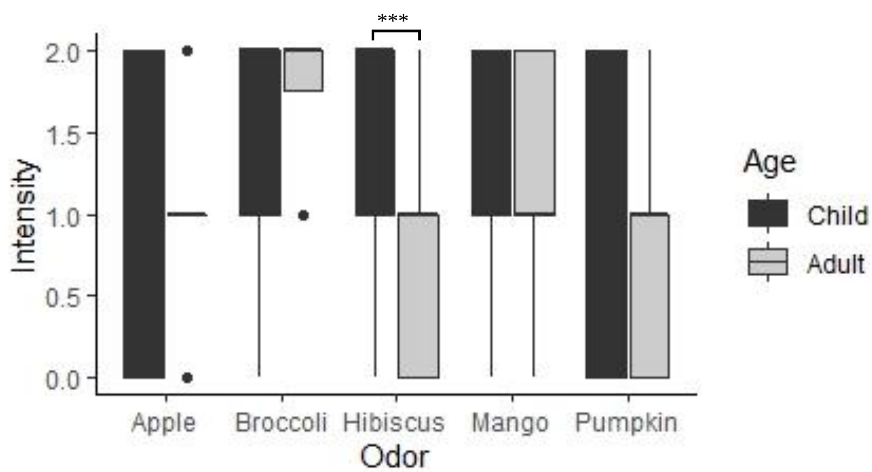


**Figure 4. Intensity for individual odors in children and adults.** The average intensity of mango, apple, pumpkin, broccoli, and hibiscus for children (n=22; in black) and adults (n=24; in gray). Rank Sum Test. ***p<0.001.

**Average intensities for odor and taste do not differ between children and adults**

Finally, I compared the average intensity for taste and odor between children and adults (Figure 5A). Intensity ratings were averaged across sucrose and quinine to determine the average taste intensity for each age group. Similarly, intensity ratings for mango, broccoli, hibiscus, apple, and pumpkin were averaged for each group. Average intensity ratings for taste were higher than for odor in both children ($p < 0.001$) and adults ($p < 0.001$). Average taste intensity and average odor intensity did not differ between children and adults. At the concentrations of flavor compounds used, subjects cared more about taste than odor independently of age. Overall, the extent to which subjects care about both odor and taste did not change with age.

Although there was not a difference in average odor intensity between children and adults, there may be changes in average odor intensity that occur within early childhood. Based on my prediction that changes in reactivity to retronasal odor affect eating behavior between ages 2 and 6, I wanted to determine if children experience age-related changes in average odor intensity as a measure of individual retronasal odor reactivity. The average odor intensity scores were obtained by averaging the intensities of mango, apple, hibiscus, broccoli, and pumpkin for each child. This is equivalent to the valence independent odor reactivity score used in the CEBQ analysis. A linear regression model was used to assess the relationship between average odor intensity and age (Figure 5B). There was a significant negative relationship between age and average odor intensity ($F_{(1, 19)}$= 4.86, $p < 0.05$, $R^2 = 0.20$). Younger children may experience heightened reactivity to retronasal odors which decreases with age. This suggests plasticity in odor intensity within children, despite the stability of odor intensity between children and adults.

