The Development of Novel Methods for Assessing Human Olfaction Ability and

the Odor Intensity of Samples

By

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ABSTRACT

Olfactory perception is a complex and multifaceted process that involves the detection of volatile organic compounds by olfactory receptor neurons in the nasal neuroepithelium. Different odorants can elicit different perceived intensities at the same concentration, while direct intensity ratings are vulnerable to framing effects and inconsistent scale usage. Odor perception is genetically determined, with everyone having a unique olfaction "footprint" and sensitivity levels. Genetic factors, age, gender, race, and environmental factors influence olfactory acuity. The olfactory system's complexity makes it challenging to create a standardized comparison system for olfactory perception tests. The COVID-19 pandemic has underscored the importance of olfactory dysfunction, particularly the loss of smell and taste as common symptoms. Research has demonstrated the widespread occurrence of olfactory impairment in various populations, often stemming from post-viral origins, which is the leading cause of permanent smell loss. Utilizing quantitative ranking on a qualitative scale enhances the precision and accuracy when evaluating and drawing conclusions about odor perception and how to mitigate problems caused by external factors. Pairwise comparisons enhance the accuracy and consistency of results and provide a more intuitive way of comparing items. Such ranking techniques can lead to early detection of olfactory disorders and improved diagnostic tools. The COVID-19 pandemic has shed light on the significance of olfactory dysfunction, emphasizing the need for further research and standardized testing methods in olfactory perception.

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CHAPTER 1

ABSTRACT

Olfactory perception begins with olfactory receptor neurons in the nasal neuroepithelium, which are responsible for detecting volatile organic compounds. Even when exposed to the same concentration of different odorants, individuals can perceive varying levels of intensity. However, relying solely on direct intensity ratings can be problematic due to susceptibility to framing effects and inconsistent scale usage.

The genetic component of olfactory perception adds an extra layer of complexity. Each person possesses a unique olfaction "footprint" and sensitivity level, shaped by their genetic makeup. Beyond genetics, age, gender, race, and environmental factors further influence an individual's olfactory acuity. This multifaceted nature of the olfactory system makes it challenging to establish a standardized system for comparing olfactory perception across different individuals.

The COVID-19 pandemic has thrown a spotlight on the importance of understanding olfactory dysfunction, with the loss of smell and taste emerging as prevalent symptoms. Studies have underscored the widespread occurrence of olfactory impairment across diverse populations, often attributed to post-viral causes, which is the primary contributor to permanent smell loss.

To address these complexities, measuring quantitative ranking on a qualitative scale presents a hopeful avenue. Pairwise comparisons, for instance, offer

a more robust method for ranking odors than numerical ranking systems, leading to more accurate and consistent results. Implementing such techniques not only enables the early detection of olfactory disorders but also facilitates the development of improved diagnostic tools.

CHAPTER 2

INTRODUCTION

Olfactory perception, the sense of smell, plays a pivotal role in the sensory experiences of various organisms. It is a highly intricate and multifaceted process, encompassing the ability to detect, discriminate, and interpret a vast array of odorants present in the environment. One fundamental aspect of characterizing olfactory perception is the perceived intensity of odorants, which typically increases with the concentration of the odorant (Purves et al 2001). This intensity can be assessed using numerical rating scales, where individuals assign a numerical value to represent the perceived intensity of a given odorant, but this seemingly straightforward approach to quantifying olfactory perception is not without its challenges and complexities.

One of the central challenges in characterizing olfactory perception is the inherent variability in how individuals perceive and respond to different odorants Jaeger et al 2013). Research by Ignatieva et al. in 2014 demonstrated that different individuals may exhibit varying perceptions in response to a range of odorants, even when presented with the same concentration of the odorant.

The use of numerical rating scales to assess perceived odor intensity leaves assessments susceptible to framing effects, where the context or presentation of the odor can influence how it is perceived and rated. Secondly, inconsistencies in the usage of the rating scale across multiple trials can introduce measurement errors. Lastly, there is the possibility of innumeracy, where

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individuals may struggle to accurately and consistently assign numerical values that adequately represent their olfactory experiences. Imagine a child and adult providing their answers–based on life experience, their versions of a mild score of 2 may be drastically different.

When smelling an odor, volatile organic compounds are detected in the nasal neuroepithelium by millions of olfactory receptor neurons; these are sensory cells that are bipolar, have a dendrite that extends into the nasal lumen and an axon that connects to the olfactory bulb in the brain (Swiegers et al 2005). At the end of each dendrite is an olfactory knob that contains several cilia.

Odorants bind to specific olfactory receptor proteins located on the cilia of the olfactory receptor neurons which triggers the opening of ion channels in the neuron's cell membrane (Swiegers et al 2005). This results in the generation of action potentials, which are transmitted by the neuron to the olfactory bulb which is responsible for processing the odor signal and transmitting it to the primary olfactory cortex, where perception of the odor occurs (Swiegers et al 2005).

The discovery of a large family of genes encoding olfactory epithelium transmembrane proteins by Buck and Axel in 1991 led to the understanding of the molecular mechanism of odorant recognition. Each OR cell expresses only one type of odor receptor, allowing for each odor to send an individual signal to the brain, but individual odor receptors can bind multiple odorants, and individual odorants can stimulate several different receptor cells (Purves et al 2001). This mechanism allows for potentially infinite combinations of odors, enabling the

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olfactory system to discriminate between millions of chemicals. Some odorants can also inhibit olfactory receptors, adding to the complexity of the system (Purves et al 2001).

In this study, we delve into the complexities of olfactory perception by determining a framework for odor intensity by converting numerical rating scales into a qualitative analysis. To achieve this, we employ a binary comparison approach between different odorants to establish a robust ranking system, contrasting it with the traditional numerical rating system. We use a novel research methodology that involves binary odorant comparisons. This approach entails presenting individuals with pairs of odorants and requesting them to rank these odorants based on their perceived intensity, as opposed to assigning numerical ratings.

By directly comparing two odorants, participants are guided by the inherent differences in odor intensity between the two options. Unlike numerical rating scales, which can be influenced by the context or presentation of the odor, binary comparisons provide a more context-neutral assessment, reducing the potential for framing effects to skew results. Inconsistencies in scale usage, a common issue in numerical rating systems, are mitigated through the binary comparison method (specific examples provided in Chapter 3). Participants are guided by the pairwise comparisons, promoting more consistent responses. Lastly, some individuals may struggle with assigning numerical values to their olfactory

perceptions accurately. The binary comparison approach circumvents this challenge by relying on relative rankings rather than absolute values.

CHAPTER 3

HYPOTHESIS

Problem and Motivation

Qualitative analysis for comparing odorant concentration leaves room for confusion, misinterpretation and misrepresentation of the relationship between two odorants. The use of a numerical scale to quantify odor intensity is subject to interpretation by various stakeholders, including the subjects themselves, researchers, and external observers. The central question that arises is how to standardize a testing method to ensure that every participant employs the same baseline for making odorant comparisons. This issue is further complicated by the well-established understanding that the intensity and sensitivity levels of the olfactory system vary significantly among individual human beings. In essence, the problem revolves around the need for a standardized, objective, and universally applicable method to assess and compare odorant concentrations accurately.

