

THE TEMPORAL SUPPRESSION EFFECT OF BINARY MIXTURE  
ON HUMAN

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of  
Master of Professional Studies in Agriculture and Life Science

Field of Food Science

by

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May 2020

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## ABSTRACT

In Laing's study in 1994<sup>4</sup>, he indicated that there has latency of recognition of odors of a mixture in human brain. The "fast" one opt to act as a suppressor of the "slow" one. Also, Laing<sup>5</sup> and Xu<sup>3</sup> both proved that mechanism exist to modulate the perception of odorants in a mixture. This study tends to figure the existence of latency on odor perceptions in a mixture. Human as subjects were analyzed responses by answering a binary forced question after presented to two puffs with 200-ms latency. PsychoPy<sup>TM</sup> controlled Sniff Olfactometer (SO), providing a double-blind experiment to deliver odorants to subjects and then measuring their responses. The 12 different single odorants prepared separately in 10% polyethylene (PEG) measured thresholds for estimating the Equal Odds Ratio (EOR), which was explained by Chloe<sup>1</sup> as: the concentration ratios of two compounds at which they are perceived with equal probability in a forced choice identification task. Once EOR has been determined, puffing games delivered every single odorant and the mixture with Equal Odds Ratio separately with 200-ms latency in between. The result shows 2,3,5-Trimethylprizaine does suppress perception of Hexanal regardless it happens before or after Hexanal presents. Otherwise, this experiment proved the second puff plays more essential influence on human responses. For determining more precise thresholds and EOR, an additional experiment is applied to see differences when orders presentation changed. The order in alternate provided more precise data, which is more fit in S curve with smaller standard error and narrower confidence interval.

## BIOGRAPHICAL SKETCH

Qi Tang was raised in Guangzhou, China. She fulfilled her Bachelor's degree from Purdue University in the field of Food Science in 2018. In undergraduate period, she worked as a research assistant in Dr. Arun Bhunia's molecular food microbiology laboratory. She assisted the study of exploring the inhibitory effect of different probiotics on *Listeria*. Now, she joins Dr. Terry Acree's lab when studying at Cornell as Master student of Professional Studies. She, with luck, joined two projects focusing on human sensory systems. As member of researcher group, she assisted to study temporal effects on odorant mixture perception. Another one is about how temporal suppression happens in binary mixture. With generous support from Dr. Robin Dando, she received opportunity to be a teaching assistant in Food Sensory class. She learned academic knowledge and gained more courage during this experience. With professional knowledge backup from her grateful professors and business skills and information from Cornell University, she decided to own her business in the future step.

## ACKNOWLEDGMENTS

I want to thank my advisor, Dr. Acree Terry, for his great support from the bottom of my heart not only for my knowledge enrichment but guidance of my life choices. I received more than I had expected at Cornell.

I also want to thank my wonderful research team members, Leto, Kaifeng, Xiaoyuan and Jiayue. They are not only my sincere friends but great technical, knowledge and emotional supporters.

Moreover, I would like to appreciate my friends and boyfriend, for their support by releasing my negative emotion during the whole journey. Additionally, without the generous help from faculty and alumni, I cannot enjoy the great time at Cornell.

## TABLE OF CONTENTS

BIOGRAPHICAL SKETCH	iii
ACKNOWLEDGEMENTS	iv
LIST OF FIGURES	vi
LIST OF TABLES	vii
LST OF ABBREVIATION	viii
INTRODUCTION	1
METHODS	2
RESULTS	11
REFERENCES	19

## LIST OF FIGURES

<b>Figure</b>	<b>Description</b>	<b>Page</b>
1	A scenario showing an experiment in progress and the Teflon <sup>TM</sup> bottle shown in sample triad controlled by three actuators	2
2	The auxiliary hardware in Sniff olfactometer for fixing subjects' positions: chinrest and aiming system	4
3	A complete 8-second single-puffed trial controlled by PsychoPy <sup>TM</sup> Program	6
4	the order switching in a group and three bottles in a sample triad	6
5	A complete trial for two puffs controlled by PsychoPy <sup>TM</sup> Program and the possible groups shown in the experiments	7
6	Thresholds of Hexanal of two subjects	10
7	Thresholds of 2,3,5-trimethylpyrazine	11
8	EORs of two subjects	12
9	Probabilities of Grass in different types of puffs in Puffing game	13
10	Comparison thresholds of Hexanal with different orders presented to subject 2	14
11	Comparison EOR results with different orders presented to subjects	15

## LIST OF TABLES

<b>Table</b>	<b>Description</b>	<b>Page</b>
1	Orders displace on comparison experiment	8
2	Correlation between different types of puffs	13