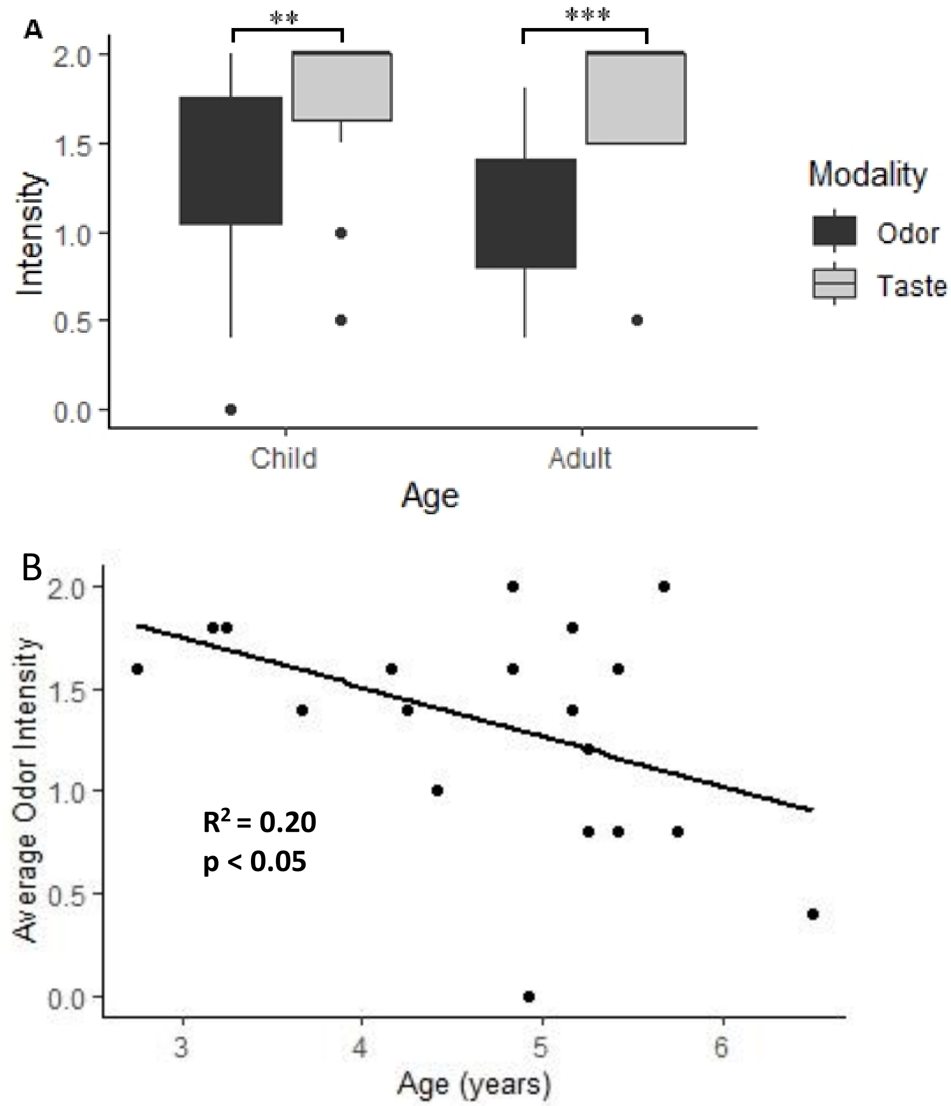


**Figure 5. A. Average intensities for odor and taste in children and adults.** The average intensity of all odors (black) and tastes (gray) for children (n=22) and adults (n=24). Wilcoxon Signed Rank Test. ***p<0.001. **B. Average intensity for odor within children.** The average intensity of all odors for each child (n=22) versus age in years. Linear regression model. F-test.

**Odors valences may become more established with age**

Although taste valences are well-characterized, the valences of retronasal odors are unknown. As with the taste valences, odor valence scores were used to calculate the percentage of negative ratings out of total nonzero ratings for each odor in each age group (Figure 6). Based on the average odor valence for children and adults there was no overall change in odor valence with age, but this does not dismiss the possibility of changes in valence for individual odors. There was a difference in valence for broccoli; adults rated broccoli as negative more frequently than children ($p < 0.01$). This analysis did not provide a comprehensive representation of the data because it excluded zero ratings, so I also examined at the full distributions of valence ratings. When I inspected the distributions of valence ratings for each odor more closely, I observed a more complex picture of how valence changes with age (Figure 7). For mango, the distribution shifted from being skewed to the right in children to being skewed to the left in adults. The distribution of apple was bimodal in children, but the peak in adults fell in between the peaks for children. Hibiscus ratings were skewed to the left in children but peaked at zero in adults. The distribution of ratings for pumpkin were skewed to the right in children but became flat in adults. The distribution for broccoli was skewed to the right in both groups. KS Tests revealed significant differences between the valence distributions for children and adults for hibiscus ($D = 0.375$, $p < 0.05$) and broccoli ($D = 0.412$, $p < 0.05$). Taken together, this suggests that there is plasticity in valence for individual odors.