The challenges surrounding the standardization of odorant concentration assessments persist for several reasons. Olfactory perception is inherently subjective, as it is influenced by individual variations in olfactory sensitivity, past

experiences, and even psychological factors. What one person perceives as a strong odor may not be the same for another. This subjectivity makes it difficult to establish a universal baseline for odor intensity.

Unlike other sensory modalities like vision or audition, where objective metrics like lumens or decibels can be used, olfaction lacks a universally accepted and measurable unit of odor intensity. Without a standardized metric, it is challenging to quantify and compare odorants objectively. When using numerical scales, participants may interpret the scale differently. For example, what one person rates as a "7" on a scale of 1 to 10 might be perceived differently by another person. This semantic confusion can lead to inconsistent and inaccurate data.

Previous attempts to address this problem have faced limitations such as numerical rating scales and magnitude estimation scales, where participants provide intensity ratings relative to a reference odor, have been used. However, they are susceptible to bias based on the choice of the reference odor, making comparisons challenging.

Consider an experiment where participants are asked to rate the intensity of two different odorants, A and B, on a numerical scale from 1 to 10. Participant X rates odorant A as a "4" and odorant B as a "7." Participant Y, with a more sensitive olfactory system, rates odorant A as a "7" and odorant B as a "9." In this scenario, it is evident that the numerical scale fails to account for the individual differences in olfactory sensitivity. The interpretation of a "4" or "7" varies widely

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between participants, making it challenging to draw accurate conclusions about the relative intensities of odorants A and B.

Without a comparison system, participants' answers would vary greatly depending on their unique smell perception and other framing effects such as sickness or emotional distress which can lead to inaccurate and unreliable data. A binary comparison system, on the other hand, provides participants with a choice between lesser, greater, or equal strength in odorant intensity by eliminating subjective variations and standardizing responses. This methodological approach, rooted in the principles of sensory perception, contributes to the production of precise and consistent olfactory data, essential for scientific research and empirical investigations within the field. By offering participants a clear reference point for discerning differences in odorant intensity, the binary comparison system minimizes the potential confounds stemming from individual differences in olfactory sensitivity, health-related factors, or emotional states. Using a ranking scale to compare odorants makes it easier to conclude whether an individual may experience any olfactory disorders, such as anosmia, because it provides a more consistent way to assess and compare olfactory function across individuals because they are presented with just two ways to rank odorants versus a range of numbers. The direct comparison of odorants enables researchers and clinicians to identify patterns and subtleties in individuals' olfactory experiences, aiding in the diagnosis and understanding of olfactory disorders. If the answers of one participant do not follow the trend of the others involved in the study, this

could be due to a disorder or genetic factors and can lead to early detection of olfactory disorders or more accurate diagnosis and treatment (Ignatieva et al 2014)**.** Suppose a person cannot smell most aldehydes and this is revealed by them ranking all of the aldehydes lower than some reference odorant in the ranking task, whereas most people (without that anosmia) would not show this pattern. This technique is also useful in the validation of conditions or disorders in longitudinal studies. By repeating the ranking scale after a period of time, it is possible to validate the presence of a condition or disorder. This can be extremely helpful in monitoring the progression of the condition and in evaluating the effectiveness of treatment.

The null hypothesis is that intensity and sensitivity levels do not significantly affect smell preferences and regardless of these factors, there are no trends in preferences for subjects with similar sensitivity and intensity levels, environmental or health circumstances, such as experiencing COVID-19.

By comparing the results obtained through this method with those obtained through traditional numerical rating scales, we can evaluate the reliability and validity of each approach. This research holds the potential to deepen our comprehension of how organisms perceive and interpret the intricate world of scents that surround them. By improving our methods of odor intensity quantification, we can gain a more precise understanding of the nuanced interplay between subjective and objective factors in olfactory perception, thereby advancing both basic and applied olfaction-related sciences.

COVID-19 and Smell Loss

Over the past two decades, humanity has encountered two significant occurrences of coronavirus infections, namely the severe acute respiratory syndrome (SARS) outbreak in 2002 and the Middle East respiratory syndrome (MERS) in 2012. The emergence of the COVID-19 outbreak can be traced back to December 2019, specifically in Wuhan, China, where patients with complex pneumonia exhibited initial symptoms. By January 2020, angiotensin-converting enzyme 2 (ACE2) was identified as the functional receptor for the SARS-CoV-2 virus, found in various human organs, including the central nervous system (Mullol et al 2020).

The typical manifestations associated with COVID-19 comprise nonspecific feelings of discomfort, elevated body temperature, respiratory distress, and a persistent cough. Additional indications would include musculoskeletal and articular discomfort, inflamed throat tissues, episodes of queasiness or emesis, gastrointestinal disturbances, as well as nasal symptoms, particularly involving olfactory and gustatory dysfunction (Mullol et al 2020). Analogous to other upper respiratory tract viral infections (such as the common cold or influenza), anosmia is a common symptom among individuals affected by COVID-19. However, in some instances, individuals who are asymptomatic may still experience an abrupt, severe, and exclusive loss of olfactory and/or gustatory perceptions (Mullol et al 2020).

The process of olfaction involves the passage of odorants carried by nasal airflow, which eventually reach the olfactory neuroepithelium. This neuroepithelium spans approximately 8-10 cm2 of the olfactory cleft, located in the upper regions of the nasal cavities (Ignatieva et al 2014). Within this neuroepithelium, odorants bind and activate specific olfactory receptor (OR) proteins. The olfactory neuroepithelium comprises a substantial population of receptor neurons, estimated to be between 5 to 30 million. These receptor neurons express a diverse range of olfactory receptors, with up to 350 different ORs identified to date (Mullol et al 2020).

The OLFaction in CATalonia (OLFACAT) survey conducted a comprehensive investigation into the prevalence of olfactory dysfunction within the general population of Europe. The survey revealed that nearly 20% of European citizens, equivalent to approximately 82 million individuals in the European Union (EU), experienced some form of olfactory impairment at some point in their lives (Mullol et al 2020). This impairment manifested as either partial loss of smell, known as hyposmia (occurring in 1 out of 5 individuals), or complete loss of smell, referred to as anosmia (occurring in 1 out of 300 individuals).

Similarly, a recent epidemiological study conducted in the United States examined the prevalence of smell and taste impairment among the adult population. The findings indicated a prevalence rate of 13.5% for smell impairment, 17.3% for taste impairment, and 2.2% for combined impairment of both smell and

taste (Mullol et al 2020). These results shed light on the significant impact of olfactory dysfunction on a substantial portion of the US population.