## LIST OF ABBREVIATIONS

SO: Sniff Olfactometer

PEG: Polyethylene Glycol

EOR: Equal odds ratio

Hex: Hexanal

TMP: 2,3,5-Tripmethylprazine

SCAPE: Swept Confocally Aligned Planar Excitation

## Introduction

In Laing<sup>4</sup> experiment done in 1994, 127 subjects were trained to recognize different odorants. After training, they are assigned to identify odorants in different arrangements and compositions. The identification score dropped from 81.8%, score of single odorants to 35.2%, the score for binary mixtures. The percentage for correctly recognizing four odors in a quaternary mixture was around 4%. Thus, human is difficult to recognize odors in an even simplest combination. In the previous study done by Chloe<sup>1</sup> from Acree's Lab, she found that no matter the two odorants were mixed in a single bottle or separately held in two separated bottles, they interfered each other. This evidence implies that this interruption happened at least at olfactory receptors level or above to the cortex. There has a mechanism to "filter" a part of odors. Laing<sup>5</sup> indicated that recognition of odorants in mixture has latency range from 0 to 580ms. The "fast" odorants are more apt to act as an antagonist of "slow" odorants. Thus, the study focused on two stimuli, to measure human responses when exposed to these odorants with latency. Xu's mice study<sup>3</sup> further verified the assumption that there has a mechanism to regulate the perceptions of odorants in mixtures. Xu applied Swept Confocally Aligned Planar Excitation(SCAPE) microscopy to detect entire olfactory epithelium of mouse. She found that at the level of olfactory receptors, the modulated effects like suppression and enhancement already occur. Two odorants interact with each other even one of them was non-detectable by the mouse. The upper level regions of the brain identify odorants by pattern recognition. Thus, modification by suppression or enhancement can make

small change for mixture combined code for specific identification in the brain. The purpose for this study is to figure the existence of latency on recognition of odorants in a mixture and influence of each odorant on another one. A double-blinded method was applied in Sniff Olfactometer where subjects were asked to answer a forced question, “What do you smell, Grass or Nut?” after sniffing two puffs in series separately by 200-ms break. Hexanal is described as a grass scent and 2,3,5-Tripmethylprazine is described as nut scent. Why these two odorants were selected is because two scents are easily recognizable for subjects. During main study, an additional study was done to understand the influence of different sequences on precision of results on Sniff Olfactometer.

## **Methods**

### **Chemical preparation:**

The odorants presented to subjects were Hexanal (CAS Registry No. 66-25-1, >98%) and 2,3,5-trimethylpyrazine (CAS Registry No. 14667-55-1, >99%). The stock solutions are 400 ppm (part per million), diluted from pure chemical compound with 100% polyethylene (PEG, J.T. Baker U160-9). All samples were diluted from stock solutions using 10% PEG which was prepared with 90% deionized water.

### **Sniff olfactometer:**

This machinery is used to present the odorants to human beings. This SO-5<sup>10-11</sup>(from DATU, Inc. Geneva, NY 14456) is constituted with three actuators. Each actuator circuit has its own power supply, serving for a separated single puff or simultaneous

two puffs from separated two different bottles. These actuators are activated by PsychoPy™. The Teflon™ sniff port can connect maximally 3 Teflon™ bottles each time. Each 250ml Teflon™ bottle contains 50 ml solution. The actuators will be triggered to puff the Teflon™ bottles when the subjects click “Ready to inhale”. When humans sitting in front of monitor, there have a chin rest and a visual aiming system to fix their position based on their own physical condition to keep their noses right above the sniff port. Guarantee that every single time subjects can receive same volume of gas (15ml) containing odorants. Figure 1 shows the scenario of an experiment in process. After putting chin on the chin rest and aiming the target through visual system, the subject can follow the instruction shown on the monitor to start the experiment. During the whole process, subjects should wear headphone to reduce interference from environment. The whole experiment is done without disturbance from researchers. A complete single puffing process is 8-second duration, including a 75-ms chemical stimuli, shown in Graph 3. A complete binary puffing process is 8.2s, in which a 200-ms break occur between two puffs, shown in Graph 5. The orders for puffing bottles are controlled by computer.

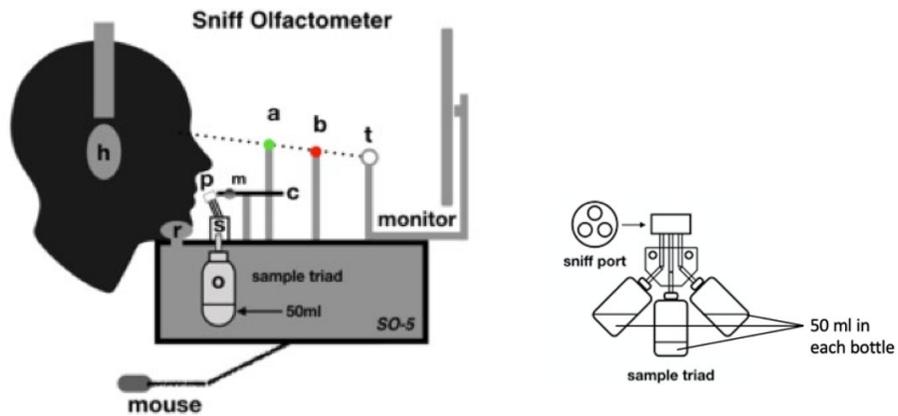


Figure 1. A scenario showing an experiment in progress and the Teflon™ bottle shown in sample triad controlled by three actuators