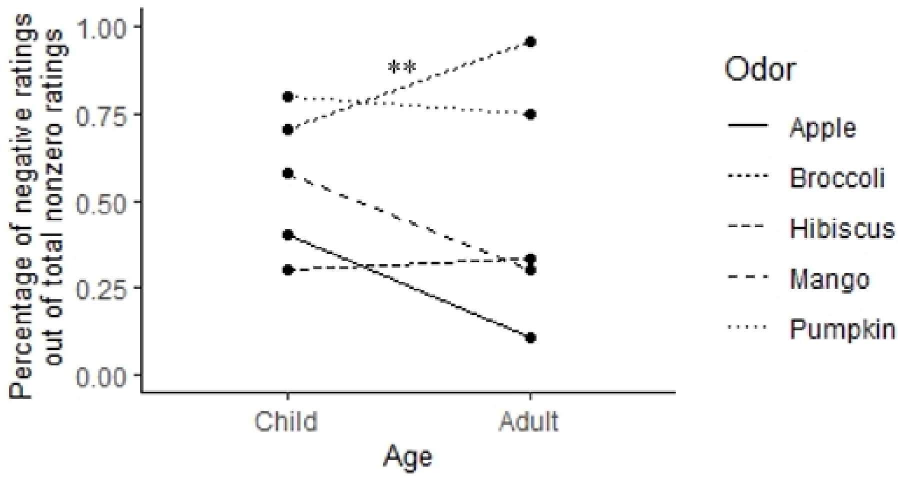


**Figure 6. Valence of individual odors with age.** The percentage of negative ratings out of total nonzero ratings for mango, apple, pumpkin, broccoli, and hibiscus for children (n=22) and adults (n=24). Chi-squared test for contingency. **p<0.01.

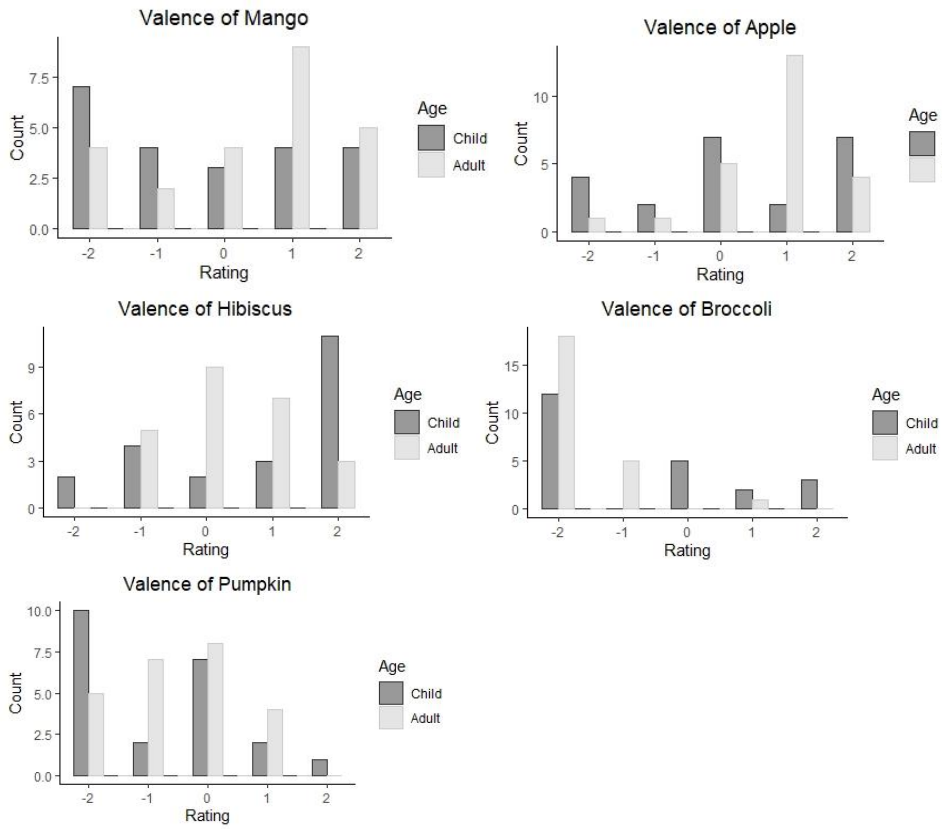


**Figure 7. Distribution of valences of individual odors.** Histograms of valence ratings for mango, apple, pumpkin, broccoli, hibiscus for children (n=22; dark gray) and adults (n=24; light gray). KS Test.

## Child Eating Behavior Questionnaire

After examining the intensity and valence of retronasal odor in young children, I investigated how individual differences in reactivity to retronasal odor relate to eating behavior. I calculated two measures of reactivity to retronasal odor: one that did not depend on valence (valence independent odor reactivity; VIOR) and one that depended on valence (valence dependent odor reactivity; VDOR).

**Valence independent odor reactivity and eating behavior**

I first examined how individual differences in VIOR predicted differences in eating behavior (Figure 8). This measure of odor reactivity was based on intensity scores and captured how much each child cared about odors on average. There was a significant negative relationship between VIOR and slowness in eating ($F_{(1, 19)}$= 13.61, $p < 0.01$, $R^2 = 0.42$). As valence independent odor reactivity increased, slowness in eating decreased. VIOR did not explain variation in desire to drink, satiety responsiveness, food responsiveness, enjoyment of food, or food fussiness. The relationship between VIOR and slowness in eating suggested the potential role of retronasal odor reactivity in shaping eating behavior. However, because VIOR was based on odor intensity alone and did not include information about valence, it might not comprehensively capture individual variation in odor responsiveness. Therefore, I also analyzed another measure of odor reactivity that would account for valence data.

