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# **The Effect of Retronasal Odor Adaptation on Flavor Perception**

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# The Effect of Retronasal Odor Adaptation on Flavor Perception

## Abstract

In order to measure the effects of odor adaptation on flavor perception, a study involving the retronasal olfactory system was conducted on ten subjects. Each subject was exposed to an odor (lime, lavender, or control) for a specific amount of time followed by consumption of a lime flavored gummy immediately after odor habituation. The retronasal odor was provided by an odorized pullulan film stuck to the subject's roof of mouth. Subjects rated the intensity of the retronasal odor over time and the intensity of the gummy flavor. After each subject attended three sessions consisting of different odors, data was collected and reviewed to provide results. Results of this study show that exposure to an odor over time decreases that odor's intensity. Results also show that exposure to a specific odor significantly affects flavor perception through adaptation.

## Introduction

One of the most complex human behaviors is flavor perception. Through the studies of neurogastronomy, the importance of how the brain perceives flavor is studied. Sensations in the oral cavity, such as temperature, texture, and odor, are what lead to flavor perception. Retronasal perception is commonly confused with taste sensations, and often, people who have lost their sense of smell usually describe this as a loss of taste. When referring to odor perception, one generally thinks of odors recognized by sniffing. This is called orthonasal perception, but odors are also recognized retronasally. This is provided by the odors one experiences while eating. When food is placed in the oral cavity, odors enter the nasal cavity through the pharynx (Bojanowski & Hummel, 2012).

The gustatory system, in combination with the olfactory system, is responsible for one's perception of food. When something is placed in the oral cavity, receptors located on taste buds interact with the stimuli to relay information to the brain. Although the only "tastes" that the taste buds perceive are salty, sweet, bitter, sour, and savory, the brain receives information based on the pleasantness, identity, texture, temperature, and concentration of the object placed in the oral cavity. Ultimately, this information ends up in the thalamus where it is then transferred to the orbitofrontal cortex to provide information for sensory integration (Purves D, 2001).

In order to perceive a specific smell, input must be transmitted to the brain through the olfactory pathway. When one sniffs a particular odor, this odor molecule travels to the posterior of the nasal cavity and binds to an olfactory receptor. Each of these receptors can be activated by many different molecules, and molecules can activate many different receptors. The unique combination of the two is what allows us to perceive such a wide range of odors. Once an odor molecule binds to a receptor, an electrical signal is given off, which travels to the olfactory bulb, and ultimately the piriform cortex and the thalamus. The piriform cortex aids in identifying the

smell, while the thalamus relays sensory information to the orbitofrontal cortex. Once in the orbitofrontal cortex, olfactory information combines with gustatory information into what we usually assume is our sense of taste (Marin, 2015).

Function Magnetic Resonance Imaging has shown that ortho- and retronasal olfactory activation are different at a cerebral level. Retronasal stimulation activated the base of the central sulcus which correlates to the oral cavity's primary representation center. This provides evidence that retronasal perception is often referred to mouth (H. Yamashita, 1999). For a food related odorant provided orthonasally, brain regions such as the orbitofrontal cortex, insula, and anterior cingulate cortex were deactivated. In contrast, when smells were recognized by retronasal olfaction and taste combined, these areas provided supra-additive responses (Small, Voss, Mak, Simmons, Parrish, & Gitelman, 2004).

The two olfactory pathways, retronasal and orthonasal, provide different cortical responses depending on the route of odor presentation. Studies have shown that a response provided by an odorant unrelated to food (e.g., lavender) was stronger when recognized retronasally compared to orthonasal detection. Food related odors were stronger when recognized orthonasally compared to retronasal detection (Bojanowski & Hummel, 2012). Studies have also shown that in the presence of food related odors, retronasal odor referral to the mouth significantly increased (Lim & Johnson, 2012).

A decreased behavioral response is known as habituation and is caused by a repeated exposure to certain stimuli. The neural process which causes the decrease in response is referred to as adaptation. In a study called "Habituation and adaptation in Humans," it has been reported that odor habituation is relatively quick with adaptation occurring slower at a peripheral level than compared to a cerebral level. The study also states that many characteristics of habituation have been linked to human olfaction specifically (Pellegrino, Sinding, Wijk, & Hummel, 2017). In the following study, retronasal adaptation is measured overtime in order to perceive the effects of flavor perception in humans. It is hypothesized that over a specific amount of time of exposure to a specific odor, flavor perception regarding the same odor should be less intense due to the effects of odor adaptation.

