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Measuring hedonic behaviors to food odors in children

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Running head: Hedonic behaviors to food odors

Abstract

Olfaction is crucial for the hedonic appreciation of food. However, measuring hedonic value of food odors remains a challenge, especially in young children or in individuals with altered verbal abilities. The protocol described in this chapter consists of a method adapted to children and people with limited verbal abilities, and which combines subjective measures of the hedonic value of odors with an analysis of motor behavior in response to these same odors. This protocol provides a way to study the perception of hedonic value of smell for non-verbal population, and allows studying the motivational components of smells.

Keywords: Olfaction, food smell, hedonic value, subjective measurement, behavioral response

1. Introduction

Olfaction plays a key role in the emotional food experience: odors can signal the presence of edible food even before it is visually recognizable and they can also signal the spoilage of food (1). Since food flavor comes from the integration of olfactory, gustatory and trigeminal sensations, and that 80% of flavor perception may actually rely on olfaction (2), losing the sense of smell has significant impact on food behavior (3,4).

Besides its link with food perception and behavior, olfaction has a special link with emotions. Experiments using verbal description of odors revealed that hedonic value is the first dimension used to categorize smells (5-8). At the anatomical level, smells recruit olfactory areas (see 9 for a review) but also emotional areas (9-12) and the reward system (13).

Scientists in the field have long tried to develop the most reliable methods of measuring these hedonic responses to food and non-food odors. A direct approach to measure odor hedonic value consists of asking participants to evaluate pleasantness on visual scales (14). These subjective ratings and declarative data are informative, but can be biased by differential use of the rating scale across participants because it relies on participant's subjective rating. Furthermore, this method limits the study of odors' hedonic value to participants with fluent verbal and reading abilities, and thus does not include all young children with or without pathological conditions. Other methods have been investigated to measure hedonic value including electrodermal activity (15,16), heart rate recording (15,17), or motor nasal exploration of odorants (18). These methods provide more objective measures of hedonic value but require placing sensors in contact with participants' bodies and/or faces and thus could hamper participants in their hedonic perception of odors. In other words, exploring hedonic responses to food and non-food odorants in an unbiased way, and especially in children, remains a methodological challenge. Moreover, progress has been made in past decades in understanding the neural basis of the reward system. New studies brought evidence to disentangle reward and affective response to stimuli with a distinction between the hedonic value (liking) and the incentive salience (wanting) of a stimulus (19). This distinction needs to be taken into account in the study of the emotional response to smells.

Here, we present a protocol aimed at investigating hedonic responses and motivated behaviors to food odorants in children that circumvents the above issues. This protocol was first developed for adults and has been published in a dedicated paper (20). We present in this chapter an adaptation of the same protocol for children aged 3 to 17 years. The protocol combines subjective evaluations of hedonic (odor liking) and motivational value of odorants (odor wanting), with implicit analysis of motor exploratory behavior of odorants. As the protocol is based on video analysis of behavior during olfactory exploration and does not require any sensors positioned on the participant's body or face, it is particularly well suited for children. The sections below outline the materials needed for this protocol and the procedure to follow when studying children.