**Valence dependent odor reactivity and eating behavior**

After observing the link between VIOR and eating behavior, I investigated how an alternative measure of retronasal odor reactivity might relate to eating behavior (Figure 9). VDOR scores were based on valence scores and represented the percentage of odors rated as negative by each child. The assumption was that children who are more reactive to retronasal odor might be more sensitive and likely to rate odors as negative. Based on the linear regression model, there was also a significant negative relationship between VDOR and slowness in eating ($F_{(1, 19)}$= 5.908, $p < 0.05$, $R^2$=0.24). VDOR did not explain variation in desire to drink, satiety responsiveness, food responsiveness, enjoyment of food, or food fussiness. Both VIOR and VDOR were negatively associated with slowness in eating. The similar relationships between two different measures of odor reactivity and the same eating provides stronger evidence that there are relationships between individual differences in retronasal odor reactivity and child eating behavior that require further investigation.

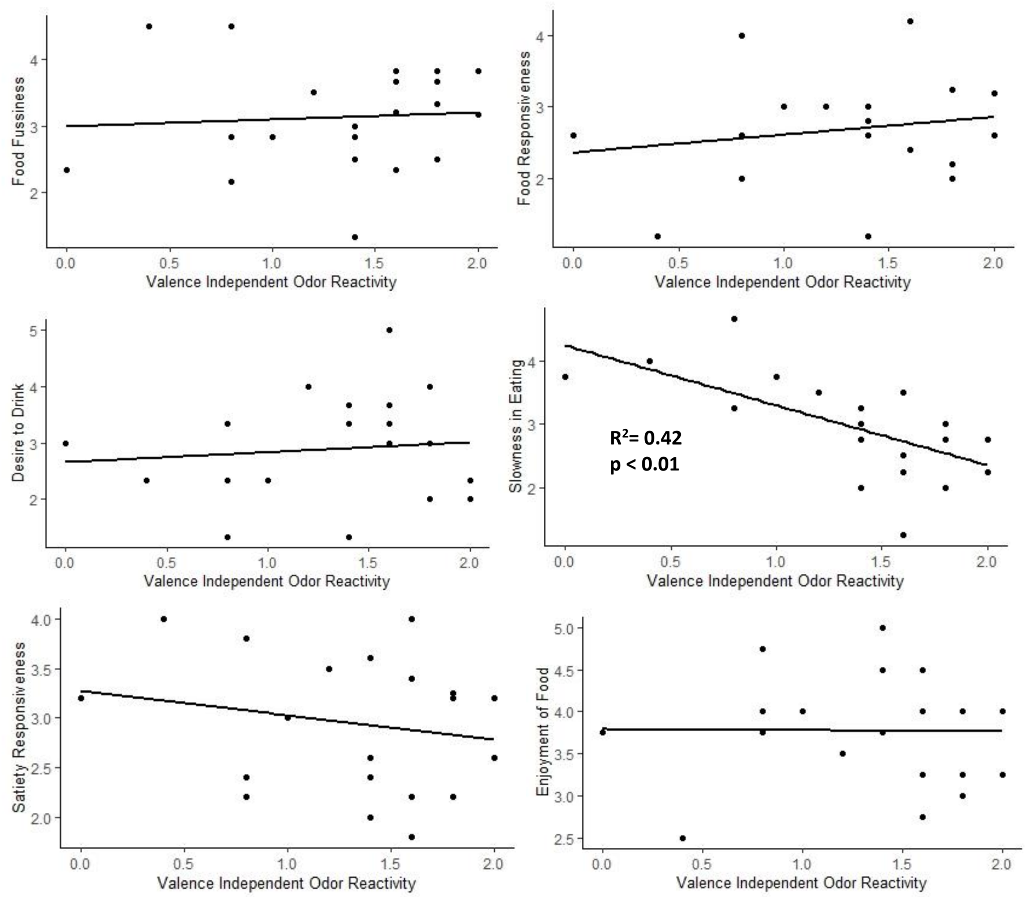


**Figure 8. Child eating behavior and valence independent odor reactivity.** Scores for subscales of Child Eating Behavior Questionnaire versus valence independent odor reactivity in children (n=21). Linear regression model. F-test.