Loss or impairment of the sense of smell can have far-reaching consequences on an individual's quality of life. It not only affects the ability to perceive pleasant aromas but also diminishes the capacity to detect potentially harmful environmental elements such as fires, gas leaks, or spoiled food. Additionally, it can lead to a reduction in appetite, eventually resulting in malnutrition. The immune system can also be compromised, leaving individuals more susceptible to infections, and worsening existing medical conditions. Importantly, studies have found an association between loss of smell and increased mortality rates, highlighting the serious implications of this sensory dysfunction (Mullol et al 2020).

Acquired loss of smell, also known as anosmia, can be attributed to various causes, with respiratory viral infections such as coronaviruses, traumatic brain injury, upper airway inflammation (rhinitis, rhinosinusitis), and neurodegenerative diseases being major contributors. Minor causes include intracranial/sinonasal tumors, drug-induced effects, exposure to toxic substances, irradiation, or iatrogenic factors. It is noteworthy that loss of taste during upper respiratory tract infections has not been extensively studied.

In the context of viral infections, such as the common cold or acute rhinosinusitis, loss of smell is a prevalent symptom, affecting over 60% of individuals (Mullol et al 2020). Typically, this olfactory impairment is transient, lasting for about 3 to 7 days. However, in some cases, post-viral etiology can lead to

permanent loss of smell. The underlying mechanisms behind smell loss during viral infections are multifactorial. They involve a combination of factors such as mechanical obstruction of odorant transmission within the olfactory cleft due to mucosal inflammation (often associated with a cytokine storm) and shedding of the olfactory neuroepithelium, resulting in neurodegeneration (Mullol et al 2020). These factors disrupt the binding of odorants to olfactory receptors (ORs).

CHAPTER 4

METHODS

Binary Comparison Validation Test

In this study, a total of 2 subjects were recruited to participate in olfactory perception assessments. Each subject was presented with a set from 215 pairs of stimuli. These randomly selected pairs were constructed from a pool of 6 unique odorous molecules. Additionally, an odorless control stimuli was included (mineral oil and water). The stimuli were prepared at 6 ten-fold serial dilutions, resulting in a total of n=6 concentrations for each odorant. The odorants were 2-decanone, 2-hexanol, acetophenone, ethyl butyrate, linalool and geraniol.

For each stimulus pair, the subjects were asked to rank the perceived intensity of the first odorant relative to the second odorant (odorant "A" and odorant "B", respectively). The ranking responses allowed the subjects to use the following descriptors: ">>" indicating that odorant A was perceived as significantly stronger, ">" indicating a moderate difference in intensity with odorant "A" being stronger, "=" indicating equal intensity, "<" indicating a moderate difference with the odorant "B" being stronger, and "<<" indicating that the odorant "B" was perceived as significantly stronger. To validate the ranking responses, direct numerical ratings trials were also included, where the subjects provided numerical intensity ratings for each stimulus.

The collected data from both the ranking responses and direct numerical ratings were pooled together. The data were then analyzed using PyTorch, a

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machine learning library, to fit a modified Bradley-Terry model. The modified model incorporated sigmoidal concentration versus intensity functions, which aimed to accurately predict the distribution of ranking relations across the trials for each stimulus pair. By fitting the data to the model, optimal parameters were obtained, enabling the estimation of the intensity functions for the stimuli.

There is an S-shaped sigmoidal curve graph that follows the intensity of just one odorant. If two odorants are at the same intensity values in their respective curves, it is difficult to distinguish the two based on intensity. However, if one was at a higher value on its curve than the other was, it would be easier to distinguish on the basis of intensity. In the early part of the S-shaped curve, odorants have weak intensity and are thus hard to detect, which is indicated by the first flat part of the S shaped curve (see Figure 2). At the point at which the curve starts to go up, this is the concentration level where the individual first detects significant intensity of the odorants.

When the curve levels out, this is the point of maximum sensitivity. The individual would not be able to distinguish between two odorants if both odorants reached a similar maximum level of intensity levels of concentration. Measuring the strength of each odorant at various concentrations allows the analysis of the overall concentration and intensity relationship between two pairs.

3 Part Smell Test Experiment

The study recruited a total of 13 participants who live in the Phoenix area. Participants were informed about the study and signed a consent form before

participation. Participants were given three tests in the order of Aroma-T, Scent-CheckPro and Sniffin Sticks (see below for details). Before the Aroma-T test, participants completed a survey to collect data about age, demographic, health history, current health status, COVID-19 history, emotional well-being and a personal assessment of their own smelling abilities. The survey was self-administered and took approximately 10 minutes to complete. Data collected from the survey was recorded anonymously.

The Aroma-T test was administered immediately after the survey. Each participant was presented with 16 odorants on a card with plastic film over each odorant. Participants were asked to smell the odorant labeled "0" first to get a baseline scent, then proceeded to do a smell test comparison between two given smells. The test provided two odorants which the participant would smell, then answered which of the two was a stronger smell in their opinion. Participants continued answering questions comparing two odorants until the test was finished.

The ScentCheckPro test was administered immediately after the Aroma-T test. The ScentCheckPro is a "scratch-n-sniff" test and is designed to assess an individual's ability to smell, their success in correctly identifying a smell when presented with multiple options.

The Sniffin' Sticks test was administered last. The Sniffin' Sticks test is a three-way odorant comparison test and is designed to assess the sensitivity of an individual's sense of smell. Sniffin' Sticks test is a test that ranges from baseline

odors that are weak and difficult to detect odors that are strong and obvious.

Data collected from the survey, Aroma-T, ScentCheckPro and Sniffin Sticks tests

were recorded in an Excel spreadsheet and analyzed and interpreted using a

heat map that was designed in Python using the Seaborn library and the graphs

and figures below.

ScentCheckPro
- Definition: Scratch-n-Sniff (odor detection)
- Technique: using multiple choice answers (cinnamon, lemon, pine, etc) to identify the odor after scratching off the film
- Strength: Multiple choice and gives participants the option of "none"
- Weakness: Lowest amount of questions (only 4) and based on participants recognizing specific smells, relies on the subject's specific knowledge of the names of odor

Aroma-T
- Definition: Binary comparison (Odor discrimination)
- Technique: flipping up two panels of odorants and smelling both to determine which of the two is stronger
- Strength: Gives a baseline smell to compare the two odorants to and determine which is stronger and low production costs
- Weakness: Compares low concentrations that may be hard to distinguish

Sniffin Sticks'
- Definition: Three-way comparison test (Odor Threshold)

- Technique: using 3 different pens and ordering them from weakest to strongest in odor

- Strength: Allows participants to order from greatest to least, designed for one of the three to be strongest and reduce participant confusion

- Weakness: Took more time to arrive at a final answer and more subject to participant guessing