2. Material

2.1. Olfactory stimuli

1. Selection of stimuli. The list of food odorants presented here is only an example (see Note 1). A total of 6 food olfactory stimuli are used: 2 monomolecular odorants and 4 aromas. The two monomolecular odorants are Cis-3-Hexenol (CID 5281167, “grass” odor), and Butanoic Acid (CID 264, “butter” or “cheese” odor). The four aromas include Chocolate, Mint, Lemon, Cotton Candy (respective references: CH-L1, ME-120, CI-08, BP-13 ; « La maison des chefs », Cannes la Bocca, France). Another aroma is used as a training stimulus: Strawberry (FR-466, « La maison des chefs », Cannes la Bocca, France)
2. Preparation of stimuli. For each stimulus, 5ml of odorous solutions are put in an opaque flask of 15 ml (opening diameter 1.7 cm, height 5.8 cm) (see Note 2). Preparation should take place under a hood and with gloves to be changed between each stimulus to avoid contamination between odorants. For Cis-3-Hexenol solution, add odorless mineral oil with a micro-pipette in the flask, and then using a new pipette tip add 60 µl of pure Cis-3-Hexenol to reach a total volume of 5 ml. Mix the solution with a vortex for at least 30 seconds. Then, put a porous polypropylene absorbent paper (3cm × 7cm; 3M, Valley, NE, USA) into the flask (see Note 3). For Butanoic acid solution, add odorless mineral oil with a micro-pipette in the flask, then 5,5 µl of pure Butanoic Acid, and follow the same procedure as for Cis-3-Hexenol (vortex, and polypropylene paper). For the 4 aromas, put 5 ml of each aroma in a flask, then mix it with a vortex and put a porous polypropylene absorbent paper (the same dimension as for the other solutions).
3. Each flask is then closed with a black plastic cap.
4. Each solution is numbered with a 3-digit number printed on a white sticker stuck on the flask. Digit numbers are chosen randomly. The experimenter has a file with the identification key of each odorant.

2.2. Flask support

1. Some children, depending on their age and/or developmental particularities, may put the flasks in their mouths or have difficulty holding them. To homogenize the olfactory exploration between these different subpopulations of children, and to allow a better grip of the flask, a cardboard box holder that meets these constraints can be used.
2. Cardboard holder dimensions: 20*20*5 cm (**Figure 1**).
3. Make a small hole with scissors of the size of the flask in the center of the cardboard holder (10 cm from the edge).
4. Cover the box with plain and neutral-colored paper. Then cover it with plastic protection to make it waterproof and cleanable to avoid odor contamination.

2.3. Video camera

1. A standard camera with a minimum resolution of (1280x720 pixels) is used to record participant exploratory behavior (24 images/second). The camera is placed at one meter on the side of the participants, in order to see the participant's profile (see Note 4).
2. The field of the camera must be wide enough to see the flask on the table, and the back, shoulders, and head of the participant when approaching the flask or moving back.

2.4. Subjective measures

1. As young children may vary in their verbal and reading abilities, the material used to measure subjective evaluations of odors is adapted. In the present protocol, subjective evaluations consist of 2 types of tasks: odor liking task and wanting task. If one wants to relate liking/wanting responses with the ability of the children to identify the odors, one can propose an odor identification task.
2. Liking/wanting task: in these tasks, children answer yes or no to the question "Do you like this odor?" (Liking question), and to the question "Do you want to smell this odor again?" (Wanting question). For these questions, non-verbal children can provide an answer with pictograms: the green smiling face for a yes (I like/I want), or the red unhappy face for a "no" (I dislike/I do not want) (**Figure 2**).
3. Odor identification task (optional): a series of images is presented to the children. For each odor, 4 images are proposed: one of the pictures represents the odor source, and three pictures represent distractors.

3. Method

3.1. The experimental procedure

1. Information and consent forms. The protocol must be validated by an ethics committee for the protection of individuals and conform to the Declaration of Helsinki. At the very beginning of the session, children and parents are informed of the aim and procedure of the experiment, and if the child and parents agree to participate, the parents complete the consent form for her/him. Then, they fill out the inclusion questionnaire (children cannot participate if one of the non-inclusion criteria are met, see Note 5).
2. Participant installation. The participant sits down in front of a table and the experimenter sits in front of her/him. To ensure that the child can smell the odors easily, the size of the chair and of the table are adapted. The camera is put on the table, on the side of the participant. A specific mark on the table enables the experimenter to see where the odor cardboard has to be put (**Figure 3**).