## **Materials and Methods**

### *Subjects*

A total of 10 subjects (5 females and 5 males) were recruited to participate on the campus of the University of Tennessee. All participants were nonsmoking and non-pregnant. Individuals who qualified for the study had no dietary restrictions, no food allergies, and rated their ability to smell higher than average. Participants were compensated to participate. Subjects were asked to refrain from eating or drinking at least 1 hour prior to the testing. Subjects were also asked to not use menthol products the day of testing. The subjects gave a signed informed consent and experimental protocol was approved by the Institutional Review Board.

### *Pullulan Film*

Pullulan was utilized to produce a tacky surface for the retronasal perception study. Pullulan, a linear homopolysaccharide of glucose, is synthesized from the fungus *Aureobasidium pullulans*. It

is sometimes known as  $\alpha$ -(1 $\rightarrow$ 6) linked maltotriose and has an array of distinctive traits such as adhesive properties, fiber forming capacity, and the ability to form compression molding and strong, impermeable films due to its unique linkage patterns. Pullulan products are biodegradable, water soluble, and can make translucent, tasteless edible films (Leathers, 2003). Pullulan (molecular weight = 200,000) was supplied by Hayashibara. A 5g/100mL aqueous solution (200mL) was cast on to 8.5x11.5in trays and allowed to evaporate at room temperature (23 °C) for 24 hours. A sheet of filter paper was then added to the partially dry film. Once the film was completely dry, it was peeled off the trays and cut into rectangular strips (1x3cm). The strips were then stored in a snap-seal bag at room temperature. Ten minutes before testing, 10  $\mu$ L of either lime or lavender odor solution was added to each strip to provide the retronasal odor stimuli. The control group consisted of strips with no odorants added.

#### *Odor and Taste Stimuli*

Lime and lavender natural oils, supplied by LorAnn Oils, were added separately to propylene glycol to order to prepare the odors used for retronasal testing. The lime solution was made at 33.3% (v/v) concentration. Due to the pungency of the lavender odor, the lavender solution was reduced to a 26.6% (v/v) concentration. In order to evaluate similar pleasantness and intensity between the two odors, a pilot test was conducted involving 6 student participants from the University of Tennessee. Each participant was required to rate each of the odors separately on a sliding scale of 1-10 based the odor’s pleasantness and intensity (1 being least intense or least pleasant). The results between the odors in both categories showed the differences between the two odors were insignificant. This provided reassurance that the two odors were similar in intensity and pleasantness in their current concentrations.

#### *Gummies*

A mixture containing glucose syrup, sucrose, sorbitol, and citric acid was heated until forming a homogeneous solution and then added into gelatin dissolved in boiling water. Both mixtures utilized a double boiling system. Lime flavoring (6  $\mu$ L per gummy, 300  $\mu$ L total) was added and the solution was then cast into cornflower dusted, hemi-spherical silicone molds with a volume of 11.2 cm<sup>3</sup> (Table 1). The mold was then allowed to harden in a refrigerator at around 3°C for 24 hours.

<b>Ingredient</b>	<b>Amount (g)</b>
Unflavored Gelatin (Knox Gelatin)	60
Water	155
Glucose Syrup (Caullet)	300
Sucrose (Great Value)	150
Sorbitol (4molar)	15
Citric Acid (Mallinckrodt)	1.5

**Table 1.** Exact amount and brands of each ingredient utilized in gummy preparation.

### *Sensory determination of adaptation testing*

Testing was done in three sessions, all on different days. Subjects were placed in single, secluded booths proctored by his or her own computer screen. Questions with sliding scales directed at the specific task-points in the session were asked in regards to odor adaptation and flavor perception. Total time for testing took around 30 minutes for each day.