CHAPTER 5

RESULTS

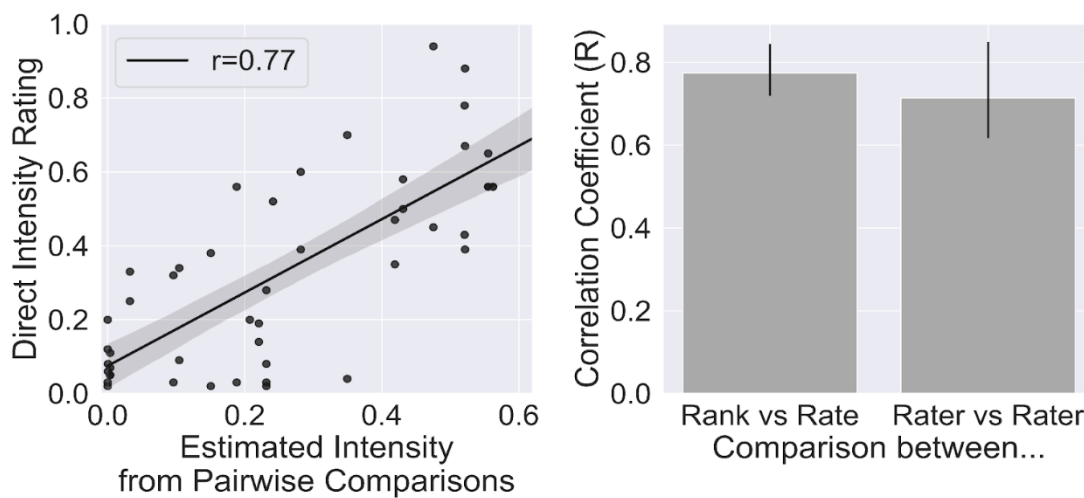## *Binary Comparison Validation Test*

Figure 1. Binary Comparison Validation Results in Qualitative Form. The figure

presents the results of a binary comparison validation study, aiming to establish

the relationship between "Estimated intensity from pairwise comparisons" and

"direct intensity ranking" for a range of sensory stimuli. The X-axis represents the

estimated intensity values obtained through pairwise comparisons and fit to a

modified Bradley-Terry model to obtain latent intensity estimates. The Y-axis de-

picts the corresponding direct intensity rankings, ranging from 0 to 1. The second

panel represents the correlation coefficient for the comparison between the rating

vs ranking system. Raters followed a similar pattern for olfactory intensity for both
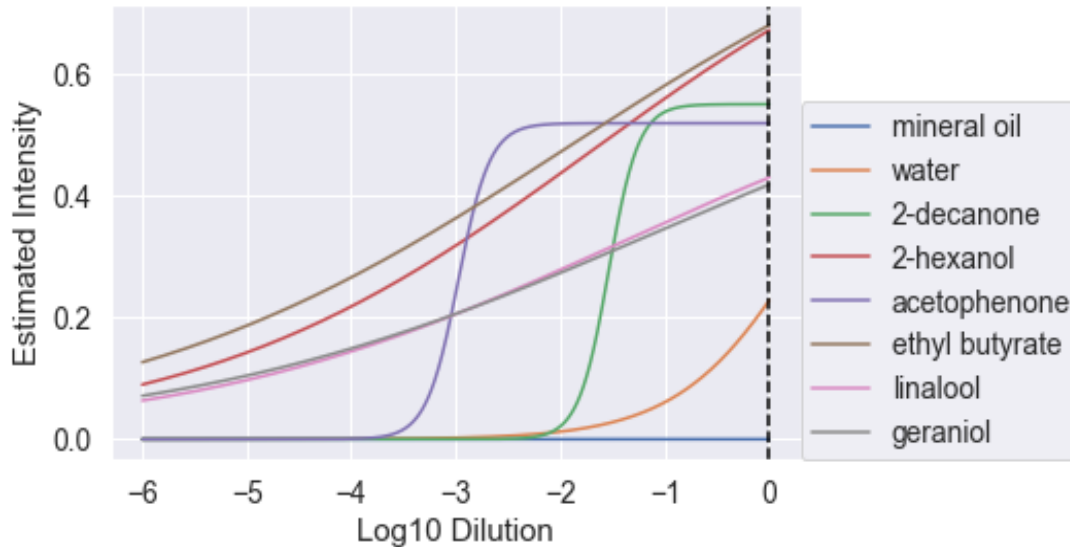
systems.

Figure 2. Estimated Odorant Intensity at Various Levels for Binary Compari-

son. *This figure presents the sigmoidal dose-response curves depicting the esti-*

mated intensity of eight distinct odorants, namely 2-decanone, 2-hexanol, aceto-

phenone, ethyl butyrate, linalool, geraniol, water, and mineral oil. The x-axis rep-

resents the dilution levels of each odorant, while the y-axis shows the corre-

sponding estimated intensity as perceived by a panel of odor assessors. The

data points in Figure 1 exhibit a positive relationship between the two variables,

suggesting that as the estimated intensity increases from pairwise comparisons,

the direct intensity ranking also tends to rise.

3 Part Smell Test Experiment and Sniffin Sticks' Validity

Table 1: *Smell Test Participant Statuses (Previous POSITIVE COVID-19 Diagnosis)*

	Alcohol Use	*Tobacco/ Nicotine Use*	*Health concerns*	*SNOT-22 Score*	*Percentage Correct for Scent Check-Pro*	*Aroma-T Lowest Concentration Detected*	*Sniffin' Sticks Thres-hold (log value)*
F, Asian 20 (1)	*No*	*No*	*Asthma*	*40/110*	*100%*	*0.001*	*-2.00*
M, Mixed 23 (2)	*Yes*	*Yes*	*None*	*0/110*	*75%*	*0.003*	*-2.60*
F, Hispanic 23 (3)	*Yes*	*No*	*Cannot taste/smell as well as before*	*51/110*	*75%*	*0.3*	*0.5*
M, White 66 (4)	*Yes*	*No*	*None*	*9/110*	*75%*	*0.01*	*0.75*
M, Hispanic 24 (5)	*No*	*No*	*Cannot taste/smell as well as before*	*13/110*	*63%*	*0.0003*	*0.50*

Table 2: *Smell Test Participant Statuses (Never Experienced COVID-19)*

	Alcohol Use	*Tobacco/*	*Health concerns*	*SNOT-22 Score*	*Percentage Correct*	*Aroma-T Lowest Concentr-*	*Sniffin' Sticks Thresh-*

		Nicotine Use			for Scent Check-Pro	ation Detected	old (log value)
M, Asian 18 (6)	No	No	None	2/110	88%	1×10^{20}	-0.70
M, Hispanic 24 (7)	Yes	Yes	None	24/110	63%	0.1	-0.40
M, White 24 (8)	Yes	Yes	Asthma	7/110	50%	0.0001	0.50
F, Black 24 (9)	Yes	Yes	Asthma	34/110	88%	1×10^{20}	-1.65
M, Black 44 (10)	Yes	Yes	Asthma	2/110	38%	0.01	-0.40

****The Snot-22 test considers various symptoms, the severity of the problem and how often it happens. Each symptom is rated on a scale of 0-5. For a complete list of symptoms, please check the appendix.**

Table 3: *Participant Average Rankings Among Tests (Summary of Rankings)*

F, Asian 20 (1)	1	4	2
M, Mixed 23 (2)	4	6	1
F, Hispanic 23 (3)	6	10	7
M, White 66 (4)	5	8	9
M, Hispanic 24 (5)	7	3	10