3. Once the installation is done, the experimenter turns the camera on a few seconds before the beginning of the experimental session.
4. To ensure that instructions are well understood by the child, the first trial is a training trial. Put the “training” odor (ex. strawberry in our case) in the cardboard box (see Note 6). The purpose of the practice trial is to familiarize the child with the test procedure. The surprise effect with the discovery of the protocol can modify the exploratory behavior of the child.
5. Put the box in front of the child (on the cross mark on the table) for a training trial (see Note 7).
6. Ask the child to smell the odor: “You can smell the odor now.” (onset of the trial) (see Note 8).
7. The child has a maximum of 10 seconds to smell the odor, and can smell the odor several times in the time window. After 10 seconds, the cardboard box is removed. If the child is still smelling it, explain that the time is up, and ask the child to give the cardboard box back. To analyze odor exploration behavior, it is important to leave this phase of olfactory exploration completely free. During these 10 seconds, participants are free to smell the odor as many times as desired and for the desired duration. The child can approach their body to the flask, or grab the box and bring the flask closer to their nose.
8. After 10 seconds, remove the cardboard box.
9. Then, ask the child “Do you like this odor?” (Liking question)
10. Show the green and red pictograms. Explain to the child “Put your finger on the green smiling face if you like the odor or put your finger on the red unhappy face if you dislike the odor” (see Note 9).
11. Afterwards, or after 1 minute without any answer, ask the second question “Do you want to smell this odor again?” (Wanting question)
12. Again, show the green and red pictograms. Explain to the child “Put your finger on the green smiling face if you want to smell the odor again or put your finger on the red unhappy face if you do not want to smell it again”.
13. If the participant says yes to the wanting question, present the odorant again. If participant says no (or after smelling the odor a second time) remove the cardboard box.
14. Then, let a resting period of 30 seconds to the child.
15. Present a new odorant, the first one of the 6 experimental trials. Follow exactly the same procedure as for the training trial. Note that the order of odor presentation is randomized between participants.
16. After the presentation of the 6 different stimuli, odorants are presented again for the identification task (optional). Instructions to the children are as follows “Thank you for your answers. Now, your next task will be to smell each odor again, and to identify it”.

17. Put the training odor in the cardboard box, put it in front of the child and ask the child to smell it.
18. Show the 4 different images to the child (see Note 10).
19. Ask the child “Put your finger on the image that corresponds to the odor you just smelled”. The odorant is left to the child, who can smell it again if needed to identify it.
20. Remove the cardboard box once the child completed the task, or after 1 minute (see Note 11).
21. Allow 30 seconds of rest for the child.
22. Present a new odorant. The first one on the list, and follow the same procedure as for the training odor (see Note 12).
23. At the end of the session, explain that the test is over, thank the child and escort the child to her/his parents.
24. At the end of the experimental session, remember to air the room.

3.2. Analysis of the video to extract odor exploration behaviors

For each odorant and each participant, videos are analyzed to extract behavioral variables. Participant’s nose trajectory and top of the flask’s trajectory are drawn manually with Volcan software (A2V module) developed under LabView (National Instrument) (14, 21, 22). These trajectories are then analyzed to extract motor behavior variables (see Note 13). For each odorant, children had the opportunity to smell the odor several times in the 10-sec time window. A trajectory is drawn for each odor sampling, i.e. each time the participant brought the flask closer than 5 cm from the nose and then withdrew it more than 5 cm from the nose (or each time they approached their nose closer than 5 cm from the flask). The trajectory drawing starts as soon as the participant begins to approach the odorant, and ends when the participant stops moving away from the odor. The step-by-step procedure to analyze the video is developed below:

1. File format. If needed, the video file should be converted with dedicated software (e.g. VLC, from VideoLan, version 3.0.6) to switch from MP4 video format to AVI format (WMV1 debit of 800 kb/s).
2. The video scale is calibrated by measuring a distance in the video and in real life (for example the size of the support or the flask).
3. For a given participant and odorant, watch the entire videotape. The analysis of the video begins when the experimenter asked the participant to smell the odor (trial onset) (see Note 14). Report the number of odor samplings. For each odor sampling, note the exact moment when the participant began to approach the odor, and when she/he ended moving away from the odor.

