Methods and Protocols in Food Science

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Moustafa Bensafi *Editor*

Basic Protocols on Emotions, Senses, and Foods

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Methods and Protocols in Food Science

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Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science.

Volumes and chapters will be organized by field and presented in such way that the readers will be able to reproduce the experiments in a step-by-step style. Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol will be clarified.

Basic Protocols on Emotions, Senses, and Foods

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Foreword

How can we measure the emotion of the eater when presented with potential food? We can't use the old stimulus-response paradigm.

First, we cannot consider food to be a well-defined stimulus; food is a multisensory object, and its perception is highly dependent on familiarity, habits, and long and short-term memories. Second, we cannot expect a precise and reproducible response to food because it is modulated by hunger or appetite, memories, etc.; in addition, the overt responses coexist with hidden and subtle bodily changes. They involve, among other networks, the reward system, and pure pleasure seeking coexists in our brain with conscious and rational systems of regulation: how to decide which of these responses is the most relevant?

Third, the eater cannot be considered in isolation, because eating is most often a social activity, even surpassing individual necessity. Fourth, "natural" or "ecological" consumption situations are diverse and difficult to control or reproduce.

To know which thread to seize in this skein of knowledge, accumulated by anthropologists, geneticists, psychologists, neurophysiologists, and linguists, one could turn to the hypotheses of emotion elaborated since Darwin. Unfortunately, the philosophical models of emotion may give the novice the impression of a theoretical tower of Babel.

In contrast, this book is intended to be a methodological guide that should make it possible to carry out reliable measurements, provided that the field of research is restricted to certain aspects of the food and that one chooses which layer of the phenomenological mill-efeuille is to be investigated. The eater is not the best witness to his eating behavior and what motivates his food choices: it is difficult to remember what we have eaten, and even more so to say why we appreciate it. The observer for their part must be all the more aware of the multiplicity of determinants as they will have to choose which ones are accessible to them.

How objectively to record emotional responses of non-verbal eaters? Should we use images instead of words? How can we access the unique phenomenological experience of each person and yet quantify and compare? While linguists and psychologists have demonstrated that bad odors are better lexicalized than good ones, why rely on a lexicon with 90% positive terms? Fixed vocabulary or only words used by the individual? It is so easy to collect large files of data of various kinds, but how to orient queries through these forests of data? The rich palette of methods and techniques presented here testifies to the audacity of these authors who did not allow themselves to be intimidated by the difficulties of linking emotion and sensoriality: to the recording of the odor of food, one can add taste and trigeminal recording, as well as visual behavior. For the motor or reflex responses, one can use fMRI recording, etc.

In my opinion, the value of this book lies in the fact that these authors, despite their very different approaches and proposals, have in common a proven experience of sensory research, an awareness of the limits and biases of their methods, and a vast culture of hard and human sciences. On top of that, I guess, from knowing many of them, that they are motivated by the desire to help.

Honorary Professor, University Claude Bernard of Lyon, Lyon, France ***Catherine Rouby***

Preface

Food is a multisensory object that stimulates all our senses and makes each eating occasion an emotional event: eating is inseparable from pleasures or displeasures, desires, or loathing. The Covid-19 pandemic has demonstrated how our affective equilibrium and well-being depends on this emotional compass: loss of smell and taste of food has a deleterious influence. Although it is an established fact that food and its sensory components generate affective changes, measuring these changes in behaviors and feelings is a real issue for science and industry. The objective here is twofold: from a fundamental point of view, one wants to understand the psychological and neurobiological bases of food emotions better, and from an applied point of view, the aim is to help manufacturers decipher the preferences and emotions of consumers faced with new products. The focus is not only on the food per se but also on the emotional impact of its sensory components (especially smell and taste). Indeed, in addition to its visual appearance and its ability to stimulate hearing and touch, food is chemically characterized by its smell, taste, and trigeminal sensation (hot/cold, irritation). These three chemosensory channels have a very privileged relationship with emotions. How can we measure affective states in response to food as a whole, or to its chemosensory components taken individually and in combination? This is a real challenge for public and private research laboratories in order to conduct rigorous research and transmit these skills to students, technicians, engineers, and scientists.

In the twenty-first century, food scientists, sensory scientists, psychologists, and neuroscientists made significant progress in probing these emotional responses to food stimuli: they found measurable changes in subjective experience, in behavior, and in central and peripheral nervous system activity. Emotional responses to food stimuli appear thus on explicit or implicit levels, and very often on all together. Technical progress over the last decade has made it possible to develop tools to characterize emotion both inside and outside the laboratory. In addition, depending on the targeted population (children, elderly, healthy, or diseased persons), the methodology can be adapted. As a consequence, with the rise of new technologies for explicit, behavioral, and physiological measurements, the data acquired is increasingly complex, heterogeneous, and voluminous. It is therefore necessary to find suitable methods of analysis to enable accurate processing and interpretation of emotional states.

In line with the Methods and Protocols in Food Science (MeFS) series format, the present book is aimed to enable young students, junior researchers, or experienced scientists who wish to enter the field of food science and emotions to become familiar with well-established practical protocols in this field of research. All protocols include an introduction to the subject area, a list of the necessary equipment and materials, and the detailed methods. In addition, the authors go beyond the mere presentation of their protocol. They bring their experience to the procedure by integrating tips and tricks to help the reader avoid the pitfalls known in the field. The book thus combines both well-established state-of-the-art techniques and innovative technologies in the field of emotions, which can be applied in food science.

The term emotion in the different protocols of this book is used in its broadest sense, ranging from preferences and liking to hedonic value and pleasantness to so-called "discrete" or basic emotions to mood. The protocols are divided into two sections. The first section is dedicated to explicit measures of emotions ranging from psychophysical to questionnaire approaches. The second section details a series of protocols enabling the measurement of implicit aspects of emotions, from behavior to neurophysiology. One can also make a transverse reading of the content of this book. First, in several protocols, it will provide methodological elements to prepare the best stimuli to use in your experiments. This includes the preparation and characterization of trigeminal stimuli (Protocol 1), and beverages and aromas (Protocol 2). Relevant information on the preparation of odorant stimuli can be found in a large number of chapters (e.g., Protocols 3, 9, 10, 11...). Finally, Protocol 19 will shed light on the preparation of taste stimuli. Second, some protocols will allow the readers to move their experimental setup from the laboratory to more ecological situations. Thus, Protocol 3 proposes a methodology to study chemosensory perception and its hedonic component in an anthropological field context. Protocol 4 details a procedure for measuring food emotions in a living-lab setting. Third, the measurement of emotions via questionnaires is a central component of this book: Protocol 5 develops the lexicon questionnaire, and Protocols 6, 7, and 8 present, respectively, the CATA-type questionnaire, the participant-defined questionnaire, and the Emoji-based questionnaire. Finally, Protocol 9 presents a method to measure food-related emotions using semi-structured interviews. Fourth, the book integrates a series of behavioral and implicit measures of emotions related to the senses and food. Often, these protocols are adapted to specific populations such as people with profound intellectual and multiple disabilities (Protocol 10), children (Protocol 11), and people with autism (Protocol 14). Protocol 13 presents a method for characterizing emotional responses to various stimuli in a virtual reality context, and Protocol 12 proposes a method for modulating visual perception by odors. Fifth, the last six protocols present implicit approaches based on psychophysiological measures of emotions in the sensory and food domains. Facial electromyographic response is addressed in Protocol 15, post-auricular reflex in Protocol 16, autonomic nervous system responses in Protocols 17 and 18, electroencephalography in Protocol 19, and, finally, functional MRI in Protocol 20.

Taken as a whole, these protocols aim to provide students, technicians, engineers, and scientists in the field the most complete information possible in terms of stimuli, materials, and methods for characterizing emotions, in order to give them the possibility of taking on new projects and new challenges in food science.

Bron, France *Moustafa Bensafi*

Preface to the Series

Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science. The series is unique as it includes protocols developed, validated, and used by food and related scientists, and theoretical basis is provided for each protocol. Aspects related to improvements in the protocols, adaptations, and further developments in the protocols may also be approached.

Methods and Protocols in Food Science series aims to bring the most recent developments in research protocols in the field as well as very well established methods. As such the series targets undergraduate, graduate, and researchers in the field of food science and correlated areas. The protocols documented in the series will be highly useful for scientific inquiries in the field of food sciences, as they have been presented in such way that the readers will be able to reproduce the experiments in a step-by-step manner.

Each protocol has been characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol is clarified. Then, an in-depth list of materials and reagents required for employing the protocol is presented, followed by comprehensive and step-by-step procedures on how to perform that experiment. The next section brings the do's and don'ts when carrying out the protocol, followed by the main pitfalls faced and how to troubleshoot them. Finally, template results are presented and their meaning/conclusions addressed.

The Methods and Protocols in Food Science series will fill an important gap, addressing a common complain of food scientists, regarding the difficulties in repeating experiments detailed in scientific papers. With this, the series has a potential to become a reference material in food science laboratories of research centers and Universities throughout the world.

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Part I

Psychophysical and Questionnaire Approaches

Chapter 1

Different Methods to Assess the Trigeminal System

Gözde Filiz and Johannes Frasnelli

Abstract

The chemical senses work both together and independently when eating and drinking. When fibers of the trigeminal nerve are stimulated, they evoke sensations such as irritation, pain, cooling, or burning which can be perceived as pleasant or unpleasant; the sensory system allowing for these perceptions is the trigeminal system. Typically, chemical substances do not activate the trigeminal system alone, as they usually also evoke an olfactory perception simultaneously, and this is already at lower concentrations. It is therefore challenging to assess sensitivity of the trigeminal system.

Here, we present a protocol that includes four different techniques enabling to assess trigeminal sensation at different levels of processing: (A) perception thresholds; (B) detection through a lateralization task; (C) peripheral neural activity via negative mucosal potential recordings; and (D) central neural activity via trigeminal event-related potentials.

Key words Trigeminal system, Lateralization test, Threshold, Negative mucosal potential, Event-related potentials, Flavor

1 Introduction

The trigeminal system is one of the three chemosensory systems, next to smell and taste. This system has a very important function during food intake and in the affective states evoked by the food that stimulate it. It is linked to the trigeminal nerve, the fifth cranial nerve. This is the thickest cranial nerve with three eponymous branches that are called ophthalmic (CNV1), maxillary (CNV2), and mandibular (CNV3) nerve.

The sensory part of the trigeminal nerve is responsible for the somatosensory sensations we receive from the skin and mucosa of our face, tongue, neck, and throat [1]. It therefore also allows for the perception of sensations such as tingling, burning, cooling caused by the interaction of chemical substances with receptors located on nerve fibers [2]. Examples for this are the preference

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of cooling sensations evoked by menthol in sweets or ice cream, as well as the burning sensation from capsaicin in spicy dishes [3]. Some humans prefer to eat spicy or pungent food with an important interindividual and intercultural difference.

In fact, flavor is the combination of trigeminal, olfactory, and gustatory (and other) inputs [4]. Although the three chemosensory systems are nominally independent from each other as they rely on distinct receptors and neural pathways, during daily life activities such as the consumption of food or drinks, two or more of the three chemosensory systems are usually stimulated together. Because of the overlapping stimulus space as well as common central nervous processing areas, the three chemical senses interact mutually and enhance or suppress each other [5].

To assess sensitivity of the trigeminal system, one has to use stimuli that are either purely trigeminal, i.e., they stimulate the trigeminal system exclusively, such as carbon dioxide and capsaicin, or mixed olfactory–trigeminal, i.e., they stimulate both the trigeminal system and the olfactory system, such as menthol, eucalyptol, or cinnamaldehyde. In the latter cases, it is challenging to separate the olfactory sensation from the trigeminal one. Different methods have been put forward to overcome this challenge. First, we can (1) use substances which solely stimulate the trigeminal systems to eliminate the interference from other senses such as olfaction. Second, we can (2) use methods that solely rely on activation of the trigeminal system. As an alternative to these two approaches, if the aim is to assess the capacity of a given substance to activate the trigeminal system, one can test (3) participants with a working olfactory system who are instructed to focus on the trigeminal aspect of a chemosensory stimulus (e.g., "please rate how pungent this odor is.") [6, 7], (*see* **Note 1**), or (4) individuals with no working sense of smell, i.e., individuals with anosmia [8, 9], (*see* **Note 2**).

In this chapter, we propose a protocol that integrates four different methods to assess trigeminal sensation at different levels of processing: (A) psychophysical detection threshold measurements; (B) psychophysical lateralization task; (C) peripheral neural activity by recordings of negative mucosal potentials; and (D) central neural activity by measured evoked potentials related to trigeminal events. The collected measures can be correlated with subjective measures related to the emotional or hedonic perception of these trigeminal stimulations in order to better understand the interplay between trigeminal system and emotions in the food domain.

2 Materials

2.1 Threshold for Trigeminal Stimuli

2.1.1 Oral Testing (Capsaicin)

Prepare five solutions of capsaicin in 10% ethanol and 90% distilled water: Solution 1: 3.16×10^{-5}%, Solution 2: 1×10^{-4}%, Solution 3: 3.16×10^{-4}%, Solution 4 = 1×10^{-3}%, and Solution 5 = 3.16×10^{-3}% w/w. Prepare one blank solution containing 10% ethanol and 90% distillated water.

2.1.2 Nasal Testing (CO_2)

Carbon dioxide is usually provided in pure form in gas tanks. It must be diluted with air, typically with the help of an olfactometer (*see* **Note 3**).

2.2 Lateralization Task

Two amber bottles (60 mL); add a cotton ball. One bottle is filled with 15 mL of 50% eucalyptol in propylene glycol. The other bottle is filled with 15 mL odorless propylene glycol [10] (*see* **Note 4**). A custom-made lid that snugly fits onto the nostril is put on the bottles. It has two openings one of which is inside the nostril, the other outside [11].

2.3 Negative Mucosal Potential

Carbon dioxide [12] 60% v/v (*see* **Notes 4** and **5**). A silver wire electrode of 0.3 mm diameter. A saturated NaCl solution. A 5 V battery. A PTFE tube (0.8 mm outer diameter, inner diameter 0.4 mm). 1% Agar in Ringer solution. An AC amplifier (bandwidth 0.1–100 Hz). Olfactometer.

2.4 Trigeminal Event-Related Potentials

Carbon dioxide [12] 60% v/v (*see* **Note 5**). EEG electrodes. An AC amplifier (bandwidth 0.1–100 Hz) (*see* **Notes 6** and **7**).

3 Methods

3.1 Thresholds for Trigeminal Stimuli

3.1.1 Oral Testing (Capsaicin)

1. The participant is seated in a quiet room.
2. The experimenter starts at the lowest concentration and increases the intensity toward higher concentrations.
3. The experimenter applies the capsaicin solution (applied volume is 0.02 mL) topically onto nasolabial folds in a single-blind simultaneous split-face application in a randomized order so that the side capsaicin/blank solution is applied to are not repetitively same [13]. The blank solution is used as a control contralaterally.
4. The participant is asked to indicate the presence of a painful sensation.
5. When the subject indicates a painful sensation lasting more than 30 s, the test is stopped. At this point, their detection threshold for capsaicin is determined.