	Scent CheckPro ranking	Aro-maT ranking	Sniffin' Sticks Ranking
M, Asian 18 (6)	2	2	4
M, His-panic 24 (7)	8	9	6
M, White 24 (8)	9	5	8
F, Black 24 (9)	3	1	3
M, Black 44 (10)	10	7	5

Participant Rankings for All Three Smell Tests

Participants WITH Past COVID Positive

Participant Rankings for All Three Smell Tests

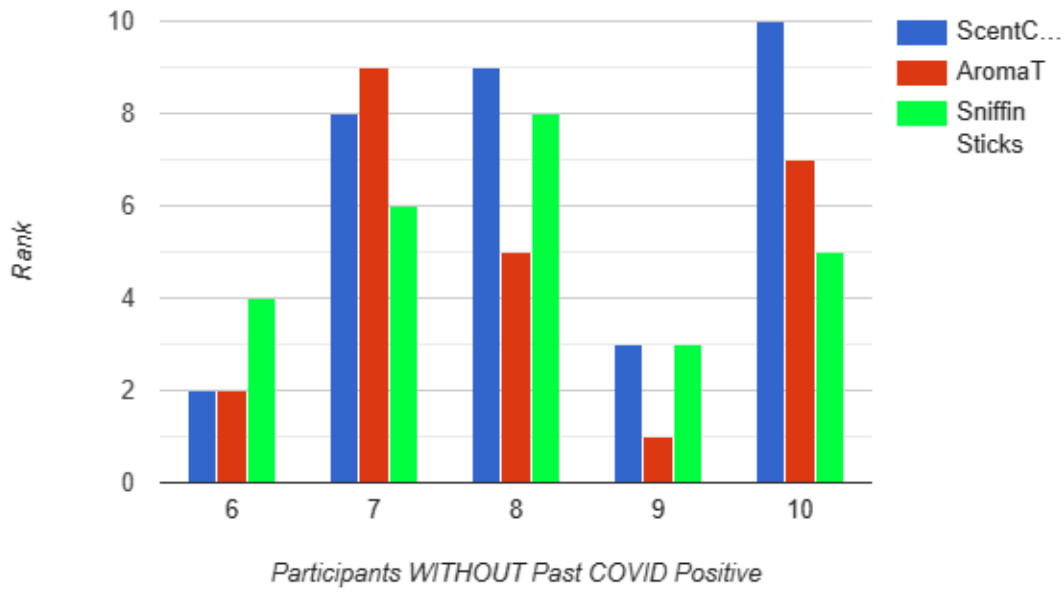*Participants WITHOUT Past COVID Positive*
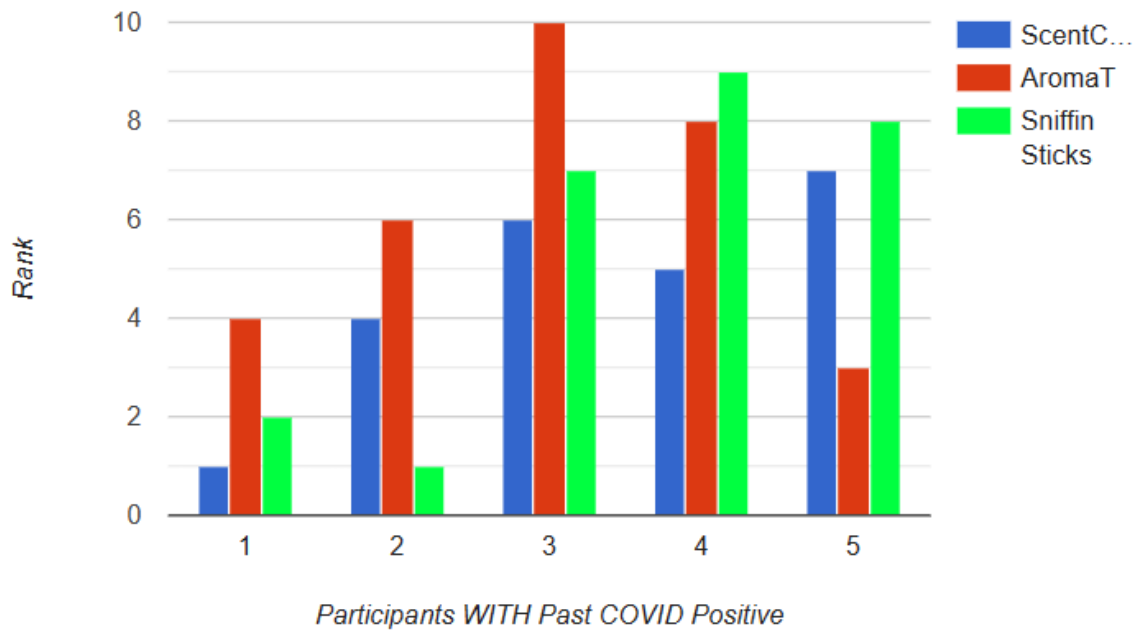
Figure 3A and B. Smell test participant rankings for each test. *The information from the table was used to formulate a bar graph that shows the rankings of each person for each test. Participants were ranked 1-10 on their performance on the three smell tests (correct answers).*

Table 1 presents the results for participants with a previous positive diagnosis of COVID-19. All participants in this group exhibited some degree of smell dysfunction, with varying severity. The SNOT-22 scores ranged from 13/110 to 51/110, indicating a wide range of symptom severity related to nasal and sinus issues. The ScentCheckPro scores ranged from 5/8 to 8/8, suggesting impairment in olfactory perception for some participants. The concentration levels at which the odor could be detected ranged from 0.0003 (lowest concentration) to 0.3 (highest). All participants reported a reduced ability to taste or smell as well as before.

Table 2 presents the results for participants who have never experienced COVID-19. The SNOT-22 scores ranged from 2/110 to 34/110, indicating a relatively lower severity of nasal and sinus symptoms compared to the COVID-19 positive group. The ScentCheckPro scores ranged from 4/8 to 7/8, suggesting some degree of olfactory dysfunction in this group as well. The ability to detect the lowest concentration of Aroma-T varied from 0.0001 to 1×10^{-20}. The Sniffin' Sticks concentration threshold was measured in log, meaning the lesser the number, the higher of a threshold the person is able to detect for the given odorants. The range was -2.60 to 0.75. After running a T-test analysis on this data, it

was found that the p-value was 1 and these tests were not sensitive enough to effectively determine if intensity and sensitivity levels significantly affect taste preferences and regardless of these factors. The null hypothesis was accepted.