Figure 2. The auxiliary hardware in Sniff olfactometer for fixing subjects' positions: chinrest and aiming system

**Participants:**

All the subjects were screened by an online survey, in which they claimed their olfactory system as “very acute”. Two selected subjects should be trained before the

actual experiment. In conditioning session, they were taught to connect labels with the odors. Hexanal is identified as “grass”, and 2,3,5-trimethylprazine is identified as “nut”. After conditioning session, they must pass a test, containing 15 randomly single puffs to examine if they can identify each odorant. They must get the scores higher than or equal to 14 out of 15 to continue the experiment.

### **Experimental design:**

The test protocol is constituted by a conditioning and training session, threshold sessions for two components, Equal Odds Ratio session and Puffing Game.

After screening two subjects, refresher session and training session are presented before the actual experiment. All the process was controlled by PsychoPy™. The Graph 3 shows how a single puffing trial works. After the monitor shows “Get ready” indication, subjects put their chin on the chin rest, aimed the target through visual system and wear headphone. Then, subjects follow the instruction shown on the screen. They inhaled when “Inhale” comes out, to make sure that the 15ml gas volume present in the middle of the inhalation. After each puff, the monitor would show the indicators related to the puffed odorants, grass, nut or PEG, which means without stimuli presenting. Subjects followed the instruction to select the same key words shown on the monitor. This session taught them how to operate the software during experiment and introduced the labels related to the component odors. A following training session is to examine whether they can identify two odorants. 15 times of

random puffs showing up, they must choose at least 14 out of 15 correctly. All the questions in the experiment are binominal, forcing to choose either side. If they cannot pass the training session, a replicated session is provided. They must pass the training session to continue the actual experiment.

In threshold measurement, 12 different concentrations of each odorant prepared from stocking solutions. 3 different concentrations were a group. Three different trials for a group by switching positions in sample triad to avoid the bias from hardware, shown in Figure 4. Same method was applied to test EOR (equal odds ratio), the ratio at which two odorants are with same potency to the subjects. Based on thresholds of two odorants, 12 binary mixtures with different ratios between Hexanal (“grass”) and 2,3,5-tripmethylprazine (“nut”) are presented to determine at which ratio the probability of selecting “grass” is 50%, the point named EOR. The different ratios alternated across subjects, estimated from thresholds.

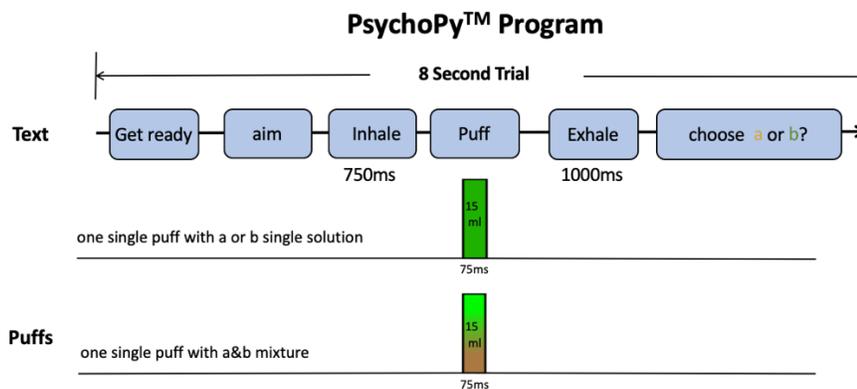
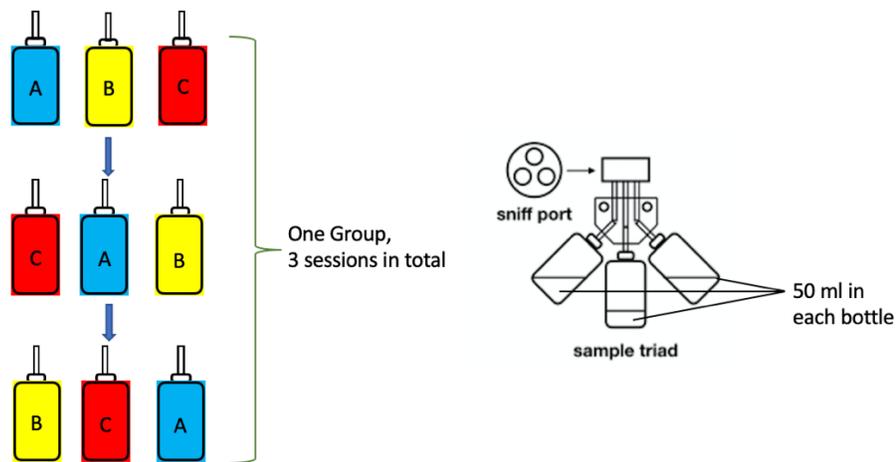
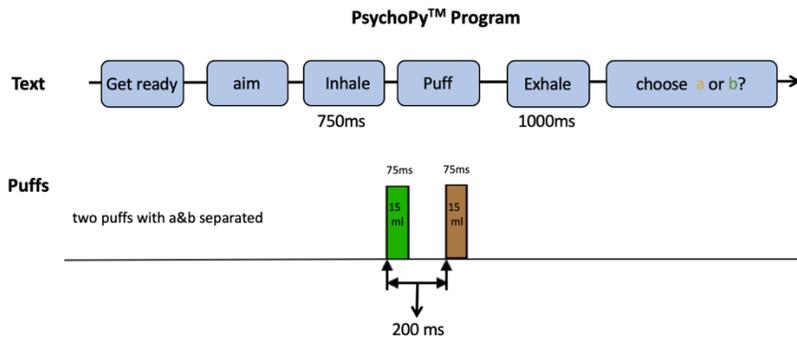