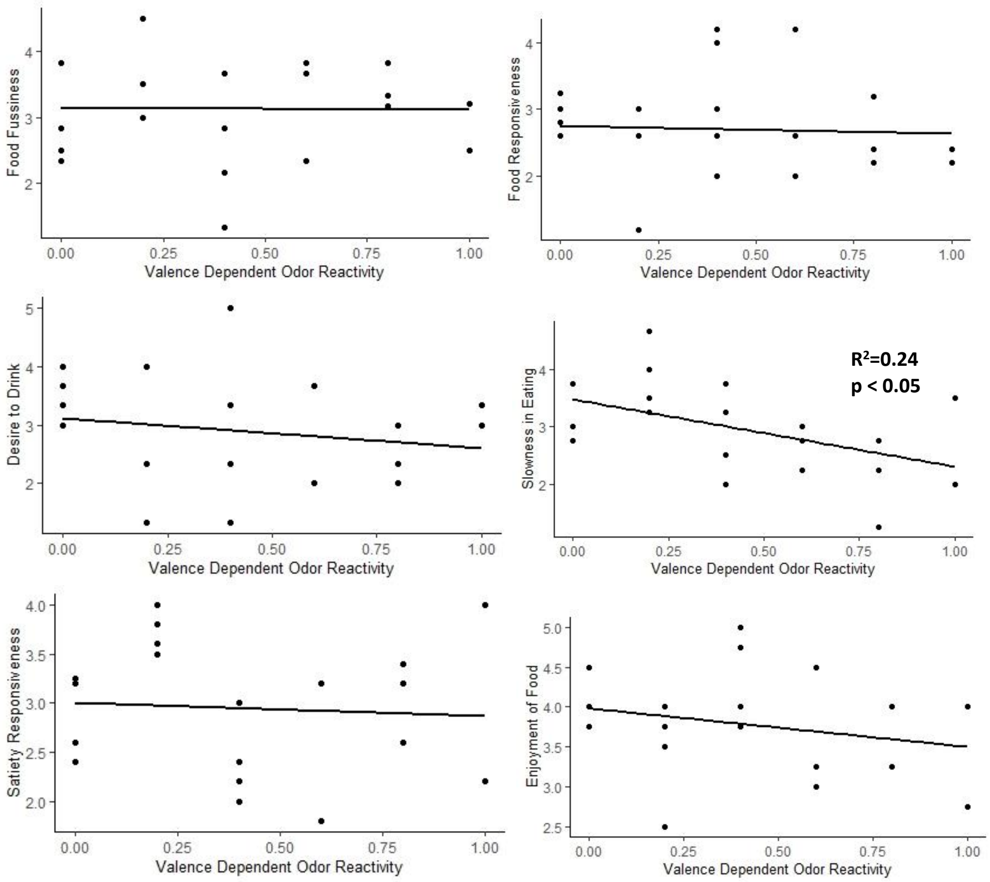


**Figure 9. Child eating behavior and valence dependent odor reactivity.** Scores for subscales of Child Eating Behavior Questionnaire versus valence dependent odor reactivity in children (n=21). Linear regression model. F-test.

# CHAPTER 4: DISCUSSION

Flavor perception is key in shaping food choices and may account for changes in eating behavior that occur in children between ages 2 and 6. Previous research on flavor perception has focused on taste and suggests that liking of sweet taste and aversion to bitter taste are innate and generally stable. However, retronasal odor perception has not been examined in young children due to the lack of age appropriate methods. This is the first study to investigate the developmental time course for sensitivity to retronasal odor in young children. Children and one of their parents sampled and rated liking of solutions consisting of tastants or odorants on a 5 point pictorial face scale. Ratings were used to determine intensity (how much subjects care about the flavor component) and valence (whether they like or dislike the flavor component) for each of the stimuli. Intensities and valences for retronasal odors were compared between children and adults to identify the how retronasal odor perception changes with age.

## Validation of rating scale based on taste stimuli

First, I validated a novel protocol for assessing flavor perception in toddlers using taste stimuli with known palatability and intensity. Intensity for sweet taste was higher in young children, but overall taste intensity and valence were stable with age. The finding that children cared more about sweet taste than the adults was anticipated based on the selection of a concentration of sucrose to optimize liking in young children. Previous research on sweet taste over the lifespan found that children prefer more concentrated solutions of sucrose than adults (Mennella et al., 2011). It is important to note that while adults cared slightly less about sweet taste than children did, both age groups still had high average intensity ratings for taste compounds. The results for taste intensity are consistent with predictions of a general degree of stability for taste intensity with age and can be used as validation for the methodology.