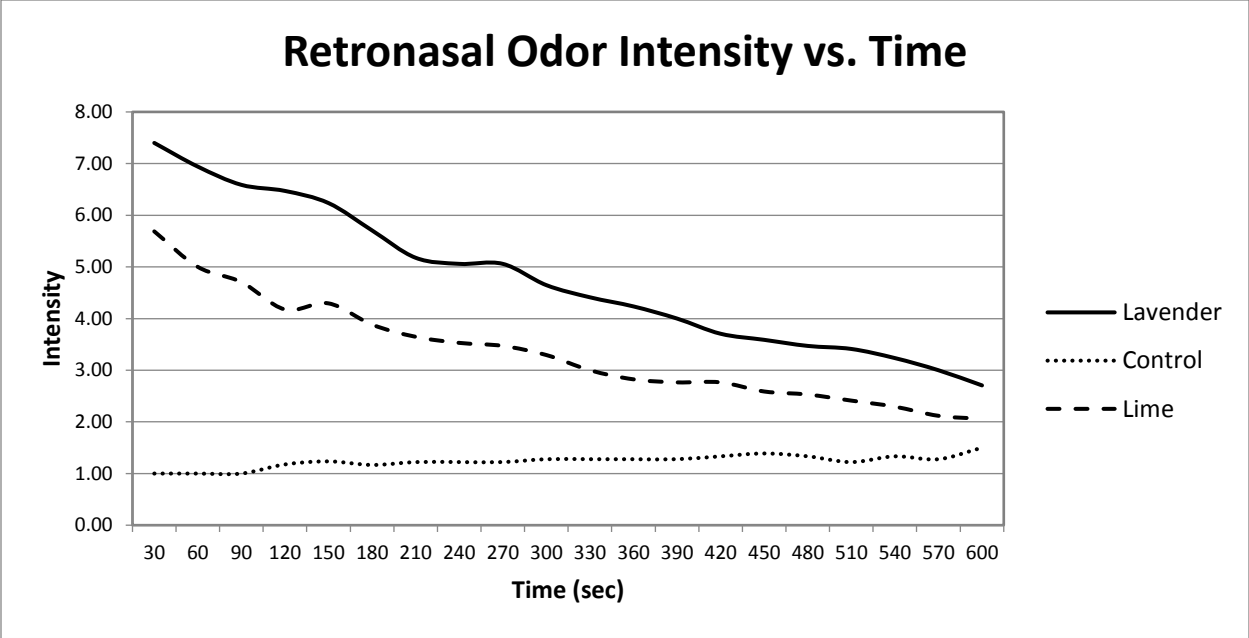
During the first session, subjects were given directions and signed a consent form. This task lasted around five minutes. In the next ten minutes, subjects stuck the lime odorized film to the roof of their mouth and rated the intensity of the odor on a scale of 1-10 as time progressed in intervals. The most intense would be rated at ten, while least intense being one. After the ten minutes were complete, subjects were given a lime gummy and told to rate the intensity of the gummy on similar scale. A five-minute break including lightly salted oyster crackers and water pursued as subjects' olfactory pathway normalized. In the next ten minutes, subjects placed the second film, the control, on the roof of their mouth and rated the intensity over time. Rating of a second lime flavor gummy followed immediately to conclude testing for the first session.

The same protocol followed for the second and third sessions with the exception of the odorants used. In the second session, subjects tested the lavender odorant followed by a control. In the third and final session, subjects tested the lime odorant followed by the lavender odorant. Gummy flavoring remained lime, the same sliding scales were used, and amount of time for each section was kept the same.