Figure 3. A complete 8-second single-puffed trial controlled by PsychoPy™ Program

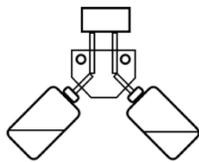


**Figure 4. the order switching in a group and three bottles in a sample triad**

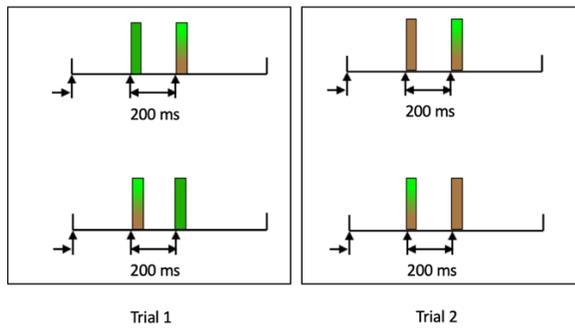
Puffing game presents two puffs in a single trial. There only left and right bottles connected to the sniff port. There has a 200-ms interval between first puff and second one. The sequence of which bottles puffed first is controlled by computer. Like what Figure 5 shown, the mixtures were 3 times of EOR and one of two odorants were puffed in a single run with a 200-ms gap in between. The experimental scheme shown in the Figure 5, there were all the probable puffing situations. As shown in trial 1, due to random order, the probable puffing situations could be Hexanal puffed first then EOR mixture, and EOR mixture puffed first then Hexanal. Same as trial 2, 2,3,5-tripmethylpazine was puffed instead of Hexanal.



### Puffing Game



- Hexanal
- 2,3,5-Trimethylprazine



**Figure 5. A complete trial for two puffs controlled by PsychoPy™ Program and the possible groups shown in the experiments**

Groups	sequential orders	alternate orders
Group 1	1	1
	2	11
	3	12
Group 2	4	2
	5	3
	6	10
Group 3	7	4