3.1.2 Nasal Testing (CO_2)

1. The participant is seated in a well-ventilated room.
2. White noise is applied on the background with or without headphones to prevent subjects from hearing the switching sound of the stimulus presentation device.
3. CO_2 is presented by means of an olfactometer. Concentrations are 30–70% v/v (in increments of 5% v/v); typical stimulus duration is 200 ms.
4. Respecting an interstimulus interval of 40s will minimize habituation; therefore, stimuli can be applied ascending/descending staircases [14].
5. Starting with the weakest concentration, experimenters present three stimuli in a row (40 s interstimulus interval) to participants (in random order, two blanks containing pure air and one CO_2 containing stimulus).
6. Participants indicate which stimulus contains CO_2.
7. The correct identification of two correct stimuli in a row triggers subsequent presentation of a weaker stimulus; the incorrect identification of a stimulus triggers the presentation of a stronger stimulus.
8. Testing continues until seven staircase reversals are obtained; the average of the last four reversals will serve as an estimate for the CO_2 threshold.

3.2 Lateralization Task

1. The participant is seated in a well-ventilated room.
2. The participant is simultaneously presented with one bottle to one nostril, while the other receives the other bottle (*see* **Note 8**).
3. The experimenter fits the bottles' lids to each nostril snugly so that the air space of one bottle reaches only this nostril.
4. The participant is instructed to take one sniff.
5. The participant indicates which nostril has been stimulated (*see* **Note 9**).
6. This is repeated 40 times in a pseudorandomized counterbalanced order (20 trials with stimulation of one nostril; 20 trials with stimulation of the other nostril).
7. The number of correct localizations is counted and used as an estimate of trigeminal sensitivity (*see* **Notes 10–12**) (Fig. 1).

3.3 Negative Mucosal Potential

1. Preparation of the electrode: The silver electrode is chloridized using the 5 V battery for 10 min in a water bath with saturated NaCl solution.
2. Insulation of the electrode: 10% Agar is sucked into the PTFE tubing; the electrode is then inserted into the tubing by ascertaining an agar bridge.

Fig. 1 (**a**) Bottle with adapted lid for dispensing odor during lateralization task. (**b**) positioning of bottles in front of the nose

3. Placement of the electrode: under endoscopic control, the electrode tip is placed onto the respiratory mucosa of the nose. A frame similar to lensless glasses is used to keep the electrode in place (*see* **Note 13**).
4. The reference electrode is placed at the bridge of the nose, contralateral to the recording site.
5. The participant is seated in a well-ventilated room.
6. Stimulus duration of 500 ms.
7. Interstimulus interval is 40 s.
8. The electrode can be replaced if there is no signal.
9. A minimum of three repetitions is needed.
10. Recordings are averaged offline.
11. Averages will show a one-phased signal; the latency and the amplitude are measured.

Fig. 1 (continued)

3.4 Trigeminal Event-Related Potentials

1. The participant is seated in a faraday cage or equivalent to prevent interference from outside, in a well-ventilated room.
2. Electrodes are placed on regions of interests over the scalp using the international 10/20 system.
3. Stimuli are presented by means of an olfactometer that does not interfere with EEG signals.
4. White noise is applied on background with or without headphones to prevent subjects from hearing the switching sound of the stimulus presentation device.
5. Stimulus duration is 200 ms (*see* **Note 14**).
6. Interstimulus intervals (ISI) of 30–40 s.
7. At least 10 recordings per condition have to be recorded in order to average out background noise (*see* **Note 15**).
8. Responses are averaged offline; the latency and amplitudes of ERP peaks are measured, either from baseline or from peak to peak. ERP responses follow a distinct pattern with positive (P) and negative (N) peaks that appear in typical order in general. Typical peaks are P1, N1, P2, and P3.

4 Notes

1. This simple approach has the disadvantage that since most trigeminal stimuli do also activate the olfactory system, there will be a non-negligible confusion between the trigeminal and olfactory percept, e.g., to separate the freshness of peppermint from its minty smell.
2. While in theory, this allows for the assessment of the trigeminal impact of a given substance with no interference from the sense of smell, this approach has another drawback: acquired anosmia is typically associated with reduced trigeminal sensitivity [15].
3. The term olfactometer, in this context, describes a tool that allows for some form of automated stimulation with volatile substances. Since the development of the first olfactometer by Dutch physiologist Hendrik Zwaardemaker in 1887, several devices have been introduced that allow for stimulation with chemical stimuli using high precision in terms of stimulus concentration, duration, and with rapid onset. Furthermore, most devices can be controlled by a computer. They are available in different degrees of complexity and therefore price ranges, but only few are commercially available.

   A sophisticated olfactometer was introduced in the 1980s by Gerd Kobal [16]. This device produces a constant flow of humidified and heated (both controlled) air that can be odorized. Its switching device allows for controlling stimulus duration in the range of milliseconds with rapid rise times and offset. A tool with these characteristics is needed if one aims to investigate electrophysiological responses to trigeminal stimulation, as it is free of mechanical co-stimulation. Modern versions of these instruments allow for birhinal stimulation with up to 8 independent stimulus channels. This device can be acquired from the German manufacturer Burghart. (https://www.burghart-mt.de/en/).

   Another olfactometer was developed by Tyler Lorig roughly 15 years later [17]. This device had a shorter onset time but does not control humidity or heat of the delivered air flow. Such a tool is mainly used in the context of fMRI, where lower airflows are needed and therefore challenges inherent to the delivery of air with room temperature and humidity are less pronounced.

   A similar olfactometer was introduced in 2010 by Johan Lundström and colleagues [18]. This device again does not provide humidification or heating and may therefore not be useful if one aims at recording electrophysiological responses. However, crucially, the publication comes with a detailed construction manual with the off-the-shelf components, so that this device can be built from scratch with a limited budget in the range of 20.000 $.

Additional olfactometers, mainly to be used for behavioral studies or in fMRI settings, are manufactured by Sensonics Inc (https://sensonics.com/) and Osmic Enterprises (http://www.osmicenterprises.com/).

4. Alternative stimuli can be used: nicotine [19], eucalyptol menthol [10], cinnamaldehyde, or others, which can be used in neat concentrations or dilutions, equally CO_2 [11]. In fact, most odorants activate the trigeminal system in higher concentrations, i.e., they are mixed olfactory–trigeminal stimuli [8]. This group of stimuli includes typically used stimuli such as eucalyptol [20], menthol, and cinnamaldehyde [21]. Even phenyl ethanol, a substance commonly used in olfactory research with a distinct rose odor activates the trigeminal system in higher concentrations [22]. For mixed olfactory–trigeminal stimuli, the concentration needed to activate the trigeminal system is usually several orders of magnitude above the concentration needed to activate the olfactory system [5]. An exception to this rule is ammonia, which activates trigeminal system even at low concentrations [23].
5. The negative mucosal potential (NMP) is an electrophysiological signal from the surface of the respiratory mucosa that responses to chemosensory (specifically nociceptive) stimulation [16]. As such, it is free of olfactory components. The NMP therefore assesses the functionality of the periphery of the trigeminal system. It is a monophasic signal and the result of the receptor potentials to painful chemosensory stimuli [24]. It is also linked to the activation of C fibers and Aδ fibers of the trigeminal nerve. This technique needs experienced researchers.
6. Event-related potentials are the result of the concerted electrophysiological response of the brain to sensory stimulation [25, 26]. This technique relies on stimulus delivery by an olfactometer with controlled humidity and heat. When CO_2 is used, pure trigeminal ERP can be recorded, alternatively, mixed olfactory–trigeminal stimuli will yield mixed ERP.
7. The long ISI yields ERP experiments long and, together with the need of sophisticated olfactometers, only feasible in highly specialized research lab settings. ERP allow to assess trigeminal processing with unmatched temporal resolution, but rather weak spatial resolution.

   Stimulus duration can vary between (100–500 ms).
8. In passive stimulation, squeezable bottles are used and participants do not actively sniff, they continue breathing normally. Instead, the bottles are compressed which delivers the air puff to the nostrils [6].

9. To avoid left-right confusion, they can be instructed to raise the hand on the side of the perceived eucalyptol.
10. In an alternative method, staircase approach might be used to decide the final threshold [27].
11. Trigeminal system acts as a mass detector rather than a concentration detector [23, 28]; therefore, the same stimulus intensity can be achieved by varying stimulus concentration and stimulus duration. Therefore, some recent approaches apply stimuli of varying duration [29].
12. Using a mixture of components might affect the thresholds since when two or more ingredients are combined, they might enhance or hinder each other's effects [30].
13. Electrode placement can be marked.
14. Stimulus duration can vary between (100–500 ms).
15. Additionally, since one challenge inherent to trigeminal stimulation is that activation of the trigeminal system may lead to blinking, sneezing, or coughing that all of which induce artifacts on the readings, eye movements can be recorded to later contaminated recordings from further analysis.

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Chapter 2

Preparation of Beverage Samples Spiked with Aroma Standards

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Abstract

Spiking beverages with aroma molecules is one of the primary methods to investigate the contribution of individual volatile compounds to key sensory attributes such as primary descriptors, intensity, pleasantness, preference, persistence, and dominance. The purpose of this procedure is to outline basic steps for preparing stock solutions from pure molecule standards in liquid and solid state, perform dilutions adapted to representative concentrations in beverages, present samples to participants, and keep reagents in viable conditions.

Key words Aromas, Beverage, Spiking, Sensory evaluation, Dilutions

1 Introduction

Spiking beverages with specific aroma molecules has been one of the primary methods to characterize the contribution of volatile compounds to the sensory attributes of beverages. Detection, preference, intensity, dominance, and hedonic appraisal are part of the main quality attributes produced in beverages by aromas that can be characterized through spiking studies [1–9]. Further, beverage flavor development and innovations rely primarily on spiking for adjusting the formulation of ingredients (including aromas) and creating specific sensory percepts [10]. Spiking also helps to characterize the sensory contribution of aroma molecules originating from raw materials or during beverage processing [6, 11]. One of the most prominent applications of spiking involves the investigation of olfactory thresholds, where pure aroma standards are added to model solutions to identify the concentrations at which tasters can detect the presence of an aroma [1]. The concept of odor activity value—which is the ratio between the concentration of a

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volatile compound and the detection threshold of the aroma—is often used as an index to correlate the chemical composition with key sensory attributes that are readily perceived by tasters in beverages such as wine, tea, and spirits [12–14]. Therefore, spiking provides a method for evaluating how specific aroma molecules such as esters, terpenes, aldehydes, and volatile phenols among others relate with positive descriptors such as *fruity* or *floral* notes [15], or off-flavors such as *moldy, rotten,* or *spoilage* aromas [15, 16]. Further, spiking has been used to evaluate the capacity of aromas to evoke emotions such as *joy, happiness, excitement, surprise, disgust,* or *sadness* [17–22]. This emerging field of research for beverages and foods will expand our understanding of how aromas drive hedonic appraisal in consumers.

Aroma spiking involves the addition of a pure aroma molecule into a beverage or a model solution that is representative of the beverage composition. The procedure to spike beverage samples with aroma standards is best represented by the preparation of serial dilutions, where the concentration of the aroma molecule is progressively decreased or adjusted to represent a concentration range of interest.

Aroma molecules in beverages characterize by having a high vapor pressure and evaporating easily at room temperature [23, 24]. These physical parameters determine the ability of aromas to *fly out* of the beverage matrix and be perceived by consumers, either by orthonasal olfaction before consumption or during ingestion via retronasal olfaction. The vapor pressure and the solubility of aroma molecules in different solvents need to be taken into account while performing sample preparation as they may require the use of specific solvents and intermediate dilution steps to successfully disperse or prepare a sample that is representative of the beverage under study.

The beverage matrix refers to the composition of substances that integrate the beverage; generally, the most abundant component is water followed by a diverse pool of molecules that may include acids, alcohols, sugars, lipids, proteins, salts, pigments, gases (carbon dioxide), and preservatives, depending on the beverage type. Some of these matrix components are known for interacting with aroma compounds and influencing the perception of quality and affective attributes [9, 25–28]. Therefore, it is important that studies using spiking take into consideration the potential for chemical changes that may occur after adding the molecule into the beverage matrix.

The purpose of this procedure is to outline basic guidelines for spiking beverages with pure aroma standards for sensory evaluation and affective studies.

2 Materials

- Micropipettes of different maximum volumes: 10 µL, 50 µL, 200 µL, 1000 µL.
- Micropipette tips of different volumes: 10 µL, 50 µL, 200 µL, 1000 µL.
- Volumetric flask of different volumes: 5 mL, 10 mL, 50 mL, 100 mL.
- Analytical scale.
- Ethanol, food grade, 98% purity.
- Deionized ultrapure water.
- Food-grade aroma standards, high purity (>95%).
- Amber glass flasks with screw caps of different volumes: 15 mL, 50 mL, 100 mL.
- Nitrile gloves.
- Safety glasses.
- Fume hood.
- Disposable transfer pipettes (2 mL).
- Weighing boats.
- Glass beakers (10–250 mL).
- Spatula (stainless steel).

3 Methods

Spiking procedures typically require diluting a pure aroma standard to a concentration that is representative of the beverage under investigation. This process is represented in Fig. 1.

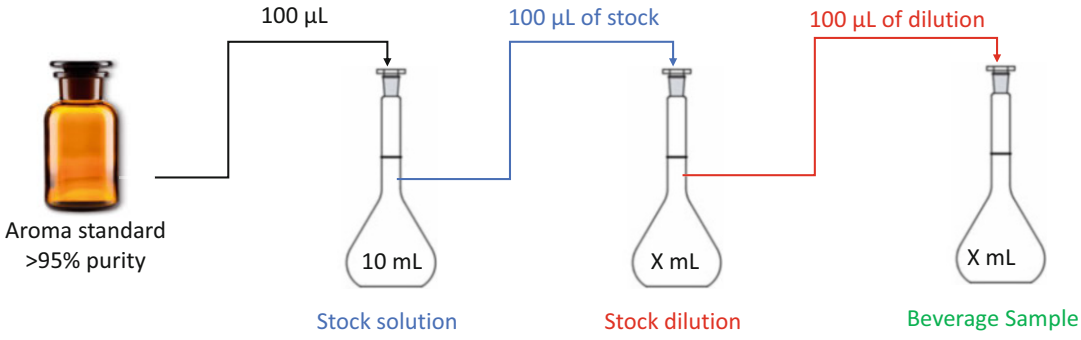

Fig. 1 Example of a serial dilution of an aroma standard to spike a beverage

3.1 Preparation of Stock Solutions

Stock solutions are the first step in the spiking procedure and they provide a way to dissolve a precise mass of the pure standard into a precise volume of solvent. In the context of beverages, aroma standards are typically diluted into solvents such as water or ethanol. Considering that most commercially available aroma standards have a purity higher than 95%, an initial dilution is typically required to further reach a target concentration in the beverage that may be in the range of only a few ng/L. Conversely, some aroma standards may be added (or spiked) directly into the beverage matrix when their concentration is attainable without dilution.