4. Then, begin the drawing of the nose's trajectory. Put a mark on the participant's tip of the nose by placing the mouse on the tip of the nose and clicking. After clicking, the video moves to the next recorded image (40ms between each image). Click on the top of the nose again. Repeat it until the participant approaches and withdraws from the flask (Figure 4). Then, save the trajectory (see Note 15).
5. If the participant smelled the odorant several times during the trial, do a trajectory for each odor sampling. The first trajectory stops when the participant ends up moving back from the flask (at more than 5cm) and the second one starts when he begins to move forward again.
6. Then, do the trajectory for the top of the flask. As for the tip of the nose, draw one trajectory per odor sampling. Trajectories for the nose and the flask should have the same length (i.e. the same number of points) and should begin at the exact same moment on the video (when participants begin to approach the odor).
7. Based on tip of the nose and flask's trajectories, the relative distance between the tip of the nose and the top of the flask was calculated to extract the following behavioral variables: 1) nasal exploration duration (total period in which the nose remains within a 5 cm-distance to the flask), 2) minimum distance between participant's nose and the flask, 3) average approach speed from the nose towards the flask, 4) average withdraw speed of the nose from the flask, 5) the number of nasal explorations (i.e. number of time participants smelled the odor at less than 5 cm of the nose) (see Figure 5). A script coded in Matlab (MathWorks) extracts these variables into a dedicated file (see Note 16).

3.3. Statistical analysis

1. For odor liking and wanting, convert the yes/no data into binary data (1 for yes, 0 for no). If the odor identification is performed, convert each response into binary data (1 for correct response, 0 for incorrect response). Statistical tests depend on the hypothesis, the number of odorants and the number of subjects (Figure 6 illustrates an example of results).
2. For behavioral data (nasal exploration duration, minimum distance, approach speed, withdraw speed, number of nasal explorations) depending on the number of participants and nature of data distribution (normality, etc.), inferential parametric or non-parametric statistics can be used (Figure 6 illustrates an example of results).

4. Notes

1. Protocol can be adapted to different odorants (e.g. other monomolecular odorants or aromas, body odors, naturally smelling objects, etc.), but take care to standardize the perceived intensity of the odors if you are looking for the difference in hedonic value and not intensity. The quantity

of odorants can also be changed, but pay attention to habituation and olfactory fatigue that could impair smell perception.

2. Dilutions vary across olfactory stimuli in order to control for intensity differences.
3. The reasons for using polypropylene absorbent paper are twofold: 1) optimize contact between air and solution, and so the evaporation of volatile molecules and 2) avoid having a liquid solution in the flask that could tip over or be drunk by children. To put the paper into the flask, roll the paper thinly lengthwise and insert it into the flask. If the paper is stuck in the opening of the flask, gently tap the flask against the table, or push the paper with a pipette tip into the flask with a light tap. Be careful, the pipette tip should not touch the edges of the flask or the solution, just the top of the paper, to avoid odor contamination. Change pipette tip between each odorant.
4. It is possible to put a second camera in front of the participant to record and analyze facial expressions.
5. To allow the child and the parents a cooling-off period, it is recommended to inform them a few days before starting the test. To be included in our study, the child had to have no allergy to odors, no asthma, and be healthy on the day of the test (e.g., no stuffy nose or cough). Inclusion criteria are provided as examples, but other criteria can be added depending on the experiment and the target population.
6. For a study with an adult population, the flask can be put in front of the participant without the use of a cardboard box.
7. Odorants are presented one after the other in a randomized order. The order of odorant presentation for each participant is set before the beginning of the first inclusion of participants.
8. For study with children with ASD, questions are asked by a familiar adult, like a parent, a teacher, or an educator. Children with ASD can be disturbed by a change in their environment. To avoid inducing disturbances, 1) the experimenter should stay with the child for days before the experimental session in order to familiarize her/him with the experimenter, 2) the experimental session is performed in a familiar place (ex. usual classroom), and 3) questions are asked by their usual teacher/educator (See **22** for further discussion on this point).
9. For children and adults with verbal abilities, they can respond verbally without the use of pictograms. It is also possible to use a visual scale to evaluate liking and wanting to improve rating accuracy.
10. For children and adults with verbal abilities, it is possible to provide a list of words with four choices for the odor identification task (one correct answer and three distractors).
11. Do not forget to record the answer.
12. You may follow the same order of presentation of odors as for the liking/wanting evaluation phase, but you can also use a different one if it is needed.
13. Other software is available in the market.