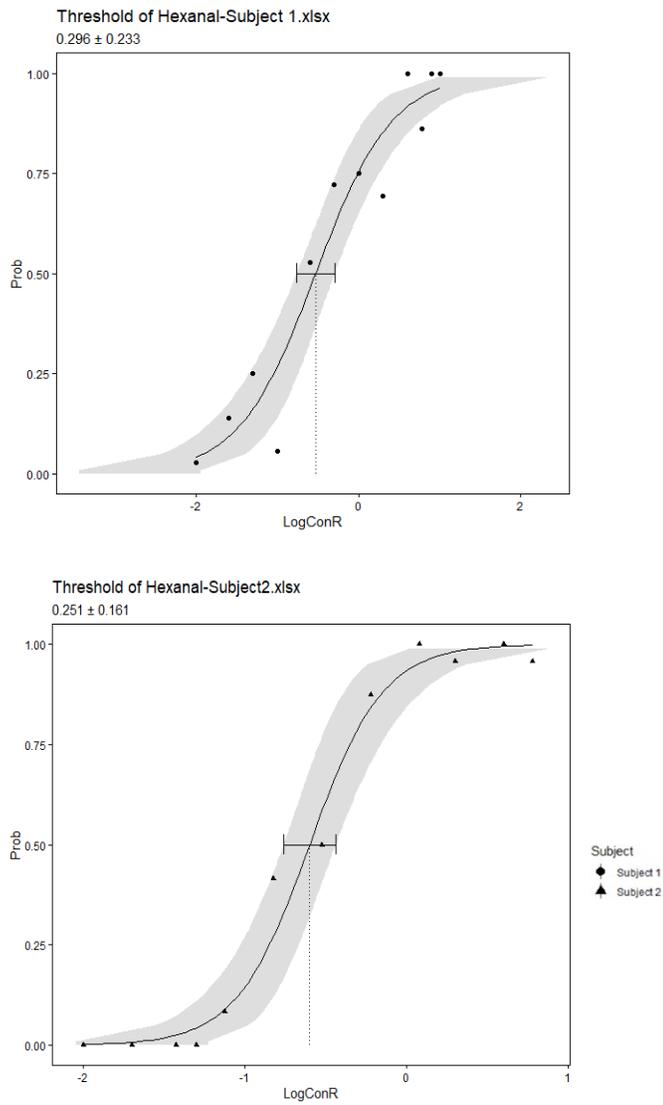
	8	8
	9	9
Group 4	10	5
	11	6
	12	7

**Table 1. Orders displace on comparison experiment**

In order to receive precise thresholds and EORs of two subjects, which will significantly determine experimental design of puffing game. Two preliminary compared trial run were applied on testing subject 2's Hexanal threshold to study if sequences of samples presented to subjects will affect the results of thresholds and EORs. Two tests with same concentrations of Hexanal made 4 hours before the trial and same procedure, were done in two consecutive days. The first day, a run with sequential order was conducted from highest Hexanal concentration (number 1) to lowest Hexanal concentration (number 12). The second day, combination of highest Hexanal concentration and lowest Hexanal concentration in the group 1 was performed to subject 2. With the same manner, the concentrations of Hexanal were closer in the following groups. Thus, in the last group, the three concentrations presented are number 5,6 and 7. After these trial runs, the compared EOR experiments were conducted to two subjects and controlled in the same day to avoid any changes of physical condition for subjects. Thus, EOR experiments were done twice with same condition and same day. The only variable is the order presented to subjects. The number 1-12 indicate different Teflon™ bottles containing odorants with different

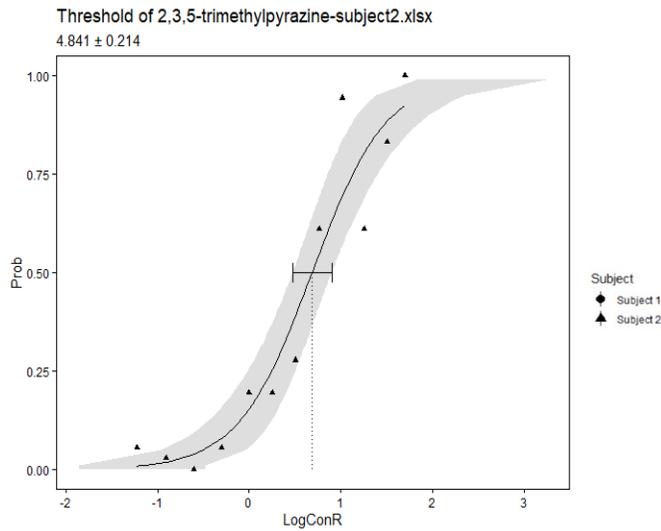
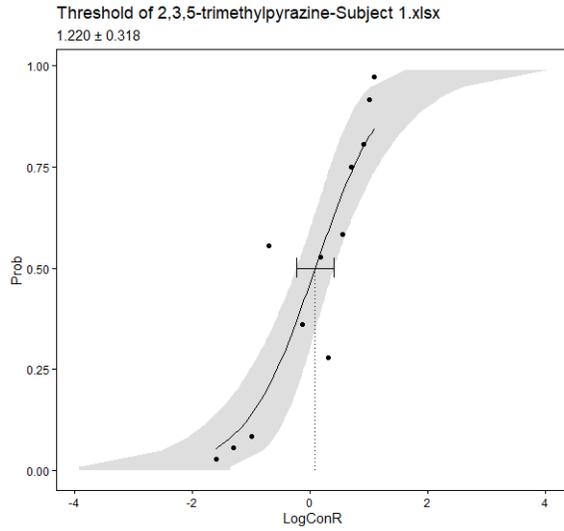
ratios. The ratios varied based on thresholds measured by each subject. 1 is the one with highest Hexanal concentration and lowest 2,3,5-Tripmethylprazine concentration while the 12 is the one with lowest Hexanal concentration and highest 2,3,5-Tripmethylprazine concentration. Thus, the experiments with sequential order were ascending sequence of Hexanal concentration and descending sequence of 2,3,5-Tripmethylprazine. Each group constitutes with three different mixtures. In comparison, the experiments with alternate orders combined 1 with 11 and 12, the rest done in the same manner. This compared trial was to avoid subjects overthinking and trying to decide the answers by observation of differences on concentrations.