The solubility of each molecule in different solvents should be identified before starting any procedures, as well as the compatibility of the solvent with the beverage. Databases such as NIH-PubChem (https://pubchem.ncbi.nlm.nih.gov/) or ChemSpider (http://www.chemspider.com/) are authoritative references for consulting the solubility of most molecules and other physicochemical parameters such as the octanol-water partition coefficient (or its logarithmic value, *log P*), or the vapor pressure. It is important to remark that hydrophobic molecules [29] will need to be first solubilized in organic solvents such as ethanol, before spiking into the beverage matrix. In these cases, using food-grade reagents is of primary importance. Further, when preparing aqueous solutions, using ultrapure deionized water is required to minimize any potential interactions between the spiked aromas and compounds such as chloride, and mineral ions commonly found in tap and bottled water that may alter the presence of volatiles in the headspace [30, 31].

Manipulation of pure aroma standards should be performed under a fume hood to avoid excessive exposure to the concentrated volatile molecules and prevent respiratory irritation and other health hazards. Further, the fume hood will provide adequate ventilation of the laboratory environment and containment of the standards in case of spills (see Fig. 2).

3.1.1 Preparation of Stock Solutions Using Liquid Standards

The aliquot (volume) of aroma standard retrieved from its primary container should be of a magnitude that ideally allows for a single draw to prevent contamination. The density and purity of each aroma standard together with the aliquot volume can be used to calculate the appropriate mass of the aroma added to the stock solution.

$$\text{Mass of aroma molecule} = \text{Aliquot volume} * \text{density of standard} * \text{purity}\%$$

The aliquot (volume) of aroma standard retrieved may typically range from a few microliters to 1 mL. Solvent volumes may range from 1 mL to 100 mL depending on the frequency of use of the solution or the dilution needs. As a standard procedure, preparing

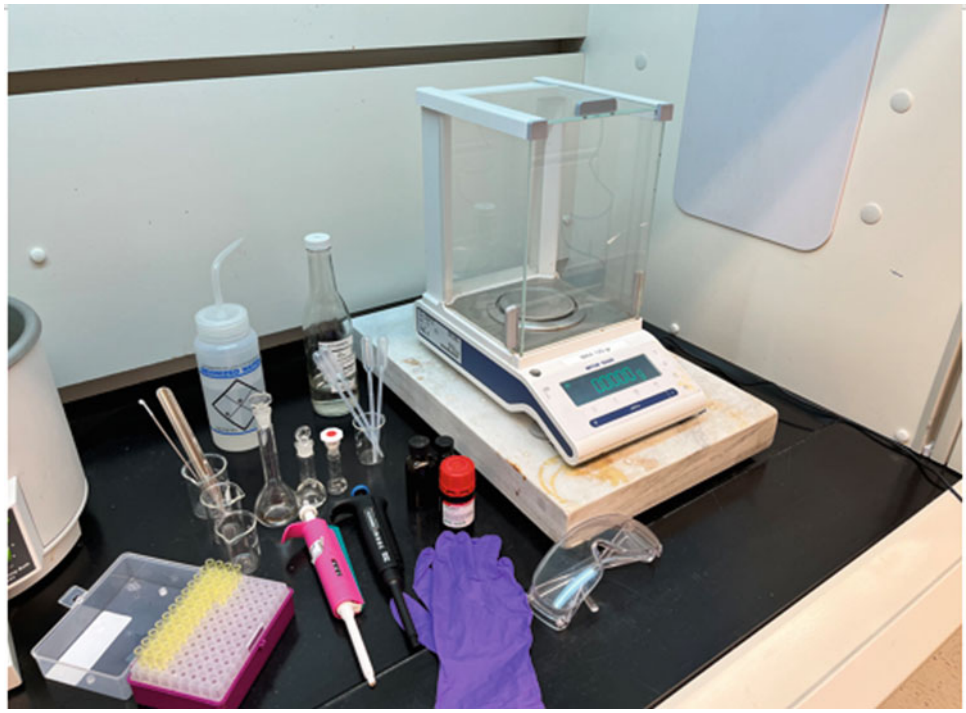

Fig. 2 Example of materials and analytical scale setup for preparing stock solutions from pure aroma standards

10 mL of stock dilution is a practical volume that provides enough flexibility to reach most of the typical aroma concentrations in beverages. Once the aliquot volume has been defined, the following steps may be followed to prepare the stock dilution (*see* **Notes 1–3**):

1. Inside the fume hood, prefill a volumetric flask (i.e., 10 mL) with the solvent of choice (up to 20% of the maximum volume) and place it into the analytical scale for taring its weight (*see* **Notes 4–6**).
2. Draw the liquid aroma standards from its original flask using a calibrated micropipette fitted with a clean tip and then transfer the aliquot into the volumetric flask. Discard tip after the transfer (*see* **Note 7**).
3. Once the weight on the scale is stable, immediately record the value (in grams or milligrams) and then recap the aroma standard flask. The recorded weight should be used to calculate the concentration of the stock solution (e.g., in 0.1 g/10 mL = 10 g/L).
4. Remove the volumetric flask from the scale and fill it to the calibration mark using a disposable transfer pipette and the solvent of choice. While filling, allow the solvent to drip over the walls of the flask to capture any particles or droplets.

Homogenize the solution by placing the stopper on the flask and shaking vigorously.

5. Transfer the contents of the volumetric flask into a pre-labeled amber glass flask with screw cap and close it tightly immediately.
6. Store the stock solution inside a refrigerator at 4 ± 2 °C to decrease volatility (*See* **Notes 8** and **9**).

3.1.2 Preparation of Stock Solutions Using Solid Standards

Opposite to liquid standards, solids require a manual extraction from the primary container, which is often less precise compared to the use of micropipettes and challenging due to the formation of lumps. As a standard procedure, attempting to extract 50–100 mg is feasible for most experimenters (*see* **Notes 1–3**).

1. Place a weighing boat inside of the analytical scale and then place a small beaker (10–25 mL) on top. Tare (zero) the weight of the materials.
2. Draw the aliquot from the aroma standard flask using a clean stainless-steel spatula and transfer the solids into the beaker. Take care not to drop any solids while transferring the standard into the beaker (*see* **Note 10**).
3. Immediately recap the aroma standard container and record the weight indicated on the scale. The recorded weight should be used to calculate the concentration of the stock solution.
4. Remove the beaker from the scale and slowly add 1 mL of solvent to facilitate the transfer of the solids into a volumetric flask.
5. Keep washing the beaker with solvent several times until no solid residue is left and adjust to the volume of the volumetric flask (*see* **Note 6**). Homogenize the solution by placing the stopper on the flask and shaking vigorously. In case the solid is not readily solubilized, submerging the flask in an ultrasonic bath for 1 or 2 min should overcome this issue. Otherwise, revise the solubility of the molecule with the solvent of choice.
6. Transfer the contents of the volumetric flask into a pre-labeled amber glass flask with screw cap and close it tightly immediately.
7. Store the stock solution inside a refrigerator at 4 ± 2 °C to decrease volatility (*see* **Notes 8** and **9**).

3.2 Beverage Spiking

Beverage spiking may be accomplished by diluting pure standards or the stock solutions described in Subheading 3.1 into the beverage matrix (model solution or a real beverage). Nevertheless, the high concentrations of most stock solutions often require creating additional dilutions. This second step is typically required when the target concentration in the beverage is in the range of only a few μg/L or ng/L. Additional dilutions may use the same solvent used

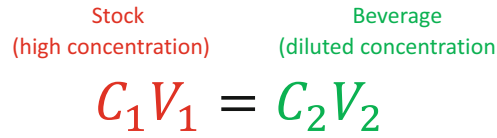

Fig. 3 General equation used for calculating dilutions volumes or concentrations

for the stock solution, or a different solvent that is more representative of the beverage matrix. For example, in wine studies, spiking solutions are typically prepared in aqueous solutions with 13–15% ethanol, which is a representation of the typical alcohol content of most wines.

Regardless of the solvent used, the volume of stock solution required to reach a specific concentration in a specific volume of beverage (or solution) can be calculated using the following formula (Fig. 3):

where C_1 represents the concentration of the stock solution (or the most concentrated solution available) and V_1 represents the volume of stock solution to be diluted (transferred) into the beverage or secondary dilution; C_2 represents the required concentration for the intermediate dilution and V_2 represents its volume. Serial dilutions from the stock solution may be prepared to have different ranges of concentrations for spiking beverages. Mass per volume units are typically used for working with these dilutions (i.e., mg/L or μg/L); care should be taken to have unit consistency when performing dilution calculations (*see* **Note 11**).

3.3 Spiked Beverage Presentation

The stability and sensory properties of the spiked beverage should be evaluated before starting any experimentation because of the possibility of chemical interactions between the added odorants and the beverage matrix [28], or degradation reactions occurring as a result of temperature changes [32, 33] or the presence of oxygen [34]. Further, the storage temperature and the time after preparation that a beverage can be used for sensory evaluation (known as holding time [35]) are sources of variation in the qualities of spiked solutions. If spiked samples need to be prepared well in advance of an experiment (days or weeks), a difference (i.e., triangle test) test with sufficient power needs to be done to verify if storage conditions lead to a noticeable change [36]. This test only needs to be executed one time to verify that there are no changes under the storage parameters.

Sample preparation for execution of the experiment is time-consuming and a sufficient amount of time shall be allocated for this purpose. Samples shall be prepared out of sight from the participants of the sensory experiment following identical steps [37]. The temperature of all samples shall be identical and be representative of the typical temperature for which the product is consumed.

3.4 Odorant Storage and Maintenance

Aroma reference standards and their dilutions are best stored in tightly capped amber flasks and inside storage cabinets featuring a ventilation system and temperature control. Additionally, aroma standard flasks may be stored in secondary containers such as plastic boxes to mitigate the aroma release inside the storage cabinet. They can be brought to room temperature 1 h before experimentation. After each use, the amber vials can be wiped with ethanol to disinfect and remove any residual particles that may cause a scent on the outside of the vials.

4 Notes

1. Researchers must be aware of risks associated with the manipulation and exposure to reagents such as pure standards of odorant molecules and solvents. Manipulation of some pure odorant standards requires personal protective equipment. Reviewing safety data sheets (SDS) for all reagents is a mandatory step before their manipulation (suppliers typically provide SDS while shipping reagents or within their websites). All personnel involved in sample preparation must be informed of potential risks and contingency measures in case of spills and unintended contact with body parts. It is worth indicating that, once diluted, manipulation of food-grade odorants is generally recognized as a safe practice.
2. Recommended personal protective equipment includes gloves, safety glasses, a laboratory coat, and wearing a respirator (when indicated in SDS). Manipulating pure odorants under a fume hood is recommended to minimize the risk of suffocation or reactions to high concentrations of aromas, besides preventing their diffusion in buildings.
3. Some aroma standards are susceptible to rapid volatilization when exposed to ambient conditions. For example, pure (>90%) acetaldehyde or acetic acid will rapidly evaporate when pipetting the pure standards under room temperature conditions. In such cases, diluted standards supplied by manufacturers might be a better alternative to avoid inaccuracies during the sample preparation.
4. Using volumetric flasks with wide necks facilities the pipetting mechanics and prevents spills. The addition of a small quantity of solvent allows for quick solubilization of the standard and helps to decrease its volatilization.
5. To protect the scale of spills, a large weighing boat may be used under the flasks or beakers.
6. Solvents and/or beverage matrices used for dilutions should be at room temperature before starting any procedures.

7. Micropipette tips should be used a single time and discarded immediately after. Care should be taken to avoid contamination of the aroma standards with dirty or used tips.
8. Storage of pure odorant standards should follow the recommendations of the manufacturer as indicated in SDS. Some standards require storage under controlled temperature conditions ranging from freezing to room temperatures. Molecules susceptible to oxidation may require storage under a vacuum to prevent degradation. Ideally, having a dedicated storage space such as a cabinet or refrigerator is a convenient solution. Using secondary containers to store pure aroma standards, such as plastic boxes, is recommended to mitigate their diffusion in storage cabinets.
9. Waste from solvents or discarded dilutions should be disposed of in sealed containers following institutional guidelines. Micropipette tips that were in contact with the pure odorants can be placed inside sealed plastic bags and disposed of with hazardous solid waste.
10. If solids dropped inside the scale, clean immediately by gently padding with a tissue soaked in solvent or water and restart the procedure from **step 1**. If solids dropped outside the scale, clean immediately by padding with a tissue soaked in solvent or water.
11. Always perform dilution calculations before the start of the procedure and double-check the consistency of units.

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Chapter 3

Studying Chemosensory Perception and Its Hedonic Component in an Anthropological Context: From Genetics to Psychophysical Measures

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Abstract

Psychophysical studies have demonstrated the impact of various biological factors on smell. In particular, it has been shown that genetic background plays an important role in the intensity of perception, the detection threshold of a molecule, or its hedonic character, and therefore, the emotions triggered. The next challenge is to explore how such findings can be generalized across a variety of populations, locations, and contexts closer to people's everyday lives since most studies are primarily conducted on Western populations in a standardized setting. It is within this perspective that we propose here a methodology to collect data and to measure the relationship between a genetic variant and an olfactory psychophysical phenotype in remote populations. Based on our field experiences in remote villages of Madagascar, and the example of a ubiquitous food-related odorant molecule ß-ionone, we present a protocol to efficiently and respectfully collect DNA and psychophysical data from individuals located in a remote area with limited resources.

Key words Smell, Food, Perception, Hedonic, Anthropology, Psychophysics, Genetics

1 Introduction

One of the characteristics of olfactory perception is the great diversity between individuals and populations [1, 2]. The study of the diversity of chemosensory perception in humans is essential to understand the variability of emotions experienced by different individuals confronted with the same food or smell. Indeed, the same smell can be pleasant for some people while it is considered unpleasant for others [1]. Some people will feel the presence of a molecule as an intense smell while others will not detect its presence [3]. Psychophysical studies have identified biological parameters that greatly influence the perceptions of molecules, such as age,

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gender, viral infections, or genetic variants [4–8]. Genetic studies concerning the perception of a molecule such as ß-ionone have shown that a single mutation can explain more than 90% of the observed inter-individual variation such as ß-ionone detection and its hedonic component [9]. βI is ubiquitous constituents in a number of fruits and vegetables and food by-products (tomatoes, white-fleshed nectarines, violet flowers, grapes therefore wine) and is also used widely as a flavoring improvement agent in the food industry [10–13]. Consequently, the genetic predisposition to βI anosmia might substantially influence food preference and food choice [14, 15] and by extension to food culture across the world.

The current challenge is to understand the role of these biological factors and genetics, in the everyday diversity of "real life." Indeed, sensory perception can be influenced by many factors such as individual lifestyle, culture, and environmental characteristics (temperature, humidity, altitude, pollution) [4, 9, 16–18]. In addition, individual genetic background could also modulate the effect of specific mutations. However, this type of study is mainly conducted in the same standardized environment on populations sharing a similar lifestyle and often on "WEIRD" populations, i.e., "Western educated, industrialized, rich and democratic" populations [9, 16, 19, 20]. Further experiments are therefore needed to account for individual diversity in terms of location, lifestyle, and genetic background and to assess the overall effect of biological factors.