14. The training trial is recorded but not analyzed.
15. To avoid any confusion in the data, name your trajectory with the participant's code, odorant's code, number of trajectories (first one, a second one, etc.), and if it is the nose or flask trajectory.
16. The variables listed here are those that were used to analyze odor exploration behavior in this experiment. Other variables such as the maximum speed of approach or withdrawal, or the maximum distance between the nose and the flask can also be extracted and analyzed (21).

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Figures

Figure 1: Cardboard box holding the odors

Figure 2: Pictogram to answer to the yes/no questions

The green pictogram for means a “yes” answer, and the red one means a “no” answer

Figure 3: Setting up the material and the participant

Figure 4: Drawing of trajectories of the nose (A) and the flask (B)

Figure 5: Extracted behavioral variables from video analysis

Figure 6: Example of results from declarative data and behavioral data

Results from declarative data (A) show that the odor of lemon was liked by more participants (N=12) than the cis-3-hexenol (liked by respectively 92% and 42% of participants, $\chi^2(1)=11.0$, $p<.001$), and the results of the duration of nasal exploration (B) show that the lemon was also explored significantly longer than the cis-3-hexenol (respectively $M=6.2\pm 2.9$ and $M=4.4\pm 2.3$, $t(11)=-3.85$, $p=.003$).

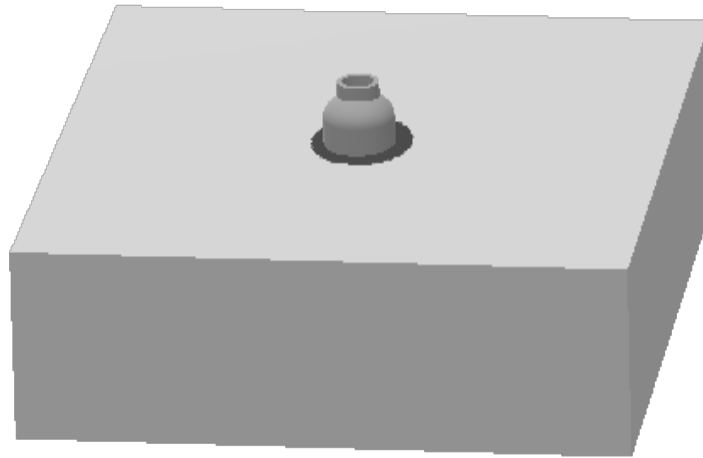


Figure 1: Cardboard box holding the odors

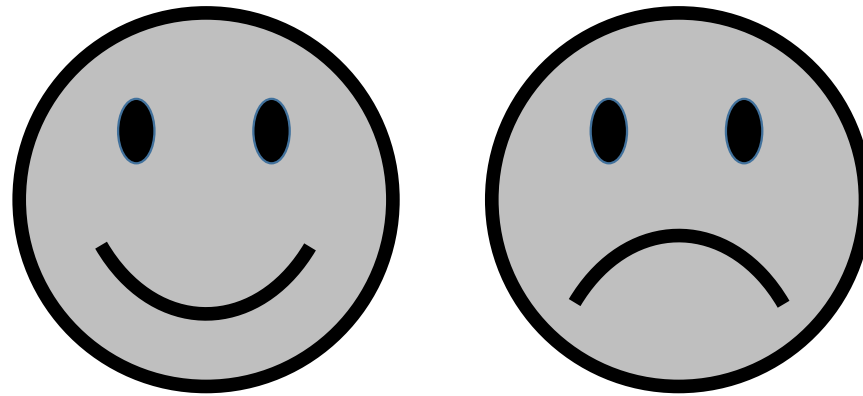


Figure 2: Pictogram to answer to the yes/no questions, green pictogram for “yes” answer, and red one for “no” answer