# Results



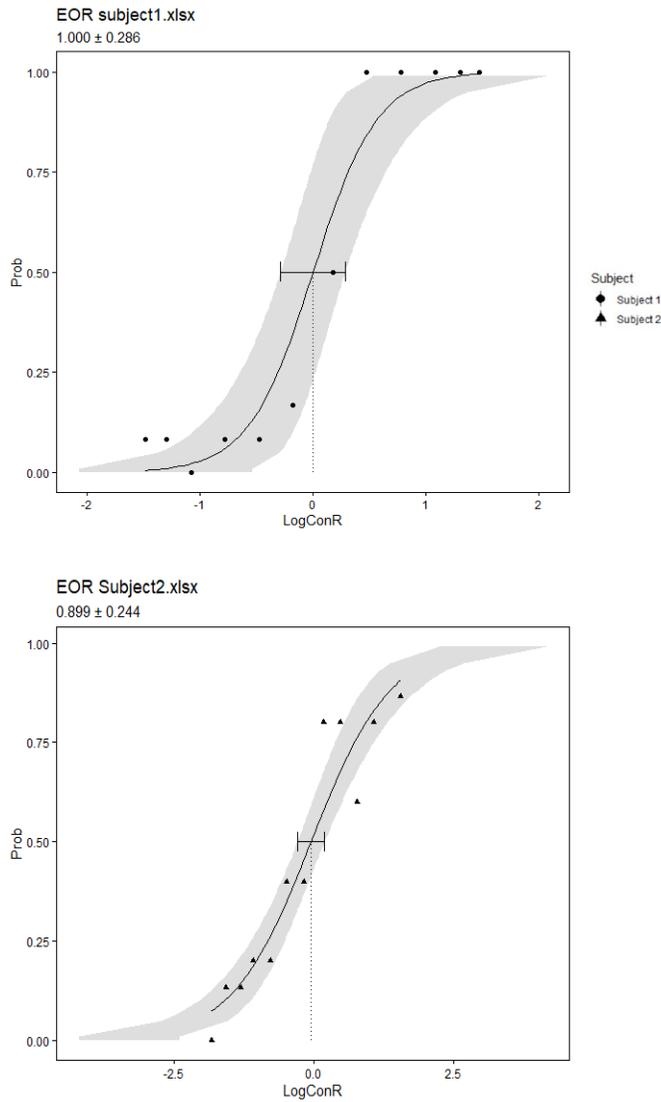
**Figure 6. Thresholds of Hexanal of two subjects**

The threshold is the point where 50% chance subjects chose “Grass”. The thresholds are showed on the top with plus or minus of standard error.



**Graph7. Thresholds of 2,3,5-trimethylpyrazine**

The threshold is the point where 50% chance subject chose “Nut”. The thresholds are showed on the top with plus or minus of standard error.



**Figure8. EORs of two subjects**

The EOR is the point where 50% chance subject chose either side when answering the question, “what do you smell, grass or nut?”. The equal odds ratios are shown on the top with plus or minus of standard error.