To meet this need, the development of experiments to measure the real influence of genetic factors on the perception of molecules in populations living in different environments is a challenge. Indeed, such a protocol requires expertise from a wide range of disciplines: (1) anthropological studies; (2) genetic–phenotype studies; and (3) psychophysical studies (Fig. 1). Based on the knowledge of specialists in these three fields, we have developed a methodology to test the reliability of genotype/phenotype relationships found in a controlled environment. We aim at retesting these relationships on people in their daily traditional way of life and in their remote environment with a minimal selection bias.

Based on the implementation of this methodology in Madagascar [17], we present here a protocol that allows for the efficient and respectful collection of DNA and psychophysical data from individuals located in a remote area with limited resources. We divided the protocol into four main steps: (1) cultural adaptation of the methodology, (2) preparation of the psychophysical test kit, (3) contacting villages, and finally (4) data and DNA collection (see Methods section).

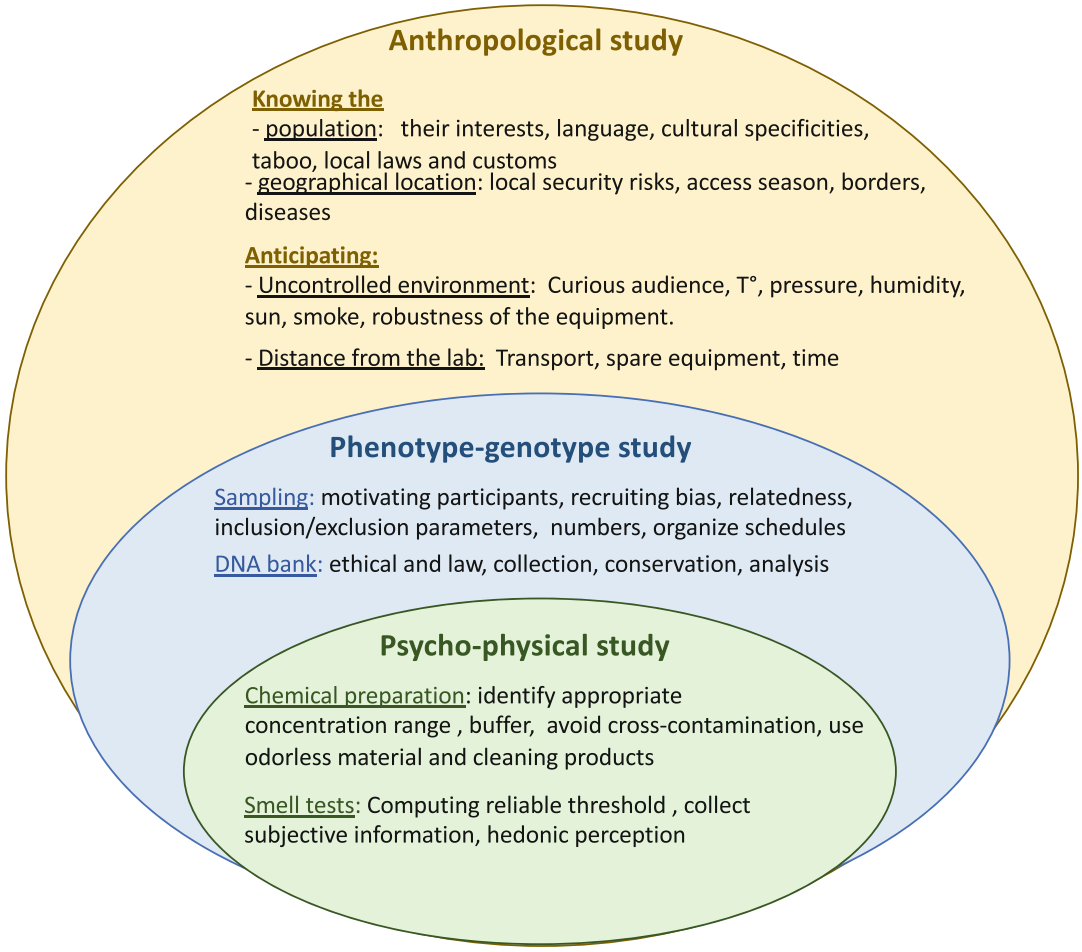

Fig. 1 Pilling up the challenges

2 Materials

Preparation of the Psychophysical Tests:

2.1 Dilution Step

1. Nitrile scentless gloves (avoid latex odorant gloves).
2. 7 series of Amber glass bottle (25–100 mL).
3. Self-adhesive unicolor labels (rectangular for the bottle and round for the cap rectangular for the body of the bottle and round the cap).
4. Pencil (odorless).
5. Chemical hood.
6. ß-Ionone (96% food-grade β-ionone (CAS: 14901-07-6) Sigma-Aldrich).

7. Eppendorf Reference® pipette, variable 100–1000 μL.
8. The solvent mineral oil.
9. Varispenser bottle-top reagent dispensers of 1–5 mL (Eppendorf™ Varispenser™ plus).
10. Self-sealing cap (PH18).
11. Lab shaker to mix the solution (i.e., 250–2500 min^{-1} speed, orbital).
12. Waists closed garbage cans.

2.2 Perceptual Test Kit

1. Nitrile scentless gloves (avoid latex odorant gloves).
2. An amber glass bottle (25–100 mL depending on the volume of the first odorant dilution).
3. 15 mL amber glass vials Ph18, 1.7 cm diameter at opening, 5.8 cm high; self-sealing cap.
4. Self-adhesive unicolor labels (rectangular for the bottle and round for the cap rectangular for the body of the bottle and round the cap).
5. Eppendorf Reference® pipette, 5000 μL.
6. Scentless polypropylene fabric cut in pieces of 3 cm × 7 cm: oil-absorbent sheets 3 M, Valley, NE, USA, Dimensions: 48 cm × 43 cm × 10 mm.
7. Rack Wheaton for 5 × 10 scintillation vials with 50 spaces for 20 mL tubes, dimensions: 32 × 17 × 3 cm made from polypropylene for laboratory preparation.
8. Plastic film.
9. Bottle rack for the tests: custom-made foam scentless rack with 32 spaces 4 rows × 8 columns, Rack dimensions: 15.5 cm × 29.8 cm × 3 cm, tube container dimensions: 2.6 cm × 1 cm.

2.3 Threshold Test Kit

1. Nitrile scentless gloves (avoid latex odorant gloves).
2. 2- Six amber glass vials of 15 mL Ph18, 1.7 cm diameter at opening, 5.8 cm high; 6 self-sealing cap (odorant vials).
3. For the buffer vials (blank): 18 amber glass vials of 15 mL Ph18, 1.7 cm diameter at opening, 5.8 cm high; 18 self-sealing cap.
4. Self-adhesive unicolor labels (rectangular for the bottle and round for the cap rectangular for the body of the bottle and round the cap (*see* **Note 1**)).
5. Eppendorf Reference® pipette, 5000 μL.
6. Scentless polypropylene fabric cut into pieces of 3 cm × 7 cm: oil-absorbent sheets 3 M, Valley, NE, USA, dimensions: 48 cm × 43 cm × 10 mm.

7. Rack Wheaton for 5 × 10 scintillation vials with 50 spaces for 20 mL tubes, dimensions: 32 × 17 × 3 cm made from polypropylene for laboratory preparation.
8. Plastic film.
9. Bottle rack for the tests: custom-made foam scentless rack with 32 spaces 4 rows × 8 columns, Rack dimensions: 15.5 cm × 29.8 cm × 3 cm, tube container dimensions: 2.6 cm × 1 cm.

2.4 Collecting Psychophysical Data

1. Odorless soap.
2. Twenty pairs of odorless washable cotton gloves (or disposable nitrile gloves without odor).
3. Printed documents (agreements, etc.).

2.5 Collecting DNA

1. Bucket of water and plastic glass.
2. Alcohol 70%.
3. Paper towel.
4. Sugar cubes for activating salivation.
5. Cleaning wipes.
6. Gloves.
7. Plastic collection box.
8. Permanent marker for DNA plastic tube.

2.6 Field Experimentation

1. Plastic cover for tables.
2. Transport boxes; plastic transport.
3. Cooler.
4. Screen, dust sheet.
5. Camping equipment (comfortable): chair, table.
6. Thermometer, GPS, hygrometer.
7. Device-making pictures (phone or camera).

3 Methods

3.1 Cultural Adaptation of the Methodology

1. In collaboration with anthropologists specialized in the target population, identify the usual language (*see* **Note 2**). Also, list the local issues, potential obstacles, and possible apprehensions that your project can encounter. Identify also scientific questions about your project that might be of interest to the target population.
2. Contact the local university to identify an experimenter. The experimenter must share the same native language as the people who will be studied. The experimenter should be able to easily interact with the general population and explain the

scientific project (typically graduate students in anthropology, sociology, etc.). It is also necessary that you share with him/her a common language for communication. Depending on the population, parameters such as gender and age might be important.

3. With local legal support (from local or national university) identify the country's legal agreements for human experimentation and committees where your project should be submitted. And enquire about the administrative requirements.
4. As in **step 3.1.1**, redact questionnaires, introductory note, and informed consent with anthropologists in order to adapt them to the studied population.
5. Modify and ensure that the documents comply with the laws and standards of the country with your legal support.
6. Translate the documents in the language of the target population. And after asking an independent person (the experimenter) to translate back into English (or in your own language) to identify any errors during the first translation.
7. Submit your research protocol and the document to the competent authorities for research on human beings in the target country (ethics committees). And make the appropriate corrections to get the official approval.

3.2 Preparation of the Psychophysical Test Kit

Basic rule regarding management and materials preparation must be adopted: Strictly separate odorant molecules and buffers: this includes all dedicated materials, storing, and preparation areas. Odorant molecule must be prepared in a chemical hood. Choose food-grade molecules (i.e., with FDA for USA for example and local authorizations). Laboratory coat and gloves are mandatory (protection for oneself and for the products). Store all odorant molecules in a ventilated refrigerator and bring them to room temperature at least 1 h before solution preparations. Diligently follow all waste disposal regulations when disposing waste materials. For odorant molecule preparations, choose airtight waste disposal in order to avoid air contamination.

3.2.1 Threshold and Perceptual Test Kits

This procedure is based on 4 alternative forced choices (4-AFC). Six blocks of four 15 mL vials are presented in an increasing order of concentration. Among each block of four vials, only one of the four contains the odorant dissolved in a mineral oil; the other three vials are blank with mineral oil. Both odorant and blank mineral oil are soaked up on oil-absorbent sheets.

Preparing the Vials

1. Cut 25 pieces of polypropylene fabric 3 × 7 cm with a paper cutter (*see* **Note 3**): 24 for the threshold test and the last one for the perceptual test.

2. Prepare numbering self-adhesive rectangular and round labels in 6 sets of 4 numbers (11–14, 21–24, 31–34, 41–44, 51–54, 61–64). All labels must be written with the same pencil and the same script. Label each of the bottles with the rectangular label. Prepare an additional self-adhesive rectangular and round label coded by a letter for the perceptual test.
3. For each of the 6 sets of 4 numbers, draw one bottle at random and separate it from the set. You will have 6 bottles separated from the others which will be the target odorous bottles in the test plus one additional bottle for the perceptual test put aside. They will be filled later on.

Filling the Odorless Vials

4. Fill the 18 remaining bottles with 4 mL of mineral oil using the dispenser (using the same batch of oil for all bottles). They will constitute the odorless bottles of the test.
5. For each bottle, roll a piece of polypropylene fabric and place in the glass vials containing the mineral oil (4 mL). Then, close the bottles and label the cap using the round sticker with the same number as the bottle. Store all these bottles in a sealed plastic bag placed in the refrigerator.

Preparing the Dilutions

6. Make 2 aliquots of mineral oil solvent each in a 100 mL bottle.
7. Under a clean laboratory fume hood (cleaned with alcohol 15 min before), put one of the previous aliquots of mineral oil and the bottle of odorant (pure ß-ionone, *see* **Note 4**). After checking that the caps are well closed, clean the bottles with alcohol and wait 15 min for any residual odors on the body of the bottle to be aspirated.
8. Prepare the initial dilution of the odorant molecule in the second 100 mL working vial (the supraliminal concentration 20,000 ppm). Store back the vial containing the pure molecule of ß-ionone.

Filling the Odorous Vials

9. Under the laboratory fume hood with the second aliquot of mineral oil, perform a series of dilutions in other 100 mL vials beginning with a concentration of supraliminal concentration of 20,000 ppm for the perceptual test, and for the threshold test ranging from 2.05×10^{-2} mol/L to 2.05×10^{-7} mol/L (refer to *see* **Note 1** for concentration). For each dilution level, vortex the vial tightly and let it stand until the smallest bubbles disappear before proceeding to the next dilution. Use filter cones for pipetting and change gloves regularly (*see* **Note 1**). Waists are directly stored in closed garbage cans (follow waste management regulations).
10. Finally, starting at the lowest concentration: place 4 mL of the concentration in the dedicated and labeled 15 mL vial. Place a

rolled piece of polypropylene in the tube then close the tube and label the cap using a sticker with the same number as the tube. Leave the bottle for 1 day to let all the liquid be completely absorbed by the polypropylene fabric. Generally, wait at least 1 day before use (*see* **Note 5**).

11. Seal separately each concentration in individual plastic bags. If you are preparing several batches of tests, separate the different plastic bags during storage and transport (to avoid contamination between vials). Store the sealed bottles in the refrigerator at 4–10 degrees.

3.3 Contacting the Villages

1. Select the best time of the year to conduct the field experiments. It might depend on the region, but in general avoid the rainy seasons because of the problems of access to the villages (blocked roads), the places of experimentation as well as the conservation of the kits. Depending on the population, identify the seasons when your target population is the least busy and is present in the village and not outside (livestock work, fieldwork).
2. Make a formal visit to the state representative (*see* **Note 2**). Arrive in the study area (in the main city): identify the representatives of the national authority, ask for an audience, and introduce yourself and the project. Present your national approvals and request a local approval. Ask for advice on how to best communicate with the local population. Ask about the traditional and current social organization, and ask who to contact in the village. Ask about potential missteps to avoid about customs and current safety conditions in order to plan travel times (i.e., night trips are often to be avoided on the road, depending on the location; rivers often have to be crossed in the morning).
3. In the city, find out about the surrounding villages from businesses and institutions (schools, hospitals). Ask about their size and date of creation, activity of the people on the spot. Prefer old villages (>50 years) of small size (<300 inhabitants). Privilege small village where everyone knows each other. Find your intercessor by identifying an individual who knows the inhabitants (ideally an inhabitant himself) and who agrees to introduce you to the inhabitants of the village.
4. Following the indications of your intercessor, and ideally in his presence, go to the village in a small team [2, 3] giving preference to local researchers able to communicate without interpreters. Introduce your project, the scientific goal, and what will happen to the village authorities. Ask for agreement to come back to the village to present the project one morning in the next 3 days. Ask for advice on the best way to proceed so

that the villagers are interested in participating and to have participants who meet your inclusion criteria. Clarify with them the criteria for inclusion of participants in the study.