This method provides multiple advantages over previous measures of flavor perception. First, the rating scale is developmentally appropriate for toddlers. Because the rating scale does not rely on verbal ability, it is more suitable for young children who lack the verbal skills to convey preferences reliably. The rating scale does not require ranking solutions in order of relative liking, which may also be too developmentally advanced for toddlers. Second, this method does not require costly, specialized equipment or invasive procedures to measure facial muscle reflexes in response to flavor stimuli. Additionally, unlike methods to assess orthonasal odor preferences in young children, such as the smell game developed by Schmidt and Beauchamp, the pictorial rating scale does not require children to learn more complex rules of a game or to participate in a pre-test with known likes and dislikes to ensure understanding (1988). This enables flavor preferences to be assessed easily and rapidly in a single session.

**Odor intensity ratings**

After validating the rating scale, I examined the developmental pattern for retronasal odor intensity. Although intensities for food odors were similar in children and adults, intensity ratings were higher for hibiscus in children. This finding demonstrates plasticity in retronasal odor perception. Experience with nonfood odors or uncommon odors may lead to altered intensity with age. This change in intensity may or may not be dependent on whether odors have been encountered specifically in the context of consumption or recognized based on orthonasal exposure.

Children might have showed higher intensity for hibiscus compared to adults because there could be less of a distinction between food and nonfood odors in children. This distinction could become clearer with age as individuals experience more odors. Future experiments might expand the number of nonfood odors included to see if this difference is consistent across other examples. It would also be important to control for familiarity with the nonfood odors involved by including common and uncommon odorants. If experience is key in the change in intensity for hibiscus between children and adults, uncommon odors may retain high intensities in adulthood. Alternatively, if the change in intensity relies on a developmental process, lack of exposure to an odor within a certain temporal window might lead to decreased sensitivity and intensity for that odor in later life. Another remaining question would be whether this change in intensity depends on experience in general or on experience specifically in the context of consumption. Testing intensity of hibiscus and other nonfood odors orthonasally and retronasally would help determine the importance of context.

In addition to investigating how individual odor intensities change with age, I compared average intensities for taste and odor between children and adults in order to discern how overall intensity for odors compared to intensity for tastes with known intensity. The deliberate selection of high tastant concentrations is likely to explain why both children and adults seemed to care more about taste than odor when intensities for tastes and for odors were averaged. Unlike sucrose and quinine concentrations, odor concentrations were not optimized to elicit maximum responses. It might be useful to conduct an experiment with various concentrations of odorants. Odorants could be presented retronasally in increasing concentrations to create a plot of average odor intensity with concentration in order to identify the concentration that maximizes intensity for each odor. It is also necessary to optimize the intensity of odor stimuli to ensure that the peaks of the valence distributions are at extremes. This would allow for clearer comparison of the distributions of odor valence scores in children versus adults.

## Odor valence ratings

Next, I investigated the trajectory of odor valences with age. Overall odor valences did not change, but the distribution of valence scores differed between children and adults, suggesting that experience may reduce variability in odor valences. Experience could also play a role in why adults rated broccoli as negative more frequently than children. Although broccoli odor initially has a slight negative valence in young children, experience may reinforce aversion. It could also be that the percentage of negative ratings for broccoli was exceptionally high because broccoli is rarely consumed in the context of a beverage. Broccoli odor being presented alone in the absence of crunchy texture or other flavor components might result in a uniquely unpleasant flavor experience. It might be interesting to have adults self-report liking of foods after testing with the liquid odorants. The reported liking of broccoli and pumpkin could be compared to the valence of isolated broccoli and pumpkin odors.

## Retronasal odor reactivity and child eating behavior

To determine the relationship between sensory perception and eating behavior, I regressed reactivity to retronasal odor for individual children on scores obtained from subscales of the Children's Eating Behavior Questionnaire. I found that retronasal odor reactivity was negatively correlated with slowness in eating. Slowness in eating is usually higher in preschool age children and is thought to decrease as children age and gain freedom in choosing the type and quantity of food they consume (Sanlier et al., 2018). In my sample, slowness in eating did not change with age, but odor reactivity significantly decreased as children aged. Although the underlying mechanism for how retronasal odor reactivity may affect slowness in eating is unknown, age-related changes in reactivity to retronasal odor may play a role. Slowness in eating is associated with lower BMI independently of age (Sanchez et. al., 2016; Webber et al. 2009). Based on the relationship between BMI and slowness in eating, it could also be that children with a higher BMI are more reactive to retronasal odors. Diets associated with higher BMI may be high in processed foods and sugary beverages, which would limit exposure to diverse retronasal odors found in fruits and vegetables. This could play a role in increased reactivity to odor. A larger sample would allow for further characterization of the relationships between retronasal odor and eating behavior, along with age and BMI.