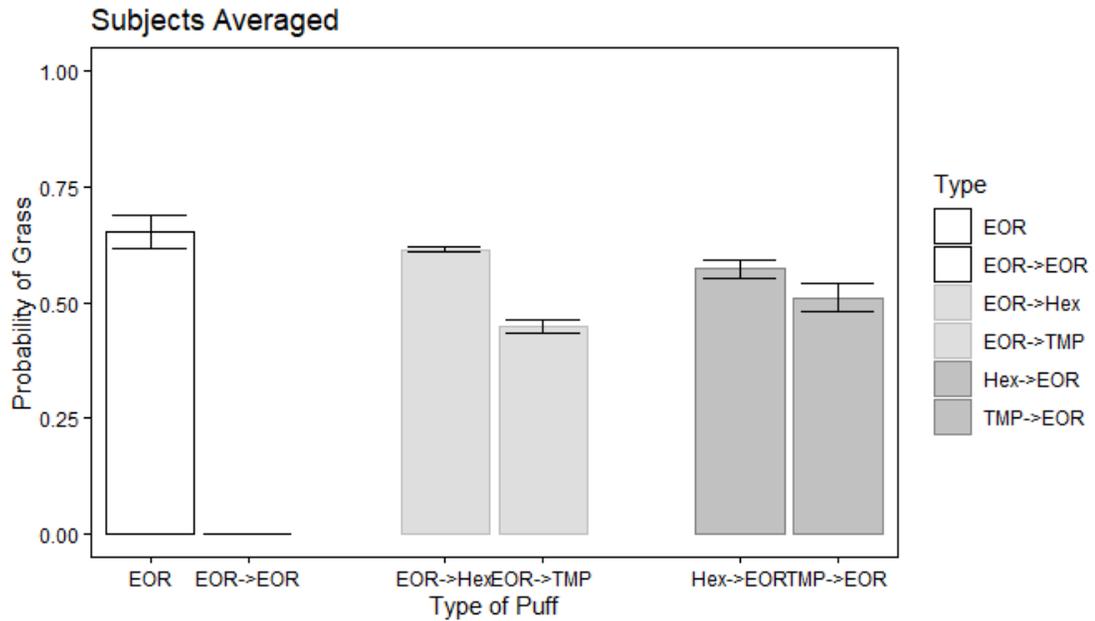


Figure 9. Probabilities of Grass in different types of puffs in Puffing game

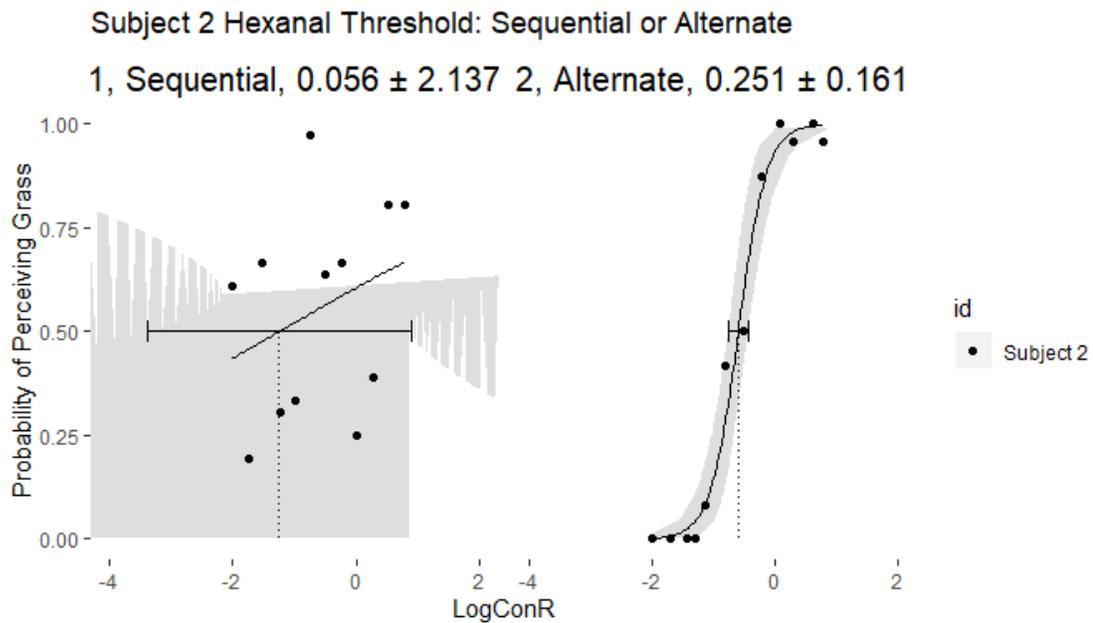
	EOR	EOR -> Hex	EOR -> TMP	Hex -> EOR	TMP -> EOR
EOR	0				
EOR -> Hex	0.2624	0			
EOR -> TMP	<0.0001	<0.0001	0		
Hex -> EOR	0.0003	0.2664	<0.0001	0	
TMP -> EOR	<0.0001	<0.0001	0.0234	0.0234	0

Table 2. Correlation between different types of puffs

Figure 9 shows probabilities of grass of different types of puffs. The EOR indicates single puff to verify if tested EOR falls at 50% probability point, N=36. EOR-EOR, puffing EOR twice with a 200-ms break, serves as a blank. The data of EOR-EOR is missing so that further study is needed. The table 2 shows p-value of significant differences between different types of puffs.

The subjects' responses are significantly different between EOR -> Hex and EOR -> TMP. However, there has no significant between EOR single puff and EOR which is followed by Hexanal. In the same situation, EOR->TMP significantly reduces probability of choosing grass.