Explain that you would like to choose an area to perform your project among several possibilities because of the constraints, find out if there is access to a room (for example a school) that is open and easily ventilated, but warn that you will have to check for ambient odor. Note the places to avoid (taboo, religion, private property). Ideally, the place should be chosen with the authorities before the day of experimentation. This will give you/them time to clean and prepare the room or the chosen place. Avoid odorant liquid soap for the cleaning.

Finally, arrange a time and place to meet the population and present the project to the whole village. Ideally, it should be the morning of the day of your experimentation. This will help to recruit participants. The largest number of volunteers is often maximum on the first day.

3.4 Collection of Psychophysical Data and DNA

1. When you arrive in the village on the day of the event, let your contacts know you are in the area. Then, identify the best place to perform the tests from the options identified during the first visit (*see* **Note 6**). Recheck the place for the tests: the first thing to avoid are the stray odors from toilets, kitchen, animals (livestock, bats, rats, etc.). Then, check and find out the wind directions and avoid areas that receive smoke from cooking areas. Move away from distractions and auditory and visual nuisance. Choose an area where you can be seen from a distance so as not to arouse curiosity. Choose a ventilated area sheltered from the sun and rain. Anticipate that all these parameters can change during the day, the sun moves, the wind can start blowing, and cooking areas can be used. It is possible that the best area is outside the village. Finally, make sure that the place is appropriate and practical for the inhabitants.
2. *Set* up a table and chairs. If you are provided with material, make sure it is odorless. Cover the tables with a plastic cover. Place a screen to avoid gusts of wind and to create a calm, peaceful environment by hiding distractions. Mark off an area so that any audience does not distract the participants: preferably away from the back and in the opposite direction of the wind so as not to catch the scent of the audience. Take the bottles out of the refrigerated area at room temperature a few hours before the experiment begins. Clean all bottles with alcohol paper (non-odorous). Allow to evaporate. Install the foam bottles. Collect the GPS coordinates, altitude, pressure, temperature, and humidity for the first time.

3. In front of the whole population that your contacts have assembled to you, thank your contacts for having allowed you to address the inhabitants of the village, thank the inhabitants for being present, and introduce yourself. Then, present your project and experiment's course. You should do a test on one of you to concretely show the course of one experimentation. Specify the inclusion criteria and explain them in the easiest way to the gathered people: leave time for the questions but do not exceed 30 min in total.
4. *Make a list of* the volunteers. Check that they meet your inclusion criteria. For example, adults between 18 and 40 years old who smoke less than 5 cigarettes a day and whose 3 out of 4 grandparents come from a defined area (50 km) might be considered as representative of the general population (*see* **Note 7**). Check the level of relatedness between all volunteers (*see* **Note 8**). Exclude people with a cold or a disorder affecting smell and taste (chronic sinusitis, nasal polyp, etc.), broken nose, nose surgery. But keep track of these exclusions to address later this putative bias (*see* **Notes 8**–**10**).
5. *Distribute and* read the informed consent with the volunteers, and make sure they understand it. With the remaining volunteers, organize a schedule for the day. Plan for each individual test to last 30 min, so you can plan for about 10 individuals per day. Specify that before the test, ideally, participants should not have cooked or handled strong food odors. They should not chew gum 1 h before the test, nor have drunk anything other than water, do not wear strong perfume or cosmetics (hair, face, etc.) before the test. Once the schedule is defined, give the individual an appointment at the test site and time. Confirm with them that everyone knows who is before and who is after so that they know when their turn will be (*see* **Note 11**).
6. *When one participant comes to the testing area*, welcome and thank the participant for coming. Ask them if they have had time to read the informed consent (distributed in **step 5**) and if they agree to participate. If they agree, ask them to sign or leave a fingerprint (if they cannot sign) sealing their agreement to participate in the study and make it clear that they can stop participating at any time. Check that the participant has respected the criteria (no perfume, cooking, etc.). Note that most of the time, participants might have cooked. In this case, discuss with them the best moments (1 h before or after the cooking time). In case of a cold, kindly suggest to the participant to not do the test. Present the project again and explain its course and what data will be collected.

7. Ask the participant to wash his or her hands with the odorless soap and to rinse the mouth with water. Ask the participant to put gloves on their hands.
8. Number the questionnaire in a way that preserves anonymity. On questionnaire, note time, temperature, humidity. Fill in the questionnaire with the participant, "nasal health" with them (*see* **Note 12**), age, sex, smoking or not, ate or not, respiratory allergy, drug treatment, for women (be likely to be pregnant, drug contraception, date of last menstrual period).
9. Explain rules to the participant. The participant's task is to sniff each of the four vials in a row for 3 s with both nostrils and to state which bottle differed from the other three vials.
10. Perform the threshold test (4 alternative forced choice, 4 AFC) (*see* **Notes 13** and **14**). Ask the participant which vial differed from the other even if they did not perceive a different smell, and whether they perceived nothing, a slight odor or a strong odor in the designated vial. The smelling kit consists of six rows of four glass vials. In each row, only one vial contains a specific concentration of βI, while the others are blanks. Each concentration is presented only once, and concentrations are presented in ascending order. Importantly, the participant must not face the experimenter during the test or during the collection of the answer (no involuntary indication). And the experimenter must clearly state that he/she does not know the answers. The experimenter should note down the answer of the participant. Alternating sets with odors in different tubes that have been in use allows the experimenter to not know the correct answer and allows the tubes time to resaturate.
11. Perform the perceptual test: This task aims at measuring four perceptions such as hedonic, intensity, familiarity, edibility. Odorant molecule are presented at their supraliminal concentration (i.e., ß-ionone 20,000 ppm). Participant are asked to sniff the vial for 3 s. Then, they are asked to rate the odor using the following 7-point scales: hedonic (1: extremely unpleasant, 2: very unpleasant, 3: unpleasant, 4: neutral, 5: pleasant, 6: very pleasant, 7: extremely pleasant); intensity (1: absent (no odor), 2: very low, 3: low, 4: medium strong, 5: strong, 6: very strong, 7: extremely strong); familiarity (1: not at all familiar, 2: very little familiar, 3: little familiar, 4: medium familiar, 5: familiar, 6: very familiar, 7: extremely familiar); and edibility (would you eat it? 1: absolutely not, 2: certainly not, 3: probably not, 4: maybe, 5: probably, 6: certainly, 7: absolutely). Finally, participants are asked to freely identify and/or describe the odor.
12. To collect DNA, ask the participant to split into the funnel of DNA Genotek tube until the amount of liquid saliva (not bubbles) reaches the fill line. Following DNA Genotek

instruction: "Hold the tube upright with one hand. Close the funnel lid by pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva. A piece of sugar can be given to the participant in order to facilitate salivation. Make sure that the lid is closed tightly. Hold the tube upright. Unscrew the funnel from the tube. Use the small cap to close the tube tightly. Shake the capped tube for 5 s. Discard or recycle the funnel." Collect the tube from the participant and wipe it with alcohol solution. Thank the participant and allow them to leave and finally write the number of the questionnaire on the tube and conversely the number of the DNA tube on the questionnaire.

13. At the end of the day, return to thank your contacts in the village, and propose to share a snack and discussion in a relaxed atmosphere. Take a group photo (*see* **Note 15**).
14. When the results are analyzed, prepare feedback tailored to the study population explaining what happens to the results and data. If you cannot travel to all populations in person, ask the experimenter to do so and send some printed photos of the residents and participants taken during the tests.

4 Notes

1. Note that the range of concentration to be used for the test will be molecule, solvent, and container dependent. It is therefore important to calibrate the concentration range even if you replicate a previously published test. Proceed on a threshold test on about ten people in order to calibrate your concentration range.

   One alternative option is to prepare a wider volume of the dilution set in order to be able to change the kit in case of problem encountered in the field (bacterial contamination of bottles, breaks...). All the material lab needed should then be included (pipettes, polypropylene, etc., cf 2. Materials).

   For the kit preparation itself, anticipate the materials regarding all the needed concentration for the test: for perceptual test (a first dilution bottle for the highest supraliminal concentration) and threshold test for ß-ionone (6 series of dilution bottle for the concentration range). Then, 7 series of amber glass bottle corresponding to 7 dilution steps should be prepared.

   Prefer solvent conditioned in glass bottles from the fabricants. Propylene bottles can transfer a plastic smell to the solvent.
2. In order to be successful with this protocol, you will have to interact with a wide range of people from the state

representative to the children in the village. Your attitude toward all these people will be very important to the success and the reliability of the collected data. Your interlocutors will often present objections, as a general rule ask them for advice to overcome these challenges and rely on the ideas of local individuals to prepare the project.

Also, a collaboration with an anthropologist can be a real plus in anticipating local communications.

3. Quantities described here are for 1 set allowing 40 individuals; several sets should be prepared to alternate the kit between individuals during the day of test.
4. The present kit preparation is a standard protocol, applied for the ß-ionone odorant molecule. Note that the choice and nature of solvent depend on the characteristic of the odorant molecule and its solubility. Refer to their characteristics to previously described protocol or to chemical data on websites such as PMIC, good scents company, etc. Also, the container could also be different and there are numerous types of tests and choices available. In our protocol, we have adapted the LCOT (Lyon Clinical Olfactory Test Rouby et al.'s test [21]).
5. It is well known that certain odors can change because of packaging, storage, and/or microbial contamination [22]. To prevent such scent evolution, ideally, a set of precautions should be taken. First, all participants were asked to wash their hands with hydroalcoholic solution before experiment. The tested scents should be stored automatically in amber glass vials, and in dark place when unserved, for chemical's protection from UV light. The kit should be replaced every week to limit the number of manipulations and thus oxidation. Finally, the efficiency of these precautions should be controlled by performing GC-MS analysis of the kits before and after field sessions.
6. The choice of the place: One way to ensure that there is no ambient odor in the chosen room for the experiments, sniff the air several times, and pause for breath or rest your nose by breathing gently in the crook of your elbow. In case of difficulty to find an appropriate place, several solutions can be found: an adapted car, choice for a more appropriate place outside the village. Note that you could provide for transport extra-costs (buses, taxis, etc.).
7. Inclusion criteria of representative individuals of the village are tricky and depend specifically on the aim of the research. In this protocol, we focus on the geographic representativity of the participants based on geographical origins. The sample homogeneity being the rule. Our first choice is that the 4 grandparents originated from the studied region. If this is too

difficult to obtain, then second choice: 3 out of 4 grandparents from the region. Other selection methods are acceptable depending on the project. Nevertheless, we consider that "ethnic affiliation" is rarely appropriate even in the case of auto affiliation. Indeed, people might tend to choose an answer thinking please the experimenter or for numerous reasons (language, current location, location of birth, one specific ancestor, physical characteristic, etc.). If you ask ethnic auto affiliation, we suggest asking the participant also "why."

8. In case of genetic studies do not include siblings, cousins, parents, children. Indeed, genetic association studies (such as GWAS with population-based design) focus on identifying the effect of genetic polymorphism on a phenotype [9, 14] and those methods are inadequate in datasets containing family structure or cryptic relatedness [23]. Indeed, kinship are confounding factors that can decrease power and increase the false-positive rate for test association [24]. Otherwise a more complex methods such as family-based design are adequate for another question including families (for example for question regarding linkage disequilibrium transmission) [23].
9. It happens that some volunteers do not meet the inclusion criteria. The most frequent scenario is that the participant has a cold the day of the experiment; the volunteer lied on their smoking state or has just decided a few months before the test to stop smoking; the volunteer wears a strong perfume (currently oil for the hair); the volunteer has just cooked a few minutes before the beginning of their test; the volunteer come with a strong smell on their clothes (smell of food, etc.), etc. It could be embarrassing to refuse these volunteers who are really willing to participate. In case of a parasite odor, you can provide a laboratory coat or/and a cap for hair in case of strong smell (food, fragrant hair oil, etc.). If this does not solve the problem, then propose to reschedule the test. If it is impossible for them or for you to reschedule the test, explain gently and clearly that the test can be done but will not be included in the final study. This will leave the volunteer the choice to stay or not.
10. Villagers can be very curious but also cautious with understandable apprehension about your project. The best way to break the ice is to have a demonstration between the members of the field team. All your team should be fully invested in welcoming the participants and trying to make them have a good time and discover new things. Thank the participants several times for their help in advancing science and more specifically your project.
11. It may happen that you fail on recruiting enough participants, on having the agreement of a village, etc. However, it may also

happen that agreement is obtained very easily and that some villages are very enthusiast, and some can be enthusiastically awaiting your arrival (news of your presence in the region travels very fast in rural areas). The protocol can be then adapted and can be flexible. You must also leave room for improvisation and the atmosphere of the moment.

12. To ensure that the measured phenotype is specific to the molecule. In addition to the health questionnaire, one option is to measure the general olfactory capabilities of each subject with an easy and quick test (such as identification or discrimination test of a very common odor).
13. This protocol is designed to measure the relation between a known genetic variant and an olfactory psychophysical phenotype in remote populations but it can be modified to identify new variant influencing a specific olfactory psychophysical phenotype in the population (i.e., discovering genotype/perception type of ß-ionone [9]). This kind of objective could be reached with the GWAS process (Genome Wide Association Study) including a more important number of participants. In this case, the perception tests could be self-administered with more time taken for instruction. To fill out their own the questionnaire, individuals should be at ease with writing and reading; therefore, performing this test inside the university proposing to the local student to be participants might be adequate. In this case, we recommend incorporating at least three experimenters and allow 10 individuals to perform the test simultaneously.
14. Other threshold procedure that can be used such as 3-AFC which would be lesser the materials and tasks for participants (ISO 13301:2018 3AFC, ASTM E679-19, ASTM E1432).
15. Renumerating participant being sensitive we leave it to the research team discretion. Whatever your choice, in a village context, we recommend sharing moment of conviviality at the end of each village visit (sharing food and drinks) and find a way to later present to them the result of your analyses.

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Chapter 4

Measuring Food Emotions in a Living Lab

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Abstract

The act of eating is known for being associated with a rich spectrum of emotions, from disgust to joy, and many contemporary methods have been proposed for their measurement. Most of them are based on a stimulus–response paradigm, considering the food or drink characteristics as stimulating variables, and collect data through face reading, physiological sensors, or questionnaires. Although food and drink properties (physical and chemical) are essential parameters of consumers' experience and emotional responses, they are not sufficient to explain emotions, feelings, and preferences in natural consumption situations. Because personal, social, physical, and environmental factors play a major role in the emotional status of consumers, complementary experiment means are needed to control most contextual variables in an eating environment. Living labs offer ideal conditions both to set a large number of environmental parameters and to measure responses in real-life usage situations. In the food domain, experimental restaurants are places where researchers and food designers can interact and observe consumer responses in natural eating environments with external variables under control. The present paper describes a short protocol for measuring participants' emotions during a meal in such an experimental restaurant where contextual cues can be controlled.