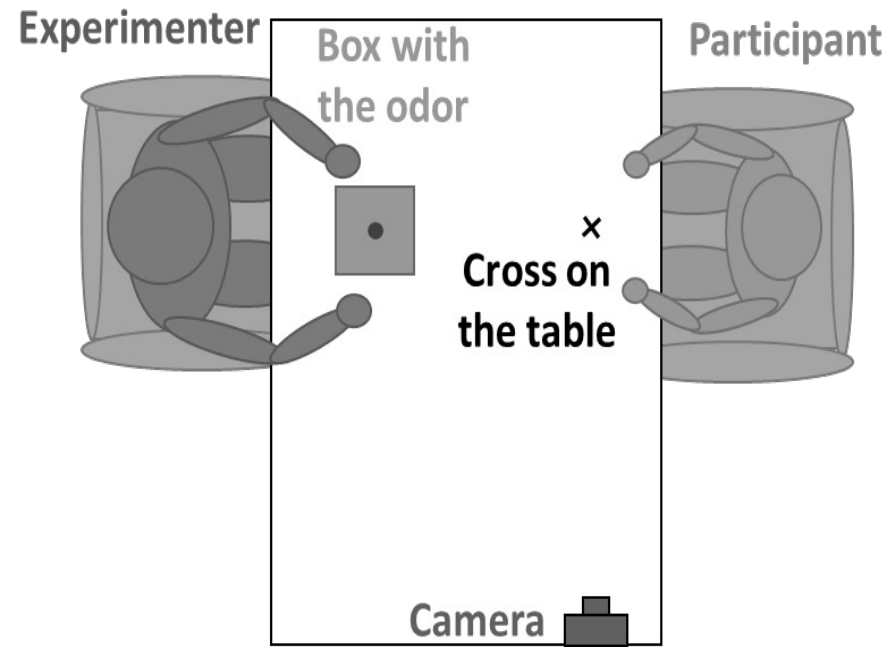


Figure 3 : Setting up the material and the participant

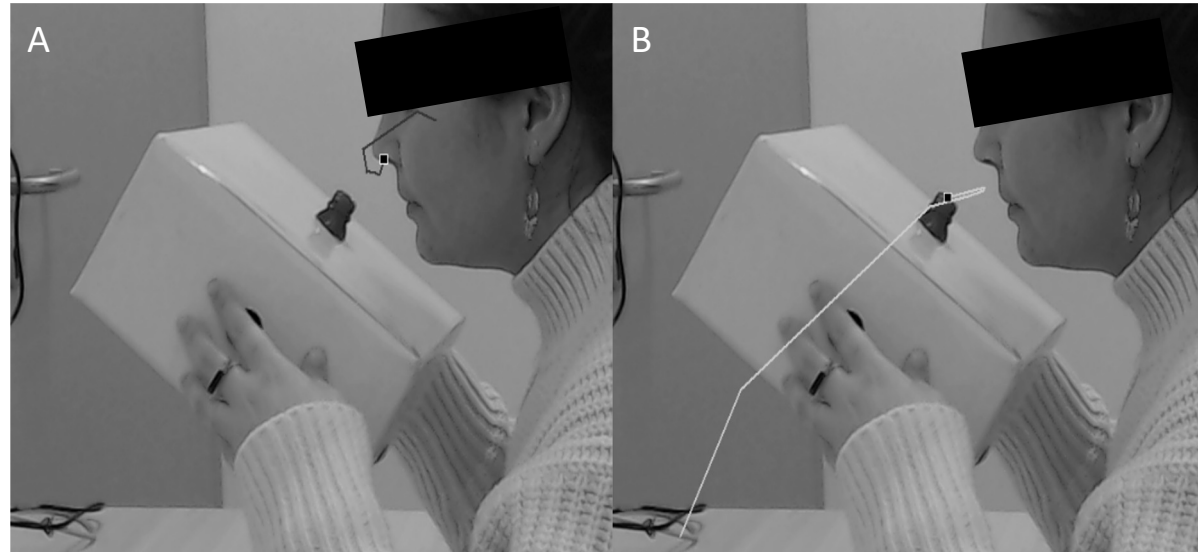


Figure 4: Drawing of trajectories of the nose (A) and the flask (B)