The findings of this study indicate a strong correlation between the participants' performance in the first two tests (Aroma-T and ScentCheckPro), and their performance in the Sniffin' Sticks test. Specifically, it was observed that individuals who scored in the top 50% in both the Aroma-T and ScentCheckPro tests also scored among the top 50% for the Sniffin' Sticks test. Similarly, participants who scored in the lower 50% in the initial two tests also scored in the lower 50% for the Sniffin' Sticks test.
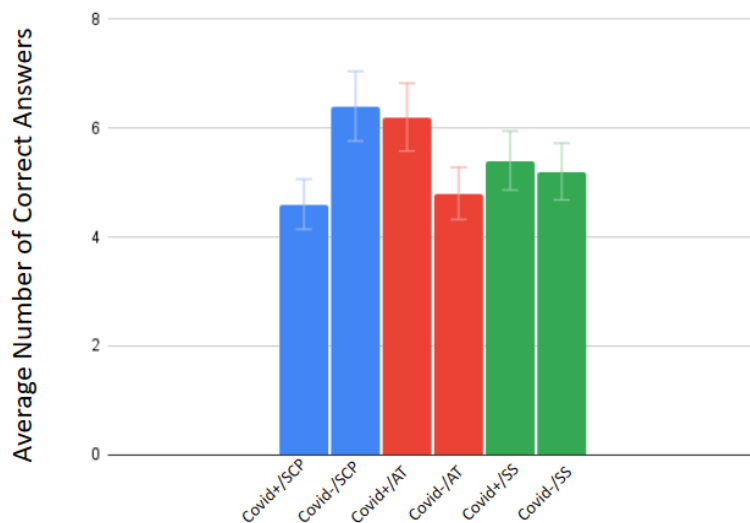

Figure 3C. Mean values for participants correct answers on each test. *This figure shows the average number of correct answers on each of the three smell tests by both groups of participants. The blue bars represent positive*

and negative COVID groups for ScentCheckPro, red bars represent positive and negative COVID groups for Aroma-T and green bars represent positive and negative COVID groups for Sniffin Sticks'. The sample size was 10 participants (5 in each group). The bars represent standard error.
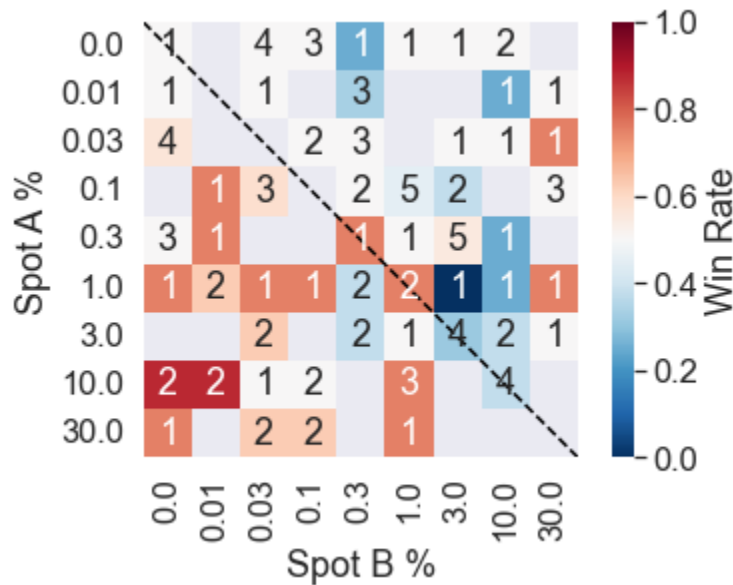

Figure 4. Aroma-T Test Validation through pairwise comparative analysis. *The odorant concentration map was generated to depict the detected concentration levels for each pair of odorants and to determine which odorant was perceived as stronger. The map utilized red squares to denote instances where "Spot A" (the first odorant in each pair) was determined to have a stronger concentration, while blue squares represented cases where "Spot B" exhibited a stronger concentration. The map also featured gray squares, which indicated pairs that were not evaluated in the specific test, implying that certain combinations were not included in the study design. White*

squares appeared in regions where both concentrations were very low, as well as in areas where one odorant was clearly stronger than the other. Seeing more red or blue would mean there were clear differences between the odorants and one was ranked as stronger than the other. White squares means the odorants exhibited similar intensities and one was not stronger than the other.

In Figure 4, the resulting map of odorant concentrations highlighted the detected concentration levels for each pair of odorants and identified which odorant was perceived as stronger. The map exhibited red squares to represent cases where "Spot A" (the first odorant in each pair) was determined to be stronger, while blue squares indicated cases where "Spot B" had the stronger concentration. The majority of red squares were observed in the bottom left half of the map, while the top right half contained a majority of blue squares. This pattern indicated that participants consistently identified the stronger odorant during the binary comparison testing.

Gray squares on the map represented pairs that were not evaluated in this specific test, implying that some combinations were not included in the study design. Dark red squares were present in the bottom left-hand corner of the map, indicating instances where Spot A had a much stronger concentration than Spot B. Conversely, darker blue squares were observed in the top right half of the map, representing cases where Spot B exhibited stronger concentrations than Spot A. White squares, on the other hand, indicated pairs where the odorants

were perceived to have equal concentrations. Interestingly, white squares appeared in some regions where both concentrations were very low, but also in areas where one odorant was clearly stronger than the other. This is not a random chance as the white squares are grouped in one specific area, where the odorants both exhibit similar intensities.

Comparing the results between the two groups, individuals with a previous positive COVID-19 diagnosis generally demonstrated more severe olfactory dysfunction compared to those who have never experienced COVID-19. The COVID-19 positive group reported higher SNOT-22 scores, indicating a greater impact on nasal and sinus symptoms, and a more noticeable reduction in the ability to taste or smell. However, it is worth noting that even individuals in the never-experienced COVID-19 group exhibited some degree of olfactory dysfunction, suggesting the presence of other contributing factors.

These results suggest that there is consistency in individuals' olfactory abilities across different olfactory tests. The Sniffin' Sticks test is meant to be used to assess olfactory thresholds, and the strong association observed in this study provides evidence for its reliability as a measure of olfactory function.

The findings also revealed an interesting exception to the overall pattern. Only 2 participants did not follow the general pattern of scoring the same 50% half for both the Aroma-T and ScentCheckPro tests. These two participants were #2 and #5, who scored in the top 50% for the Aroma-T test, but not for the other two tests.

CHAPTER 6

DISCUSSION

Binary Comparison Validation Test

The use of ranking responses and direct numerical ratings in conjunction with the modified Bradley-Terry model allowed for a comprehensive analysis of olfactory perception. The obtained intensity functions provide quantitative information regarding the concentration-dependent perception of odorants, shedding light on the mechanisms underlying olfactory perception. The integration of machine learning techniques, such as PyTorch, facilitated the fitting of the model and the estimation of optimal parameters, further enhancing the accuracy of the predictions.

The obtained parameters from the fitted modified Bradley-Terry model provided valuable insights into the relationship between stimulus concentration and perceived intensity. The sigmoidal concentration versus intensity functions derived from the model were found to best predict the observed distribution of ranking relations for each stimulus pair across the trials.