Key words Living Lab, Context, Experience, Real-life testing, Food and drinks, Emotions

1 Introduction

Various models exist to describe food emotions [1–3]: emotions have a central role in eating behavior and food enjoyment from the very first contact with the food, should it be visual or olfactory, to the tasting and ingesting experience [4, 5]. Emotions can change food intake by increasing or decreasing consumption and can lead to an overall positive or negative eating experience. Different theoretical frames support research on food emotions [6, 7] and have led to the development of various methodological approaches, sometimes also including affect or mood criteria [8, 9].

Most studies on food emotions are conducted in controlled conditions in order to focus on the effect of food characteristics on the emotional response. For instance, sensory cues, such as

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sweetness or fatty texture, influence mood and potentially mitigate the effects of stress [4]. Conversely, emotions and affective states might influence food acceptability, liking, and choice. Emotions affect hunger, thirst, and eating [10]. Chronic stress can change food intake by increasing or decreasing consumption, [11, 12] but past studies conducted in real eating situations demonstrate the importance of better controlling environmental factors to unfold food factors from context factors [13, 14]. While emotion measurement in response to foods and beverages developed rapidly, the conditions under which emotions were measured stayed within the relatively controlled testing situations typically used in both academia and in product development situations. For instance, Edward et al. asked students to rate their emotions before and after a meal in a natural eating context, in the cafeteria at Bournemouth University, UK [15]. In order to measure emotional responses to food in a real eating situation, the students had to choose and pay for their meal as usual. Subjects filled out a questionnaire before and just after their meal, using Richins' items [16]. Students reported more positive emotions than negative ones, as previously shown by Desmet et al. [3]. Nevertheless, measuring emotions in real eating situations requires researchers to clearly separate food factors from contextual factors. To do so, living lab approaches were identified as interesting solutions to provide natural eating environments, maintaining the control of both food and context variables [17, 18] and allowing the measurement of emotions as described hereafter. In this paper, we describe a short protocol for measuring participants' emotions and affective states during a meal in an experimental restaurant (Living lab) with a possibility of altering or controlling contextual and environmental factors. Of course, the protocol is not a detailed description of a closed method, but rather a procedure to follow that can be adapted to the specific experimental needs of any research project.

2 Materials

In this section, we describe the material used to measure participants' emotions during a meal in an experimental restaurant. As stated above, these are intended to be general guidelines [19, 20] but materials may be adjusted according to specific experimental needs and availability. These recommendations are based in large part on our experiments conducted at the Institut Paul Bocuse's experimental restaurant. This living lab will thus occupy a central place in the description of the protocol.

1. *Participants.* At Institut Paul Bocuse's experimental restaurant, participants are regular customers of the Living Lab. Typically, people previously reserve a table for a meal. This reservation is

often made in the context of a family meal, a meal with colleagues, or a meal with friends.

2. *The setting and contextual factors.* It is necessary to conduct experiments in a Living Lab that meets the requirements for such explorations. For instance, the Institut Paul Bocuse experimental restaurant offers a bistronomic type of cuisine. The restaurant is a 100-m^2 dining room that can accommodate up to 30 people per service. It is composed of about 10 tables (round or square) of 2–6 people. The disposition can be modified if necessary to accommodate specific group sizes. The tables are covered with white tablecloths, the lighting is subdued, and background music is adapted to the general atmosphere that goes along with the meal. On the walls are paintings by artists, which the chefs use as inspiration for the menu. This type of "mid-range restaurant" is attractive to a wide range of customers. A professional kitchen is adjacent to this dining room, allowing the preparation of menus by the chefs. This general atmosphere of the dining room can be adjusted according to the specific needs of the study (*see* **Note 1**). Moreover, the choice of decoration, tableware, music, and lighting depends on the type of menu to be studied and the context in which the study is carried out. This adaptability is one of the added values of the Institut Paul Bocuse's experimental restaurant. The implementation of this brasserie concept (mid-range restaurant) is often used.
3. *Type of meal.* Depending on the needs of the study, the menu can be imposed [21] or freely chosen [22]. The type of meal can be adapted according to the audience targeted by the study. In all cases, participants are in natural conditions of choice and consumption as in any other restaurant.
4. *Time of meal.* It may be lunch or dinner. Eating is associated with a social activity shared with friends and relatives. In the context of the studies of Giboreau et al. [21] and Porcherot et al. [22], researchers decided to focus on dinner, as it was considered more relevant for measuring emotions in a meal context.
5. *Menu on the plate.* The menus served in the Institut Paul Bocuse's experimental restaurant are high-quality bistronomic food (Fig. 1). Participants pay for their meals, as usual customers. They pay a fixed price of 32 euros per person. This price includes an amuse-bouche, a choice between two starters, a choice between two main courses, a dessert, and a glass of wine. It is of course possible to adapt the menu to special diets, allergies, or intolerances as well as set a specific food offering according to a specific study objective (*see* **Note 2**, Fig. 2).

Fig. 1 Living Lab of the Institut Paul Bocuse Research Center where guests participate to experiment during their meal. (© Eric Leroux)

Appetizer

~ ~ ~

Green asparagus and grapefruit, soubise sauce, lemon cream

Or

Soup, cucumber, beetroot, and radish macedoine, quail egg

~ ~ ~

Sea trout cooked on one side, apple and fennel coleslaw, broccoli and pea with spices, fish bisque

Or

Tournedos lacquered with honey, grapefruit and ginger, mashed apple and celery, potato millefeuille, shitake espuma, chimichurri sauce

~ ~ ~

Chocolate sphere and chestnut cream, coconut meringue and tahini mousse

Or

Poached rhubarb with vanilla, strawberry jelly and basil sorbet

~ ~ ~

Mignardises

Fig. 2 Example of a menu composed of an appetizer, a starter to be chosen out of 2, a main course (idem), and a dessert

10 positive words *(French word)*	10 negative words *(French word)*
Admiring (*Admiratif*)	Disappointed (*Déçu*)
Amused (*Amusé*)	Disgusted (*Dégouté*)
Excited (*Excité*)	Irritated (*Irrité*)
Happy (*Heureux*)	Nostalgic (*Nostalgique*)
Pleasantly surprised (*Agréablement surpris*)	Sad (*Triste*)
Reassured (*Réconforté*)	Stimulated (*Stimulé*)
Relaxed (*Relaxé*)	Stressed (*Stressé*)
Serene (*Serein*)	Tense (*Tendu*)
Sleepy (*Somnolent*)	Uncomfortable (*Mal à l'aise*)
Well (*Bien*)	Unpleasantly surprised (*Désagréablement surpris*)

Fig. 3 Emotion questionnaire

6. *Contextual variables to be controlled or manipulated*. There are multiple parameters in such experiments (*see* Subheading 2, **Items**), for instance, the effect of the colored context of the experimental room on the emotion can be analyzed (1), or the effect of the color of an aperitif drink on the emotion can also be studied (2) (*see* **Note 3**).
7. *Demographic questionnaire*. This questionnaire makes it possible to collect additional information on the participants (age, sex, socio-professional category, consumption habits, etc.).
8. *Emotion questionnaire*. Participants' emotions and affective states are measured by a questionnaire completed by the participants at times of interest. The items within the questionnaire are kept short and easy to understand in order to be as close as possible to the reality of a restaurant context. The questionnaire collects given emotional states of the participant such as relaxed and disgusted (*see* Fig. 3). The scale used may be from 1 (e.g., not at all disgusted) to 5 (extremely disgusted) (*see* **Note 4**).
9. *Questionnaire on specific evaluation of certain dishes*. This questionnaire should include short items to ask for the participant's hedonic appreciation of a particular dish (e.g., appreciation of the aperitif and/or the main course in general on a scale from 1 (I didn't like it at all) to 9 (I liked it extremely) (*see* **Note 4**).

3 Methods

In this section, we provide a step-by-step description of the procedure to follow.

1. *Evaluate the number of participants needed for your study*. According to the AFNOR XP V09–500 standard, a minimum of 60 participants per condition/treatment is required to conduct a study of this type. However, a higher number of participants (between 80 and 100) is preferable to obtain powerful statistical results. The number of participants depends on the

number of customers in the experimental restaurant at the time of the fieldwork. For instance, in Porcherot et al. [22], 280 restaurant customers took part in the study with a written consent form.

2. *Welcome and briefing*. Here, we will take the example of the studies conducted for dinner. The arrival of the customers in the experimental restaurant is between 7 and 8 p.m. in order to standardize the dinner time.
3. *Recruit participants*. At Institut Paul Bocuse's experimental restaurant, customers are invited to participate in the experiment on arrival. They are free to agree or refuse to be involved in the ongoing study. It is also possible to recruit participants before they arrive at the restaurant and follow selection criteria to target a specific group of consumers (*see* **Note 5**).
4. *Inform participants about the aim of the study*. Once they arrive at the restaurant, customers are welcomed by the headwaiter who leads them to their table. They are informed about the general aim of the study and the experimental nature of the dinner and about the fact that they might have to give their opinion on questionnaires. However, they are not informed about the very specific objectives of the study so as not to influence their responses on the questionnaires. If they agree to be involved in the study, they have to sign the consent form.
5. *Ensure consent of participants*. To be involved in the study, customers must sign a consent form. This consent form is accompanied by an information sheet. Both must inform the participants on the settings of the study and the use of the data collected. It is signed by the participants at the beginning of the experiment. Most of the time, participants are filmed and must also give their consent for this aspect of the study [21, 22].
6. *Collect demographic information*. The participants fill out the characterization questionnaire. This is usually done during the recruitment phase.
7. *Start the study*. The meal is served, and according to the scientific questions asked (evaluation of the starter, the main course, or the dinner), the questionnaires on emotions and on appreciation of certain dishes are provided after the consumption. For instance, in [21] the emotion questionnaire was asked before dinner and after dessert. The format could be paper or online (via tablets, smartphones, etc.).
8. *At the end of the study*, the collected data are formatted in a table: one row per participant and one column per factor studied (*see* **Note 6**). An example of the data format is given in Fig. 4. This format allows statistical analyses to test the significance of the difference between products taking into account inter-individual differences.

Participants code	Arrival time	Gender	Age	Nb guests at the table	Guests type	Departure time	**Conextual variable**	Admiring-Before meal	Amused-Before meal	Excited-Before meal	….	Liking of the starter	…
193	12:15	M	34	3	Family	1.50	Red atmosphere	8.5	6.2	7.6		7.3	
528													
051													
…													

Fig. 4 Example of data format

9. *Statistical analysis*: The collected data can be analyzed with parametric and non-parametric approaches depending on the type of variables and the sample size.

4 Notes

1. *Restaurant.* The details provided in this chapter refer to the equipment present in the experimental restaurant of the Institut Paul Bocuse. However, it is possible to apply this protocol to other types of restaurants such as school cafeterias or company canteens, provided that the respective constraints are respected.
2. *Choice of menu.* Depending on the objective of the study, the menu may be imposed or freely chosen by the client.
3. *Contextual variables.* For instance, in (1) the measurement of emotions is carried out within different colored atmospheres (control, red, blue, green, white) characterized by the following elements: music, light, tablecloths, tableware, table set, menu, and pictures on the walls. Morever, in (2), the measurement of emotions is carried out by answering a questionnaire during the meal, after the consumption of an aperitive drink (3 variants of Kir, a French aperitive made of white wine and fruit liqueur, were studied: with apricot, grapefruit or blackberry).
4. *Rating scales.* Besides a standard hedonic scale to evaluate the liking of the food (a 1–9 Likert scale), emotions are rated 1–5 scales using a limited set of attributes. A similar number of positive emotions and of negative emotions should be used.

Participants are asked to evaluate each attribute on a visual analogue scale how they feel at the present moment (*How do you feel right now?* E.g., *from not at all happy to extremely happy.)*.

5. *Pre-recruitment*. If necessary (specific inclusion criteria, fixed number of participants, etc.), recruitment can be planned and carried out before the experimental meal. In this way, it will be possible to target participants and focus the study on the target.
6. *Data anonymization*. In order to ensure the anonymity of the participants, an anonymous code should be used for each participant.

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Chapter 5

Lexicon Questionnaire (EsSense Profile®)

Herbert L. Meiselman

Abstract

The EsSense Profile ® is a lexicon of 39 emotion words selected to test food products in central location tests and in other contexts. The method uses a fixed lexicon of 35 positive emotions and 4 negative emotions, and a scaled response format (5-point scale: not at all, slightly, moderately, very, and extremely). The scale is usually used with a preceding hedonic scale. The emotion terms are presented in alphabetical order.

Key words EsSense Profile®, Emotion, Product testing, Lexicon, Emotion list, Wellness

1 Introduction

Emotions are studied using both explicit and implicit methods. Explicit methods are also labeled self-report methods and mainly involve questionnaires and interviews. Explicit methods are the most commonly used measures of emotion according to recent reviews [1, 2]. This is probably because they are easy and inexpensive to administer and therefore are widely used in clinical, academic, and commercial sectors; all of these sectors can involve food. The application of emotion measurement to food began with clinical research on eating, and a number of methods were widely used including the MAACL-R: The Multiple Affect Adjective Check List Revised [3] and the POMS: Profile of Mood States [4]. The MAACL-R has 132 items in its longer version and 70 in its shorter version with 2 positive subscales and 3 negative subscales. The POMS-2 has 65 items in its longer version and 35 in its shorter version, with just one positive subscale and 5 negative subscales. Both the MAACL and the POMS have a large number of negative emotions, demonstrating the early interest in negative over positive emotions. This reflected the clinical and mental health orientation of early research with food and other consumer products. This research led to the conclusion that restrained eaters react to stress

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with negative emotions that lead to overeating [5]. More recent research on consumer emotions and consumer reactions to products has broadened this view to include both positive and negative emotions.

1.1 The EsSense Profile^{R}

The EsSense Profile^{R} was designed to study food ingredients and food products in a USA-based global food company, known for their spices, The McCormick Co. Inc. The EsSense Profile^{R} was developed by a group within McCormick, headed by Silvia King, working with outside consultants Herbert L. Meiselman (who provided the psychology expertise) and Edward Carr (who provided the statistical expertise). The EsSense Profile^{R} was first presented at the Eurosense conference in 2008 and published in 2010 [6]. This was followed by later papers presenting more results of extensive research on the method [7, 8].

The EsSense Profile^{R} as published [6] is a fixed lexicon of emotion terms each of which is rated on a 5-point scale (not at all, slightly, moderately, very, and extremely). A lexicon is "the vocabulary of a language, an individual speaker or group of speakers, or a subject" (Merriam Webster online dictionary). When this scale was developed in the years before 2008, there were no other published commercial emotion scales, especially none aimed at food products. So, there were no guidelines on how long such a scale should be, whether the emotion terms should be positive or negative or mixed, and how to select emotion terms. Most other emotion scales developed since 2008 and aimed at food products used different lexicons for different food categories and that remains one of the key questions in emotion measurement; can one have a general emotion scale with a fixed lexicon, or do you have to develop a new scale for each new food category? The latter approach is time-consuming and expensive and out of reach for many food companies and academic researchers. This has led to widespread use of the EsSense Profile^{R}.