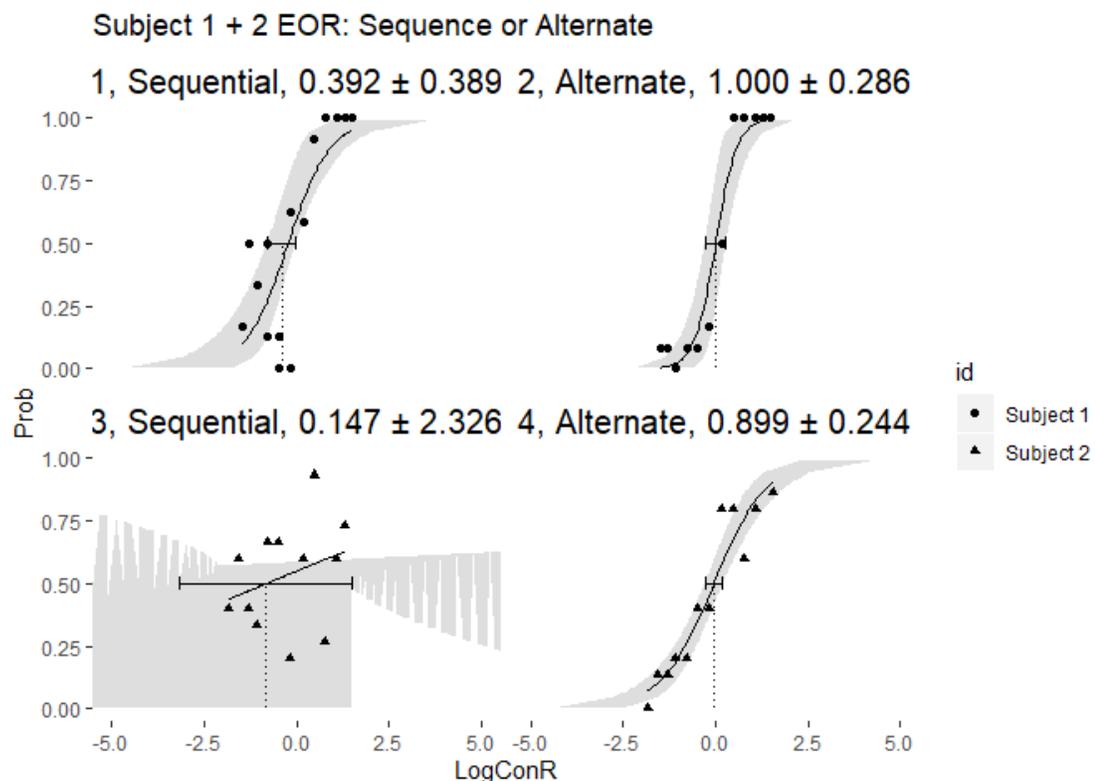
There is no significant difference between EOR -> Hex and Hex -> EOR while TMP does reduce response of "grass", no matter puffing prior or after EOR. In the contrast, Hexanal presented before EOR significantly reduced compared to single puffed EOR while there is no significant difference between EOR -> Hex and EOR. Overall, the second puffs in binary puffing game play more essential role on human than the first puffs.



**Figure10.** Comparison thresholds of Hexanal with different orders presented to subject 2

The Figure 10 shows the results of subject 2's two threshold tests for Hexanal. All the training process, duration of the test and solutions were the same. The difference

between these two were the date the subject had done the experiment and the orders of 12 solutions presented to. The left side is the result when odorants were presented to subjects in sequence from highest concentration to lowest concentration. In this case, the 12 dots did not fit the S curve. On the right side, the graph shows the result when 12 different solutions in disorder alternate. The order was shown on the Table 2. The 12 dots fit with S curve with smaller confidence interval. There are only two outliers presented. For further proving the influence of sequence of different concentrations on responses, two compared groups of EOR test applied to two different subjects in the same day, shown in Figure 11.



**Figure 11. Comparison EOR results with different orders presented to subjects**

The Figure11-1 and 11-3 show EOR tested in sequential and Figure11-2 and 11-4 show EOR tested in alternate. As to compare confidence interval, the Figure11-2 and

11-4 have higher precise results, fitting in S curve. The Figure 11-1 shows that only 6 points fall in the confidence interval with 0.389 standard deviation. In comparison, Figure 11-2 shows smaller confidence interval area with smaller standard deviation value. For subject 2, in sequential trial, 12 dots cannot fit in S curve and thus the EOR value calculated in this situation is of insignificance. While in alternate trial, the standard deviation is 0.244. Like what it shown in threshold and EOR compared trials, the subject 2 is sensitive to the changes of orders presented.

## **Discussion**

Based on the study, one conclusion is that during two consecutive puffs, the last one plays more important role on human's responses. Second, TMP significantly reduced probability for recognizing Hexanal no matter it is puffed first or last. Then, compared with sequential display, tests designed with the order in alternate could give us better fit in S curve with smaller confidence interval areas.

Whether the existence of latency in recognition of binary mixture cannot be concluded in this experiment. It shows that TMP does suppress the perception of HEX and the influence of TMP to Hex is larger than what is opposite. Thus, TMP might act as antagonist in this mixture.

Due to limitation of time, there still have some improvement should be done in the future. For the future direction, the makeup experiment for EOR -> EOR should be done as a blank to compare with. Also, the EOR test should be repeated to make sure the values closer to 50% before doing puffing games. Thus, the EOR values should be

verified to be reproducible. Also, the study can be scale-up from 2 subjects to more than 10. The odorants also can be increased from 2 to 4.

The order comparison study could go further. There should have a specific study designed to analyze the different results due to order display. It could be as result of temporal background created from previous puffs. Thus, more specific study to strictly control variables is necessary. Also, the effect might be only applied for the situation with Hexanal or EOR. No any experiment done for pure 2,3,5-Tripmethylprazine and other odorants. Thus, different odorants should be included in this experiment including some with similar chemical structures and some with totally different structure. To understand if the rules can be applied to all stimuli or only applied to those no shared commons.

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