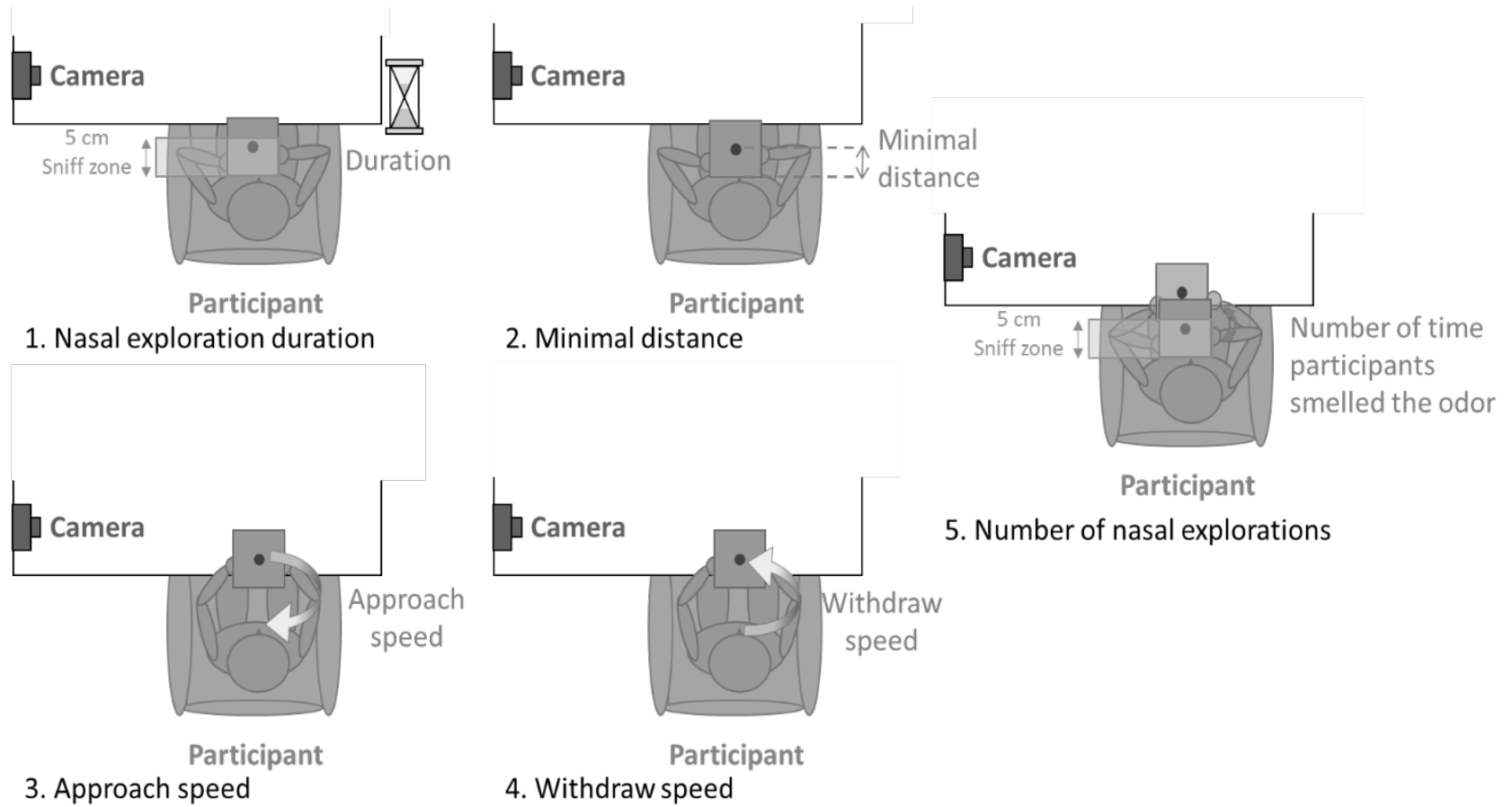


Figure 5: Extracted behavioral variables from video analysis

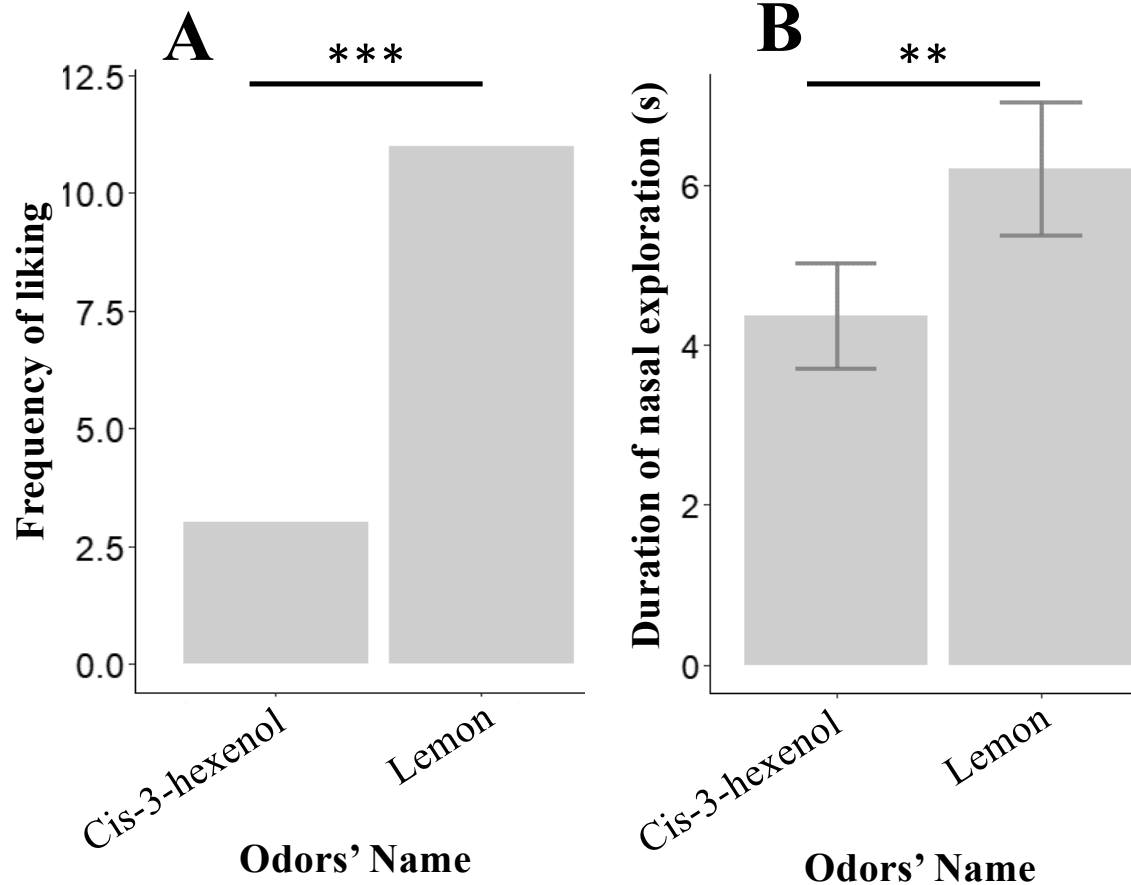


Figure 6: Example of results from declarative and behavioral data

Results from declarative data (A) show that the odor of lemon was liked by more participants (N=12) than the cis-3-hexenol (liked by respectively 92% and 42% of participants, $\chi^2(1)=11.0$, $p<.001$), and the results of the duration of nasal exploration (B) show that the lemon was also explored significantly longer than the cis-3-hexenol (respectively $M=6.2\pm 2.9$ and $M=4.4\pm 2.3$, $t(11)=-3.85$, $p=.003$)