1.2 Positive and Negative Emotions

Early product-oriented research on food and emotions also used a large number of negative emotions [9]. More recent commercial research tended to use more positive emotions based on the observation, first reported by Desmet and Schifferstein [10] and confirmed by a number of commercial studies that people tend to react to actual food products with positive emotions. For example, King and Meiselman [6] noted that positive emotions were used to describe emotion response to favorite foods, and negative emotions were used to describe emotion response to least favorite foods, but positive terms were used with higher frequency. Only four negative words were selected 20% of the time or more as opposed to 10 positive terms. Since publication of the EsSense Profile^{R}, a number of studies have confirmed the positive valence of emotions for foods, including Kanjanakorn and Lee [11] for coffee. Jaeger et al. [12],

using the CATA version of EsSense, found that both positive and negative emotion terms discriminated samples for a number of different products. The use of positive emotions for foods might be exaggerated in commercial testing where the consumers tested are usually product users.

1.3 Rating Scale or CATA (Check-All-That-Apply)

King and Meiselman [6] report on testing the EsSense Profile® in both a scaling version and a CATA version and concluded that the rating version was useful when discriminating different flavors of the same product. Thus, the scaled version is useful when testing different versions of the same product, which is often the goal in commercial food testing [13]. Others have tested the CATA version of the EsSense Profile®. Low et al. [14] used a RATA (rate all that apply) task on the EsSense25 and then further reduced the 25 emotions to 9 categories of emotion response. Jaeger et al. [12] reported ten studies using a CATA version of the EsSense Profile®. They found that the method was able to differentiate product samples that large differences in liking were often associated with more emotion words, but that some samples with similar liking scores had different emotions. They found high repeatability to a replicate sample. Jaeger et al. reported that consumers used 15–27 emotion terms in each of the ten studies. They also tested the method in a home use test (HUT). They concluded that the CATA method for the EsSense Profile® produces good results, but they warned that "study specific influences should be expected." This warns us that researchers should probably not try to compare scaled EsSense Profile® results with CATA EsSense Profile® results. In commercial research, the CATA method can be used to narrow down the number of variants in initial testing, while the scaled method can be used to optimize the variants.

Thus, the EsSense Profile® is a fixed lexicon of 39 emotion terms, mostly positive, each of which is rated on a 5-point scale from not at all to extremely.

2 Materials

1. Hedonic Scale. The EsSense Profile ® as designed by King and Meiselman [6] is always preceded by a 9-point hedonic scale from dislike extremely to like extremely (Table 1). The hedonic scale might augment the emotion response and also product differentiation (*see* **Note 1**).
2. List of Emotion Terms. King and Meiselman [6] developed the lexicon of the EsSense Profile® from existing emotion lists and from consumer feedback (Table 2). Existing questionnaires included the MAACL-R [3] and the POMS [4]. Consumer feedback was collected by Internet, central location tests, and

Table 1
The instruction to fill out the hedonic scale, followed by the scale

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
❑	❑	❑	❑	❑	❑	❑	❑	❑

Table 2
Lists of emotion terms in EsSense Profile[R] [6] and EsSense25 [16]

EsSense Profile	EsSense25	EsSense Profile	EsSense25
Active	*Active*	Loving	*Loving*
Adventurous	*Adventurous*	Merry	
Affectionate		Mild	*Mild*
aggressive	*Aggressive*	Nostalgic	*Nostalgic*
Bored	*Bored*	Peaceful	
Calm	*Calm*	Pleasant	*Pleasant*
Daring		Pleased	
Disgusted	*Disgusted*	Polite	
Eager	*Enthusiastic*	Quiet	
Energetic		Satisfied	*Satisfied*
Enthusaistic		Secure	*Secure*
Free	*Free*	Steady	
Friendly		Tame	*Tame*
Glad		Tender	
Good	*Good*	Understanding	*Understanding*
Good Natured	*Good Natured*	Warm	*Warm*
Guilty	*Guilty*	Whole	
Happy	*Happy*	Wild	*Wild*
Interested	*Interested*	Worried	*Worried*
Joyful	*Joyful*		

focus groups totaling 16 experiments involving over 5000 subjects. The EsSense Profile[R] list of emotions represents emotion terms that are applicable to the products of McCormick and Co. Inc. (*see* **Note 2** on applicability to other product categories.). The EsSense 25 was developed from the full EsSense Profile[R] using a sorting method and hierarchical clustering [16].

3. Scaled Response or CATA. A scaled response is recommended for different formulations of the same flavor, while CATA (Table 3) is recommended with different flavors (*see* **Note 3**).

Table 3
Check-all-that apply (CATA) format [6]

☐ Active	☐ Glad	☐ Pleasant
☐ Adventurous	☐ Good	☐ Polite
☐ Affectionate	☐ Good-natured	☐ Quiet
☐ Aggressive	☐ Guilty	☐ Satisfied
☐ Bored	☐ Happy	☐ Secure
☐ Calm	☐ Interested	☐ Steady
☐ Daring	☐ Joyful	☐ Tame
☐ Disgusted	☐ Loving	☐ Tender
☐ Eager	☐ Merry	☐ Understanding
☐ Energetic	☐ Mild	☐ Warm
☐ Enthusiastic	☐ Nostalgic	☐ Whole
☐ Free	☐ Peaceful	☐ Wild
☐ Friendly	☐ Pleased	☐ Worried

3 Methods

3.1 Instructions to Consumers

The EsSense Profile^{R} (Table 4) is administered to a large group of consumers, either in person or online (*see* **Note 4** for testing actual products or just product names) (*see* **Note 5** for recommended testing protocol).

Instructions for panelists are as follows [6]:

Please select the words which describe how you **FEEL RIGHT NOW**. Select all that apply.

3.2 Number of Consumers Needed

Over the years, the number of people that is thought to be necessary for a consumer test has continually grown; the latest number from Hough [15] is 112 people. Of course if you want to segment the data by age or gender or other variables, then you need that number in each cell. Commercial companies typically test hundreds of people with an emotion questionnaire when studying product effects.

3.3 Order of Emotion Terms

The emotion terms are presented in alphabetical order; King and Meiselman [6] reported that using alphabetical or random order produced very similar results ($r = 0.99$). The use of a consistent order of emotion terms should make the task easier for consumers.

3.4 Duration of Testing

The hedonic question and the 39-item emotion questionnaire can be completed in 10–15 min or less on an Internet survey, and under 30 min for a consumer test of several (up to 4) products. Those wishing to get maximum information should use the full EsSense Profile^{R}. Those interested in taking less time for emotion testing

Table 4
The scaling format for the EsSense Profile [R], showing the rating categories and the first part of the emotion list in alphabetical order [6]

Feeling	Not at all	Slightly	Moderately	Very	Extremely
Active					
Adventurous	1	2	3	4	5
Affectionate	1	2	3	4	5
Aggressive	1	2	3	4	5
Bored	1	2	3	4	5
Calm	1	2	3	4	5
Daring	1	2	3	4	5
Disgusted	1	2	3	4	5
Eager	1	2	3	4	5
Energetic	1	2	3	4	5

should consider the shorter EsSense25 [16], which has 25 emotion terms including all of the 4 negative terms in the full EsSense Profile[R] (Table 2).

3.5 Culture/ Language

The EsSense Profile[R] and/or the EsSense25 have been translated into many languages including Portuguese [17, 18], Dutch [19], Italian [20], Spanish [21], Korean [22], French [23], and several languages in South Africa (Zulu, Xhosa, and Sesotho) [24].

Rocha et al. [17] also used CATA (each item rated yes-no) for their 25-item questionnaire adapted from the EsSense Profile[R]. Rocha et al. [17] selected 25 terms from the EsSense Profile[R] for the Portuguese translation, but these terms are different from Nestrud et al. [16]. Rocha et al. used a yes–no response format rather than a scaled format. Rocha et al. left out the following terms which appear on the EsSense Profile[R] and EsSense25: aggressive, bored, disgusted, good, guilty, happy, mild, nostalgic, pleasant, tame, warm, wild, worried. Note that Rocha et al. left out all of the negative terms.

Some studies have used the whole EsSense Profile[R] and other studies have selected items from the scale. For example, Chen et al. [25] selected 6 items from the 39-item EsSense Profile[R], noting that some terms did not apply to the oral care products under study, and other terms were difficult to differentiate in the Chinese language. This highlights the difficulty of using one lexicon translated into multiple languages.

Spinelli et al. [20] found that the EmoSemio and EsSense Profile [R] methods shared very few emotion terms when the meaning of the emotion terms was examined; this underscores the challenges of taking emotion terms from one language and translating them and applying them in another language. The true meaning of the word might not be captured by a dictionary translation (*see* **Note 6**).

3.6 Age and Gender Effects

King et al. [6] examined the role of gender in the relationship between acceptability and emotions in food products from different food categories. Overall, they found few significant associations between acceptability and emotion for protein sources (beef, chicken, fish), but many significant associations for herbs and spices and for snacks. However, they noted that for males very few emotions were associated with acceptability for snacks, whereas for females the number was much larger. Thus, the role of gender might be product category specific in looking at the relationship of emotion and acceptability.

Den Uijl et al. [25] used the EsSense25 to study aging and found that the emotions of younger consumers (18–45) varied on both valence and arousal, whereas emotions of the older consumers (>65) varied mainly on valence. Also, older consumers were generally less extreme in emotion scores, especially for negative emotions. This agrees with the more general research of Svard et al. [26] who compared younger (20–30) and older (65–75) persons in non-food psychology tasks and observed that older persons perceive less arousal, potency and valence for emotions, and more so for negative emotions. Kanjanakorn and Lee [11] compared males and females in their study of coffee and found no difference in strength of emotions reported.

Spinelli (personal communication) studied effects of gender with the EsSence Profile ^{R} in a study of chocolate and hazelnut spreads [20]. For blind products without labels, men scored higher than women on emotions calm and nostalgic, while women scored higher for loving, merry, and tender. For branded products, men scored higher for bored while women scored higher for affectionate and tender. Using their EmoSemio method, Spinelli et al. [20] found that women scored higher than men for cuddled, tender, and disappointed, and men did not score higher on any emotion.

3.7 Correlation of Emotion and Liking

King et al. [6] studied the relationship of emotion and liking and noted that different food categories demonstrate different relationships of acceptability and emotion. They found greater numbers of emotions associated with acceptability for herbs and spices and for beverages, and fewer numbers associated for animal proteins. Therefore, one cannot assume a constant level of association of emotion and acceptability across product categories.

4 Notes

1. Intensity of stimulation. The EsSense Profile^{R} is sometimes used to study different intensities of stimulation, for example, different strengths of an ingredient in a product. The combination of the hedonic scale and the emotion scale will produce results on how the change in stimulus intensity impacts liking and emotions.

2. List of emotion terms. Potential users of the method need to decide whether the list of terms will work for them. King, Meiselman, and Carr note: "This list can be expanded or edited to account for specific emotions that may be appropriate in specific product categories and in specific applications." [7 p. 114].

   The list will probably work for other food products, but as one moves toward other product categories (perfumes, soaps, etc.), the applicability of the emotion list needs to be considered. The EsSense Profile ® has been used with a broad range of products.

3. CD-CATA. Ng, Chaya, and Hort [28] compared the EsSense Profile® with intensity scaling of emotions with a check-all-that-apply approach for a consumer-defined lexicon (CD-CATA) in a study of blackcurrant squash. Both EsSense Profile ® and CD-CATA discriminated the products more effectively than hedonic scores. The authors point out other differences between the performance of the EsSense Profile ® and the CD-CATA; neither method was consistently better. This is another method where the list of emotions is selected for that product category. For example, Ng et al. noted that EsSense was easier to use because it does not require extensive pre-testing of emotion terms, and it was easier and quicker to perform.

4. Emotions for names and actual foods. Cardello et al. [27] used the EsSense Profile® to study emotion responses when the food stimulus was a food name or an actual food product. This is important in food testing which is done both with actual products in a taste test laboratory or at home, or with food names either in person or online. They noted a high correlated between emotion and liking and also noted that well-liked foods receive higher emotion ratings to the food name, while less like foods receive higher emotion ratings to the actual food.

5. Instructions. When consumers are tested in person, they should be given the usual instructions to not discuss the questionnaire with other people in the room. They are usually asked not to ask for help in completing the questionnaire—("do the best you can"). Consumers will sometimes ask for the meaning of certain emotion terms.

6. Comparison of EsSense Profile® to other Methods.

   EmoSemio—The EmoSemio method is one of the emotion methods that uses a list of emotions designed for each product category. It is potentially useful to those who plan to work within one product category. Spinelli et al. [19] compared their EmoSemio method with the EsSense Profile®. The studied chocolate and hazelnut spreads served to two panels of

over 100 product users, with one group using EmoSemio and the other using EsSense Profile® translated into Italian, both panels using a 5-point scale from "not at all" to "extremely." EmoSemio does not use single emotion words, but presents emotion words in short sentences. They found that the EmoSemio discriminated better than EsSense because the emotion list was designed specifically for this product category, the EmoSemio was designed in Italian rather than being translated, and the full sentences rather than single emotion words made the task easier for Italians. Spinelli et al. [19] recommend that emotion list length needs further research.

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Chapter 6

Using Check-All-That-Apply (CATA) Questions in Emotion Questionnaires

Sara R. Jaeger and Gastón Ares

Abstract

Check-all-that-apply (CATA) questions have become the most common tool for product sensory characterization with consumers and the question format is increasingly being used in emotion questionnaires. This is because CATA questions are simple for research participants, deliver reproducible results, and reliably elicit emotional associations to products and other food-related stimuli. This protocol explains how to implement questionnaires with emotion words as the CATA terms and how to analyze the generated data. Drawing on the more extensive literature on CATA questions in sensory product characterizations with consumers, methodological issues related to how CATA question implementation can influence the results are also covered.

Key words Check-all-that-apply questions, CATA, Emotions, Consumer research, Questionnaires

1 Introduction

Food and emotions have been a topic of interest for many years, initially often through a desire to understand how feelings and moods influence decisions about what to eat and drink [1]. In the last few decades, another stream of emotion research has gained prominence—understanding how our feelings and moods are influenced by what we eat and drink [2]. The latter fits into the broad area of product-focused emotion research and is the topic of interest for the present protocol, where focus is directed to the use of emotion questionnaires in product-focused consumer research, specifically in the format of check-all-that-apply (CATA) questions.

For most academic and industry applications, CATA questions are an excellent methodological choice for uncovering product-elicited emotional associations. Their use is supported by extensive methodological research in the context of sensory product characterization with consumers, as well as a smaller body of research specifically relating to the use of CATA questions for product

Moustafa Bensafi (ed.), *Basic Protocols on Emotions, Senses, and Foods*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_6,

emotion profiling. The purpose of this protocol is threefold: to explain how to use CATA emotion questions for data generation, how to analyze the data, and, finally, to create awareness of key empirical issues to consider in applied research.

1.1 The Ascendancy of CATA Questions in Food-Related Consumer Research

The obvious starting point for this protocol is to define CATA questions: they are versatile multiple-choice questions in which respondents are presented with a list of words or phrases and asked to select all the options they consider appropriate for describing the focal stimulus.