The validity of the binary comparison method versus the numerical method for determining odor intensity was proven with a correlation coefficient of 0.77. This shows that over 50% of the variance in quantitative odor intensity ratings can be explained by qualitative odor intensity rankings. The bar graph

indicates an upward trend in "less than" comparisons and an upward trend for "greater than" with the concentration levels becoming more and more diluted. Odorants with higher concentration level were predicted to result in more "greater than" comparisons, while odorants with lower concentrations were predicted to result in "lower than" determinations when compared to other odorants.

While some odorants had a steady upward trend for the intensity level they exhibited for each concentration level (2-hexanol, ethyl butyrate, linalool and geraniol), there were a few that remained low intensity and then suddenly became stronger after high higher dilution (2-decanone and acetophenone). Reasoning for this could be related to the chemical structure or naturally light odor of the compound.

3 Part Smell Test Experiment Analysis

The study aimed to investigate the olfactory function of individuals with a previous positive diagnosis of COVID-19 compared to those who have never experienced COVID-19. Thirteen participants from the Phoenix area were recruited for this study. The participants underwent three tests in a specific order: the Aroma-T test, the ScentCheckPro test, and the Sniffin Sticks test. Data collected from these tests, along with information obtained from a self-administered survey, were recorded and analyzed.

The Aroma-T test provided insights into participants' ability to detect and compare different odors. Results from Table 1 indicated that participants with a previous positive COVID-19 diagnosis exhibited a wide range of symptom

severity related to nasal and sinus issues, as reflected in the SNOT-22 scores. It is important to note that the participant with the highest SNOT-22 score reported pre-existing asthma, suggesting that underlying health conditions may contribute to the severity of olfactory dysfunction.

The ScentCheckPro test assessed participants' olfactory perception, accuracy in identifying scents, and discrimination abilities. Participants in Table 1, regardless of the specific scent identification scores, demonstrated impairment in olfactory perception, indicating that individuals with a previous positive COVID-19 diagnosis continued to experience olfactory dysfunction even after the resolution of acute infection.

Regarding olfactory sensitivity, as measured by the lowest concentration of Aroma-T detected, participants in Table 1 exhibited a range of olfactory sensitivities. Some individuals demonstrated a higher sensitivity to odorants, detecting them at lower concentrations, while others had a lower sensitivity. These findings suggest that olfactory sensitivity among individuals with a history of COVID-19 can vary significantly.

In Table 2, data for participants who have never experienced COVID-19 were presented as a reference group. Although the severity of nasal and sinus symptoms, as indicated by the SNOT-22 scores, was relatively lower in this group compared to the COVID-19 positive group, participants in Table 2 still exhibited some degree of olfactory dysfunction. This observation suggests that factors other than COVID-19 may contribute to olfactory impairment, as individuals

without a previous COVID-19 diagnosis also demonstrated variations in olfactory sensitivity.

These findings align with previous research suggesting that COVID-19 can lead to persistent olfactory dysfunction. The variability observed in olfactory measures among participants in both groups highlights the influence of individual differences and potential underlying factors that may contribute to the severity and persistence of olfactory impairment.

It is important to acknowledge the limitations of this study. The sample size was relatively small, which may limit the generalizability of the findings. Additionally, the study focused on participants from the Phoenix area, which may introduce geographical biases. Future research with larger and more diverse samples is warranted to further explore the long-term effects of COVID-19 on olfactory function.

This study provides evidence of persistent olfactory dysfunction in individuals with a previous positive COVID-19 diagnosis. The severity of olfactory impairment varied among participants, and the presence of other health conditions, such as asthma, may influence the severity of symptoms. Individuals without a previous COVID-19 diagnosis also exhibited olfactory dysfunction, suggesting the involvement of additional factors. These findings contribute to our understanding of the long-term effects of COVID-19 on the olfactory system and emphasize the importance of comprehensive assessments and ongoing support for individuals experiencing olfactory dysfunction following COVID-19.

The findings suggest that individuals with a previous positive COVID-19 diagnosis experience more severe olfactory dysfunction. This highlights the potential long-term effects of the virus on the sense of smell. The higher SNOT-22 scores in the COVID-19 positive group indicate a greater impact on nasal and sinus symptoms. This implies that COVID-19 might not only affect the olfactory receptors directly but also lead to broader nasal and sinus complications. The notable reduction in the ability to taste or smell reported by the COVID-19 positive group suggests that the virus might influence both taste and smell senses, leading to a more profound impairment in flavor perception. The observation of olfactory dysfunction in individuals who have never experienced COVID-19 suggests the presence of other contributing factors. This finding underscores the need to investigate other potential causes of olfactory dysfunction beyond the virus.

Alternative explanations for the results may include viral damage and inflammatory response; COVID-19 is known to infect the olfactory epithelium and cause damage to olfactory receptors, which could explain the more severe olfactory dysfunction in the COVID-19 positive group and the virus triggers an inflammatory response that may affect the nasal passages and olfactory nerves, leading to a more pronounced impact on smell and taste. However, olfactory dysfunction is a multifactorial condition, and individual responses to viral infections can vary widely. Other factors like age, genetics, or exposure to environmental pollutants could contribute to the differences observed.

Conducting longitudinal studies could help assess the progression of olfactory dysfunction in individuals with a history of COVID-19 over an extended period to better understand the long-term effects. Controlled experiments with animal models or cell cultures could help elucidate the specific mechanisms through which COVID-19 affects olfactory function. Comparative studies with other viral respiratory infections could help differentiate the specific impact of COVID-19 on olfactory function compared to other common viruses. Investigating potential interventions or treatments for olfactory dysfunction in both COVID-19 positive and never-experienced COVID-19 groups could provide valuable insights into managing this condition.

Aroma-T Validity

In order to assess the validity of the ArOMa-T test, we also measured the probability that an objectively higher odorant concentration would be perceived as stronger than a lower concentration. If this probability is not closely related to relative concentrations, then it would be difficult to draw conclusions from threshold measurements, since the notion of a threshold implies that detection gets easier as nominal concentration increases. The resulting graph exhibited an S-shaped sigmoidal curve, reflecting the varying concentrations of odorants being compared. This approach provided a quantitative basis for qualitative analysis by converting binary comparisons into numerical values that could be interpreted on a qualitative scale.

The qualitative scale employed in this study used a set of values (1, 0.75, 0.5, 0.25, and 0) to represent the relative strength of odorant concentrations. This scale, which involved greater-than, lesser-than, and equal-to comparisons, provided a more structured and standardized approach than allowing participants to assign arbitrary numbers with personal meanings.

The use of binary comparisons and the construction of the concentration map provided valuable insights into the perception of odorant strengths among participants. The observed patterns in the map shown in Figure 3 demonstrate the ability of individuals to discriminate between different concentrations of odorants. This approach offers a quantitative basis for qualitative analysis, allowing for a more nuanced understanding of participants' perceptions of odorant concentration levels.