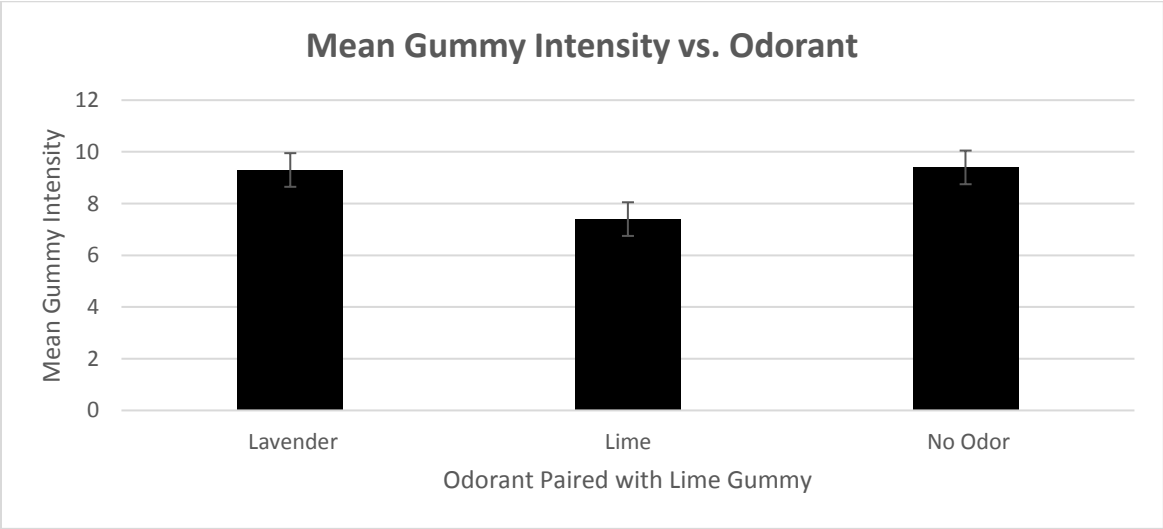
## **Results**

In the beginning of the test, subjects were asked to rank the intensity of the odor that had been placed on the roof of their mouth (lavender, lime, control). Over time, results show that the average retronasal intensity of the lavender and lime odors decreased over time for each subject based on his or her rankings. The lime odor intensity began at a rating of 5.69 and decreased to 2.06. The lavender odor intensity started at a rating of 7.40 and fell to a rating of 2.71. The control of no odor remained fairly constant and around an average of one, which was to be expected (Fig 1).

After the ten-minute period where subjects were exposed to a retronasal odor, a gummy was immediately consumed and subjects were asked to rate the lime flavor intensity of the gummy. As projected, the lime gummy tested with the lime odorant was rated less intense in flavor than the lime gummies tested with the lavender and control odorants (Figure 2). The average intensity of the gummy paired with the lime odor was 7.4. The average intensity of the gummy paired with the lavender and control odors showed a rating of 9.3 and 9.4, respectively. A t-test showed the results from the average intensity ratings of the lime flavor in the gummy were significant. A confidence rating of these results was at the high level of 96.6% (Table 2).



**Figure 1.** Intensity ranking of each retronasal odor (lime, lavender, control) over a ten-minute period in thirty second intervals using a sliding scale of 1-10.



**Figure 2.** Average of lime gummy intensities immediately following a ten-minute time period consisting of retronasal odor stimuli.

<b>p-Vale: 0.034</b> <b>Confidence: 96.6%</b> <b>Stat Method: GLM</b>	<b>Gummy - Lavender</b>	<b>Gummy - Lime</b>	<b>Gummy - No Odor</b>
<b>Mean Score</b>	9.3	7.4	9.4

**Table 2.** Quantification and t-test values from average gummy intensities vs. odorant data.

## Discussion

The present results show that odors received retronasally can cause an effect on flavor perception. The significant decrease in the lime and lavender odors perceived retronasally shows that habituation has taken place in regards to that odor specifically. Supporting evidence of this involves the data of the control odor remaining fairly constant throughout the study. The results also show that odors perceived retronasally can alter flavor perception by causing adaptation. This is proven specifically through the lime flavored gummy paired with the lime odorant. After being exposed to the odor for ten minutes, data shows that the intensity of the gummy had significantly decreased. To further support this theory, the high and constant intensity rating of the lime flavored gummy eaten after exposure to the lavender and control odorants show that the adaptation of these odors had not effected the flavor perception of an unrelated flavor. This proves that habituation had not been present when testing these two scenarios.

Due to time constraints, there are many things that could be improved upon in this study. For example, a total subject count of ten is not what one would consider a large pool for data. In addition, one subject had to be excluded due to not finishing the study. Although the reported data was still significant, a larger amount of test subjects can provide more information and further support this data.

If more testing is to be done, a wider range of odorants and gummy flavors could expand on current findings and knowledge. An example of this would be adding a cherry odor, or changing the gummy flavor to lavender, as it is usually not related to food consumption. One could also test how long the habituation actually lasts after a ten-minute period of adaptation to a certain odor. To do so, a subject would immediately consume a flavored gummy after the exposure to the odor retronasally and rate the first gummy's intensity. Then the subjects would wait varying amounts of time before eating a second flavored gummy and rating the intensity of the flavor. Orthonasal adaptation experiments could be conducted on flavor perception in addition to other suggested studies. Although, this suggested study proposes a problem regarding the ability to seclude the subjects to orthonasal stimulation before or during testing. This could cause less significant results and biased data.



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