**Limitations**

One limitation of this study is that it involved a small sample of subjects which limits the ability to fully understand the relationships between individual differences in retronasal odor reactivity and eating behavior. Additionally, because the subjects were recruited from a single community, the findings might not generalize to other geographic locations or populations. The sample might be biased to children that have had experience with more complex sensory environments because subjects were recruited from interactive museums. Prospective data collection might expand recruitment to other community locations to capture a more diverse sample. Additionally, future work should aim to validate the use of the rating scale in other populations, including other cultural groups, socioeconomic groups, and groups with other medical conditions or sensory deficits which were excluded from the present study.

**Future directions**

My study is the first to examine how retronasal odor perception develops in young children, and provides preliminary evidence for plasticity in the subjective aspects of retronasal odor perception. My method for assessing flavor liking without reliance on advanced verbal ability could be extended to study retronasal odor perception in other groups that might experience alterations in flavor perception, such as individuals with developmental disorders or in older adults. The findings also imply a relationship between reactivity to retronasal odors and individual differences in eating behavior. A better understanding of how retronasal odor reactivity impacts eating behaviors could aid in the development of childhood interventions to prevent disease in later life.

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## CURRICULUM VITAE

**EDUCATION:**

| | |
|---|---|
| 2018-2021 | **M.S. Candidate Neuroscience** |
| | Wake Forest University, Winston-Salem, NC GPA: 4.00 |
| 2014-2018 | **B.S. Neuroscience** |
| | High Point University, High Point, NC Minor: Psychology |
| | GPA: 3.99, Summa Cum Laude |

**HONORS/AWARDS/FELLOWSHIPS:**

| | |
|---|---|
| 2018-current | Beta Beta Beta, National Biology Honors Society |
| 2017-current | Alpha Chi, National College Honors Society |
| 2017-current | Psi Chi, International Honors Society in Psychology |
| 2017-2018 | Goldston Scholarship 2017 Chief Junior Marshal |
| 2015 | Alpha Lambda Delta, Freshman Honor Society |
| 2015 | Research Fellow, Competitive Summer Undergraduate Research Program in the Sciences (SuRPS), High Point University, 1 of 24 selected |
| 2014-2018 | High Point University Honors Scholar Program |
| 2014-2018 | High Point University Presidential Scholar |

**RESEARCH EXPERIENCE:**

2019-current **M.S. Candidate. Department of Neuroscience. Wake Forest University. Winston-Salem NC 27101. (Research Advisor: Dr. Joost Maier). Project Focus-** *Linking Eating Behavior to Multisensory Flavor Perception in Early Childhood. Flavor preference is a major determinant of food choice which relates to risk for obesity,* *metabolic disease, and Alzheimer's disease. Between ages 2 and 6, children undergo changes in eating behavior which may result from changes in retronasal odor reactivity. In order to determine the developmental course of retronasal odor preferences, young children and their parents rated liking of clear liquids consisting of water, tastants, or odorants on a 5-point pictoral rating scale. Ratings were also correlated with Child Eating Behavior Questionnaire scores and BMI in order to determine the relationship between reactivity to taste and retronasal odor, eating behaviors, and physical health*.

- Collaborated with community centers (the Kaleideum and Greensboro Science Center) to recruit subjects and host experiments.
- Presented findings at poster session for the International Symposium of Olfaction and Taste 2020 and the Association of Chemoreception Sciences Virtual Meeting 2021.

2017-2018 **Student Researcher. Department of Biology. High Point University. High Point, NC 27262. (Research Advisor: Dr. Kristin Ackerman). Project Focus –** *The Effects of Chronic Nicotine Exposure on Eye Development in Zebrafish. Zebrafish embryos were exposed to varying concentrations of nicotine (0 µM, 100 nM, 1 µM, 10 µM, 30 µM, 50 µM, and 100 µM), and the mean ratios for eye diameter to body length were compared to determine the effect of nicotine concentration on eye size. This could have implications for how smoking during pregnancy impacts eye development in infants.*

- Obtained images using Leica stereoscope
- Analysis using ImageJ software

2015-2017 **Research Fellow. Department of Chemistry. High Point University. High Point, NC 27262. (Research Advisor: Dr. Brian Augustine). Research Focuses -**