One possible explanation for this exception could be individual variations in olfactory perception and processing. It is known that olfaction is a complex sensory system, influenced by various factors such as genetics, cognitive processes, and prior experiences. These two participants (number 2 and 5) might possess a unique combination of these factors, leading to a disparity in their performance across different olfactory tests. Further investigation would be necessary to determine the specific factors contributing to this discrepancy and whether it represents an isolated occurrence or a distinct subgroup of individuals with atypical olfactory profiles.

Another aspect worth considering is the specific characteristics and requirements of each test. The Aroma-T and ScentCheckPro tests might assess different aspects of olfactory function, such as odor identification, discrimination, or memory. The Sniffin' Sticks test, on the other hand, primarily focuses on olfactory thresholds. It is plausible that individuals with superior odor identification abilities (as reflected in their performance on the Aroma-T test) might struggle with olfactory thresholds (as indicated by their lower scores on the Sniffin' Sticks test). Similarly, individuals who excel in odor discrimination (as suggested by their performance on the ScentCheckPro test) might not perform as well on olfactory thresholds. This discrepancy could be attributed to the distinct cognitive processes and neural mechanisms involved in each aspect of olfaction.

It is worth noting that the limitations of this method include potential individual variations in odorant perception and the influence of subjective factors on participants' judgments. Additionally, the sample size and the specific odorants used in the study may limit the generalizability of the findings. Further research with larger and more diverse samples, along with a wider range of odorants, would be valuable to validate and expand upon the findings of this study.

The binary comparison method was used to assess the AromaT validity, while AromaT, Sniffin' Sticks, ScentCheckPro and the resulting concentration map provided insights into participants' ability to discriminate between different concentrations of odorants and relate that to health history. The S-shaped sigmoidal curve observed in the graph reflects the concentration patterns, while the

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color-coded map highlights the stronger odorant in each pair. This approach offers a quantitative framework for qualitative analysis and enhances our understanding of odorant perception. Further research in this area can help uncover additional factors that influence odor perception and contribute to the development of more comprehensive odor assessment methods.

CHAPTER 7

CONCLUSION

The sense of smell plays a critical role in our perception of aromas and flavors, particularly in the context of wine tasting. Olfactory perception is a complex process influenced by factors such as genetic variations, cultural experiences, and individual preferences. The genetic makeup of individuals affects their ability to detect specific flavor compounds, leading to variations in taste and smell preferences. The olfactory system, with its intricate network of receptors and neural pathways, allows us to discern a wide range of odors and flavors. The convergence of olfactory sensory neurons expressing specific odorant receptors in glomeruli enables the spatial organization and integration of olfactory information within the brain. By understanding the genetic and sensory factors that contribute to olfactory perception, researchers can develop personalized flavor profiles and cater to diverse consumer preferences. This knowledge can benefit the wine industry by adapting to changing global markets and creating new opportunities. Ultimately, unraveling the complexities of olfactory perception enhances our understanding of human sensory experiences and how they shape our interactions with the world around us.

The use of binary comparisons and a centralized qualitative ranking scale in olfactory perception research provides a more precise and accurate method of comparing odorant concentration and drawing conclusions about the perception of odorants. This approach aligns the test for every participant, ensuring

consistent baselines for making comparisons. The technique allows for the detection of trends in intensity and sensitivity levels and can aid in the early detection of olfactory disorders. It also facilitates the validation of conditions or disorders over time and provides a better understanding of the mechanisms behind odor perception. Moreover, understanding the individual differences in olfaction and taste sensitivity levels is crucial in studying taste preferences and the perception of flavor. Environmental factors, health circumstances, and individual variation in sensitivity levels significantly influence taste preferences and the ability to differentiate between tastes and odors. The close relationship between olfaction and taste highlights the importance of considering both senses when studying flavor perception. The prevalence of olfactory dysfunction, such as anosmia, in viral respiratory infections and specifically in COVID-19 underscores the need for accurate assessment methods. Visual analogue scales and other quantitative approaches can provide valuable insights into the severity and extent of olfactory and gustatory dysfunction in COVID-19 patients.

The variability in gene expression caused by single nucleotide polymorphisms (SNPs) located in promoter regions can contribute to interindividual differences in olfactory perception and the variability of olfactory cognition. The integration of machine learning techniques and the use of ranking responses and direct numerical ratings, such as the modified Bradley-Terry model, provide valuable insights into odor intensity and perception. Additionally, individual variations in olfactory perception, influenced by genetic factors and underlying health

conditions, can impact the accuracy of likability ratings and the severity of olfactory dysfunction. The findings highlight the complex nature of olfaction and the need for comprehensive assessments and ongoing support, especially for individuals experiencing olfactory dysfunction following COVID-19. Further research with larger and more diverse samples is necessary to deepen our understanding of these phenomena and their implications in various fields, such as perfumery, food and beverage industries, and sensory evaluation.

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APPENDIX I

SNOT-22 SYMPTOMS LIST

1. Need to blow nose
2. Nasal Blockage
3. Sneezing
4. Runny nose
5. Cough
6. Post-nasal discharge
7. Thick nasal discharge
8. Ear fullness
9. Dizziness
10. Ear pain
11. Facial pain/pressure
12. Decreased Sense of Smell/Taste
13. Difficulty falling asleep
14. Wake up at night
15. Lack of a good night's sleep
16. Wake up tired
17. Fatigue
18. Reduced productivity
19. Reduced concentration
20. Frustrated/restless/irritable
21. Sad
22. Embarrassed

DEFERRAL

Richard Gerkin
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-
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Dear Richard Gerkin:

On 12/10/2020 the ASU IRB reviewed the following protocol:

Type of Review:	IRB Site
Title:	Olfactory Tools for COVID-19 Screening and Surveillance
Investigator:	Richard Gerkin
IRB ID:	STUDY00013316
Funding:	Name: other, Grant Office ID: FP00026123, Funding Source ID: U01DC019573
Grant Title:	FP00026123;
Grant ID:	FP00026123;

Documents Reviewed:	• AROMA-T scratch and sniff test, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • CITI Training Certificates, Category: Non-ASU human subjects training (if taken within last 3 years to grandfather in); • Local Context Review, Category: Other; • mPST smell test, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Notice of Award, Category: Sponsor Attachment; • Recruitment flyer, Category: Recruitment Materials; • Research Plan from Grant Application, Category: Sponsor Attachment; • SNOT-22 respiratory assessment, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

	• Social Determinants of Health questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Tanushri Bhatnagar CITI Training Certificate, Category: Other; • The Protocol, Category: IRB Protocol; • UF IRB Approval, Category: Off-site authorizations (school permission, other IRB approvals, Tribal permission etc); • UF Olfactory Tools for COVID-19 Screening and Surveillance Core ICF.pdf, Category: Consent Form; • UF SMART IRB Acknowledgment, Category: Other;

The ASU IRB deferred review of this project to University of Florida IRB and the associated IRB protocol # is IRB2020022524.

If you have questions, you can contact the IRB at research.integ-

rity@asu.edu. Sincerely,

IRB Administrator
cc:
       Tanushri Bhatnagar
       Bryan Brayboy
       Richard Gerkin