*Examining techniques to enhance vapor-deposited Au thin film adhesion onto PMMA substrates for application in biomedical devices. Projects: Novel Patterning Techniques of Vapor-Deposited Au Thin Films onto Polymeric Substrates (Summer 2015) The enhanced adhesion of gold to PMMA by chloroform treatment, evidenced in a previous study, was manipulated to develop a gold patterning technique using Inkjet Printing. Chloroform was deposited into patterns on pieces of PMMA using the Microfab 4 Inkjet Printer. After gold was deposited onto the PMMA, the excess gold was wiped away, leaving an array of gold dots where the chloroform was deposited. Atomic Force Microscopy (AFM) Study of Acrylic Polymer Thin Film Deposition (2016-2017) Based on early research, thin films deposited from solutions of PMMA (MW = 996000 g/mol) dissolved in tetrahydrafuran (THF) yielded porous membranes with multiple layers and pore diameters ranging from around 3 nm to 1 µm. The current project aims to isolate this membrane for potential applications in filtration devices or bio warfare detectors*.

- Training on Raman Spectroscopy, Single Wavelength Ellipsometry, FT-IR Spectroscopy, Atomic Force Microscopy.
- Collaboration with professors and student researchers at James Madison University.
- Experience with Microfab 4 Inkjet Printer.
- Presented research posters at High PURCS, SNCURCS, and the Joint School of Nanoscience and Nanoengineering in Greensboro.

2013-2014 **Student Researcher. Duke Pain Prevention and Treatment Research Program. Durham, NC 27708. (Project Supervisor: Dr. Laura Porter). Project Focus –** *Investigation of the Role of Emotional Expression on Physical and*

*Psychological Wellbeing in Gastrointestinal Cancer Patients. Used SAS statistical software to examine correlations between self-report measures of emotional expression, perceived pain, and physical, social, and psychological wellbeing.*

- Worked through the North Carolina School of Science and Mathematics Mentorship Program at the Pain Prevention and Treatment Research Program for eight hours weekly.
- Analyzed data acquired from a previous study in which GI cancer patients and their spouses completed various questionnaires.
- Attended weekly meetings with social workers from ongoing studies.
- Wrote literature reviews, a miniature grant proposal, and a final research paper.
- Presented a PowerPoint presentation at a final research symposium at the North Carolina School of Science and Mathematics

**PRESENTATIONS**

*presenting author

Principle Investigator (PI), Postdoc (P), Graduate Student (G), Undergraduate Student (U), Outside Collaborator (C), Technician (T)

**Poster Presentations:**

1. Sarah Colbert* (G), Joost Maier (PI). Plasticity of Retronasal Odor Perception in Young Children. Association for Chemoreception Sciences, 2021.
2. Sarah Colbert* (G), Joost Maier (PI). Feasibility of Novel Protocol for Assessing Multisensory Flavor Perception in Young Children. International Symposium of Olfaction and Taste, 2020.

3. Sarah Colbert* (U), Brian Augustine (PI), Wm. Christopher Hughes (C). Atomic Force Microscopy (AFM) Study of Acrylic Polymer Thin Film Deposition. HPU Research and Creativity Symposium, High Point University. 2016.
4. Sarah Colbert* (U), Brian Augustine (PI), Wm. Christopher Hughes (C). Atomic Force Microscopy (AFM) Study of Acrylic Polymer Thin Film Deposition. Nano Manufacturing Conference, Joint School of Nanoscience and Nanoengeneering, Greensboro, NC. 2016
5. Sarah Colbert* (U), Graham Rich (U), Watson Stahl (U), Brian Augustine (PI), Wm. Christopher Hughes (P), Harry Hu (P). Novel Patterning Techniques of Vapor-Deposited Au Thin Films onto Polymeric Substrates. State of North Carolina Undergraduate Research and Creativity Symposium, High Point, NC. 2015.

**OTHER RELATED WORK EXPERIENCE**

2018-2019 **AmeriCorps Member. Cooking Matters, Wilkes Community Partnership for Children. North Wilkesboro, NC**

- Initiated Cooking Matters programs in Wilkes County to help in the fight against hunger.
- Taught thirteen 6-week cooking courses for low-income parents to empower families to shop for and prepare healthy meals on a budget.
- Coordinated all Cooking Matters programs in Wilkes County.
- Collaborated with local organizations to host programs, and recruited parents from the community to participate in courses.
- Managed budget for groceries and supplies.

- Analyzed surveys and reported data to maintain grant funding.
- Attended national training at Share Our Strength in Washington, DC.