In sensory and consumer science, the ascendancy of CATA questions began in the 2000s following work by Adams et al. [3] who used CATA questions as a tool to obtain information about consumers' perception of products. Adams and colleagues were not the inventors of CATA questions, which were already used in marketing research, but they significantly contributed to interest in this question format. A concurrent development which contributed to uptake was that the field of sensory-consumer science at this time was transitioning away from the reliance of trained sensory panels for sensory product characterization and moving toward such product insights being delivered by consumers [4]. CATA questions became a popular for this consumer-centric product-focused sensory research, and applications are now very common.

The essence of data collection using CATA questions is that consumers are presented with a set of products and a list of sensory terms to characterize them. One-by-one, samples are tasted, and each participant selects all the terms that are considered appropriate for describing each of the samples, without any constraint on the number of terms that can be selected (Table 1).

Table 1
Basic format of a check-all-that-apply (CATA) question with *N* different terms (emotion words)

	Tick all that apply
Term 1	□
Term 2	□
Term 3	□
. . .	□
. . .	□
. . .	□
Term $N-1$	□
Term N	□

Assuming a tasted sample, instructions given to participants are: Please taste this sample. How do you feel?

The task of completing sensory CATA questions has been found to be easy and not tedious for participants [5], and this aligns well with the popularity of this question format among marketing researchers who value it because of low participant response burden [6–8]. The repeatability of results from sensory CATA questions has been established, for example by Jaeger, Chheang et al. [9] and Ares, Antúnez et al. [10] who demonstrated that results from sensory product characterizations with CATA questions with consumers in different sessions were highly similar (*see* **Note 1**).

1.2 Emotion Questionnaires in Product Research

In the food and beverage domain, questions about what emotions are and how to measure them are enduring and ongoing. To even begin to cover these questions is beyond the present scope and we refer to, for example, Meiselman [11] or Cardello and Jaeger [12]. Suffice it to say that questionnaires are currently very popular in emotion research, so much so that they are regarded as the default method for measuring consumers' product-elicited emotional associations [13]. This may change over time as implicit methods in emotion measurement [14, 15] find greater application, either alone or in combination with questionnaires or other explicit methods.

A likely reason why questionnaires have gained such popularity in emotion research is their familiarity, flexibility, and ease of implementation. Questionnaires were used by many of the pioneers in product emotion research including King and Meiselman [16] and Chrea [17] and interested readers can find a detailed account of the measurement of consumer product emotions using questionnaires in Cardello and Jaeger [12]. An accompanying chapter [12] in the same edited book—Meiselman [13]—focuses on methodological issues in consumer-centric product emotion research using emotion word questionnaires. We cover many of the same topics below, but not in similar depth. Furthermore, we restrict focus in this protocol to emotional associations, which ignores the fact that product-elicited emotional associations are often obtained in conjunction with hedonic product evaluations or as part of a multi-response approach where individual stimuli are characterized using several types of response variables. In addition to liking and emotions, the latter can include sensory attributes, product conceptualizations, situational appropriateness, attitudes, and behavioral intentions [18–23].

In the remainder of this protocol, we describe how to use CATA questions in product-focused emotion research. It includes information regarding stimuli selection, consumer participants, questionnaire design, data collection, and data analysis. However, because each study is different, materials and methods need to be adapted to these unique situations. Hence, the protocol below is generic rather than specific.

2 Materials

2.1 Consumers

1. Approved practices for conduct with human subjects according to the principles in the Declaration of Helsinki should always be followed [24]. Anonymity and informed consent are two of the cornerstone aspects.
2. Consumers taking part in product-focused emotion research are typically adult product users. Depending on research objectives, participants may be non-users of the product/brand/category. It is also possible to conduct product-focused emotion research with children/youths and elderly people.
3. There are no specific guidelines for the number of consumers to be included in studies focused on product-elicited emotional associations, and guidelines for hedonic tests are usually considered. A minimum of 100–120 consumers is generally regarded as appropriate [25–28]. When consumer segmentation is intended, the minimum number of consumers should be much higher and exceed several hundred.

2.2 Stimuli

1. The stimuli used in product-focused emotion research are physical samples (e.g., foods, beverages, personal care, and household care products) or written/visual stimuli including descriptions of consumption/use situations (*see* **Note 2**).
2. The number of stimuli used for product emotion characterization by CATA questions can range from one to a few to many. The decision about how many stimuli to use in a specific study depends on the stimuli format (*see* **Notes 3–6**).
3. Pilot work should be conducted for all types of stimuli. The purpose is to ensure that the stimuli are well aligned to the selected emotion words and that the task performs as anticipated (*see* **Note 7**).

2.3 Emotion Words

1. Selection of the emotion words used to populate the CATA question (i.e., the emotion vocabulary) is extremely important when conducting product-focused emotion research.
2. Three options exist: (1) using a generic emotion vocabulary previously developed to be applicable to a broad range of product categories (*see* **Note 8**), (2) using a product-specific emotion vocabulary previously developed specifically for the product category they are working with (see **Note 9**), or (3) developing a new emotion vocabulary specifically for the focal set of stimuli / product category (*see* **Note 10**).
3. Conduct pilot work on the emotion word vocabulary, especially if it is a new vocabulary. The purpose is twofold: to ensure product category relevance of the emotion words and that

these are appropriate for the target group of consumers. Otherwise, the task can appear odd and weird [29].

4. Dimensional coverage of the emotion vocabulary used in the CATA question should be considered. Valence (pleasure to displeasure) and arousal (activated to deactivated) are the two core dimensions of human affect, spanning, respectively, pleasure to displeasure and activation to deactivation [30, 31]. The inclusion of emotion words that span the arousal dimension (e.g., alert, passive) or express combinations of valence and arousal (e.g., secure, uninspired, excited) can generate additional stimuli insights [32] (*see* **Note 11**).
5. CATA questions in emotion research are typically populated with single words (e.g., annoyed, happy, excited, relaxed), but two notable exceptions exist. One is the use of pairs of similar emotion words (a.k.a., composite terms) as done in the valence × arousal circumplex-inspired emotion questionnaire (CEQ) [33], for example, happy/satisfied, dull/bored, and active/alert. The other exception is the EmoSemio approach by Spinelli and colleagues [34] where words are replaced by sentences. It is largely a matter of researcher preference which option is used (*see* **Note 12**).
6. Translation of emotion words from one language to another represents a difficult challenge [35] and this can be problematic for researchers who are interested in global and cross-cultural research. There have been some attempts at translations, including the EsSense Profile (Spanish) [36] and the valence×arousal circumplex-inspired emotion questionnaire (Mandarin) [33]. Cardello and Jaeger [20] include other examples from various product-specific studies.
7. It is possible to use emoji instead of words in emotion questionnaires [37] and benefits of doing so may extend to cross-cultural research (*see* **Note 13**).

2.4 CATA Questions Variants

1. CATA questions have several limitations and question variants have been developed to overcome some of their limitations. The most popular variants are as follows: (i) rate-all-that-apply (RATA) questions, (ii) the circumplex-inspired emotion questionnaire (CEQ), and (iii) forced-choice yes/no questions.
2. Rate-all-that-apply (RATA) questions increase the discriminative ability of CATA questions when working with similar samples [38]. Participants are asked to check all the emotion terms that apply to describe a sample and, for those that apply, to rate their intensity using a structured scale with a limited number of points (3-point or 5-point scales [38, 39]). Empirical evidence has shown that RATA questions do not always have a higher ability to discriminate among samples than CATA questions [38, 40]. According to Jaeger et al. [37],

RATA questions are only recommended when the aim of the study is to discriminate among samples with similar emotional profiles (*see* **Note 14**).

3. The valence×arousal circumplex-inspired emotion questionnaire (CEQ) is a single-response emotion questionnaire with a circular layout where 12 pairs of emotion words are placed in fixed order along the perimeter of a circle [33]. Subsequent research has shown that it can be used in a regular CATA question format, with the 12 pairs of emotion words as the CATA question terms [32]. A benefit of the CEQ is that it purposefully spans the two core dimensions of human affect—valence and arousal. This coverage can increase sample discrimination compared to questionnaires that primarily use emotion words linked to degree of valence.
4. Forced-choice yes/no questions increase consumers' attention to the task by being asked to answer yes/no to each one of the response options [7, 8]. Although this question variant has been used for sensory characterization [41, 42], its application for product-focused emotion research has shown a decrease in sample discrimination [43], likely due to requiring participants to engage in a more systematic cognitive processing style.

3 Methods

3.1 Data Collection

1. Data collection in product-focused emotion research should align with best practices for sensory-focused consumer research as described in textbooks, for example, Heymann and Lawless [44].
2. When product-focused emotion research is conducted in central location test (CLT) settings, participants are typically seated in sensory booths or equivalent. Traditionally, stimuli are presented in monadic sequence and labeled with 3-digit random numbers. Best practice prescribes the use of experimental designs to minimize bias linked to stimuli presentation and first-order carry-over (William's Latin square designs). For tasted samples, serving size—at a temperature reflecting typical product use—should be enough for two to three bites (*see* **Note 15**).
3. When the stimuli are written or images, data collection is done using a questionnaire, either via pen-and-paper or (bespoke) software/web-based platforms. The use of experimental designs to control stimuli presentation order is not always possible in online surveys, and randomization must be accepted (*see* **Note 16**).
4. To avoid response bias, the order of the CATA terms within the list should be balanced following a between-subjects

randomization to minimize the influence of primacy bias on consumer responses to CATA questions [45, 46]. This implies that the order of the emotion terms within the CATA question is modified across participants (*see* **Note 17**).

5. When CATA questions are used for characterizing product-related emotional associations, participants are presented with the same question more than once. Therefore, balanced presentation order for the CATA terms within participants may reduce response bias. The use of a balanced presentation order of the terms across samples for each participant is recommended when working with list of less than 30 emotion terms [47]. However, balancing the presentation order of the terms across samples for each participant may reduce discrimination when working with more than 30 terms [48]. In such cases, practitioners are recommended to only balance presentation order of the terms between participants.
6. Because the order of the terms may influence responses to CATA questions, detailed information about how the terms of the CATA question were presented to participants should be provided.

3.2 Data Analysis

1. Responses to CATA questions consist of binary data describing whether each consumer selected each of terms of the CATA question to describe each of the samples or not (0 and 1, respectively). Data are inputted in a data matrix containing the emotion terms in columns, as shown in Table 2.
2. The frequency of use of each of the emotion terms included in the CATA question for describing each sample is calculated.
3. Data are summarized using contingency tables which show the percentage of participants who selected each emotion term for each sample [49]. This summary information enables the identification of the most relevant emotional associations of each of the samples (*see* **Note 18**).
4. The existence of statistically significant differences among samples for each of the emotion terms included in the CATA question is evaluated using Cochran's Q test, a parametric test used for binary response variables [50]. When differences among samples are significant according to Cochran's Q test, post hoc pairwise comparisons are performed using the sign test [49] (*see* **Note 19**).
5. A graphical representation of the similarities and differences in the emotional associations of the samples is obtained using correspondence analysis (CA) [51]. This analysis is performed on the contingency table containing the number of participants who selected each of the emotion terms included in the CATA question for each of the samples [52].

Table 2
Example of a matrix with data from 120 consumers using check-all-that-apply (CATA) questions with N emotion terms for 6 samples (A–F)

Consumer	Sample	Emotion 1	Emotion 2	...	Emotion *N* − 1	Emotion *N*
1	A	0	1	...	0	1
1	B	0	1	...	0	0
1	C	1	1	...	1	1
1	D	1	1	...	0	0
1	E	0	0	...	0	0
1	F	0	0	...	1	1
2	A	0	0	...	1	0
2	B	1	0	...	0	1
2	C	0	0	...	1	0
2	D	0	0	...	0	1
2	E	1	1	...	0	0
2	F	0	0	...	0	1
...	...	...	...	...	...	...
120	A	1	0	...	0	1
120	B	0	0	...	0	1
120	C	0	1	...	1	1
120	D	0	1	...	0	0
120	E	0	0	...	0	0
120	F	0	0	...	1	1

Note. The value "1" indicates that the participant checked the emotion term for describing the sample, while the value "0" indicates that the emotion term was not checked

4 Notes

1. Repeatability is expected to be high when the results from groups of consumers are considered, albeit not at the individual level, where repeatability of CATA questions is expected to be lower [9]. This is not unique to CATA questions but generally expected when consumers are the research participants [53].
2. Stimuli can be branded or unbranded depending on the purpose of the study. In academic research, the use of branded samples is less common, but not absent from the publication record [54, 55]. It is expected that branded samples will be

more common in applied/commercial research. In home-use tests (HUT), samples may be given to participants in commercial packaging [22].

3. When stimuli are tasted samples, sensory fatigue needs to be taken into consideration, and it would be uncommon to exceed 10–12 samples in a single session. Typically, the number of samples is lower, say 4–7, fitting with applications such as prototype testing and competitor benchmarking. If the stimuli are aroma samples (e.g., personal care or household products), olfactory overload is a risk factor and enforced breaks between samples can be necessary. Breaks should be sufficiently long that sensorial carry-over is no longer present, and pilot work may be needed to establish duration, which will be product dependent.
4. When stimuli are images, product names, or written concepts, sensory fatigue is not of concern, and the number of stimuli that each participant can evaluate can be larger than when tasted samples are used. However, participant boredom may be a concern, especially if the task is very long (e.g., many samples in combination with a CATA list with many emotion words).
5. The number of stimuli to include in a study with tasted samples may be the same or reduced in home-use tests (HUT) relative to central location tests (CLT), for example to focus on the most promising new product variants. It can be relevant to only test one sample each day, to better resemble actual produce use in consumers' homes.
6. Consider data analysis during study planning. Certain types of multivariate data analysis require that five or more stimuli be included in a study [56].
7. It is good practice that the people who develop a test complete it themselves before commencing full-scale data collection with consumers.
8. The EsSense Profile™ [16] which comprises 39 words, largely skewed toward positive emotions, is probably the most well-known generic emotion vocabulary for foods and beverages. A shorter variant—EsSense25—with 25 words was presented by Nestrud et al. [57]. For aroma stimuli, alternatives exist like the GEOS and ScentMove [58]. Completely generic vocabularies, not developed with the intention of being used for foods, beverages, personal/household care products, also exist and typically stem from clinical research. Cardello and Jaeger [12] offers a comprehensive account, including listings of the different emotion vocabularies.

9. Cardello and Jaeger [12] contains many examples of previously developed product-specific emotion vocabularies, including, blackcurrant juice, beer, coffee, and chocolate.
10. When developing a new emotion vocabulary, it is a good idea to draw inspiration from the academic literature, even if it pertains to product categories others than the one of particular interest. This can be particularly relevant in relation words that describe emotions versus words that are perhaps better considered as conceptual. For example, Prescott [59] has suggested that some of the terms in the EsSense Profile™ [16] are not emotions—whole, polite, and mild. Thomson and colleagues can also be consulted for insight on this issue, for example, Thomson and Crocker [54].
11. We recommend that practitioners consider inclusion of words that relate to degree of arousal. The valence×arousal circumplex-inspired emotion questionnaire (CEQ) illustrates an approach to doing so and was developed to purposefully spanning the two-dimensional valence×arousal emotion space [40]. The dimension of dominance (dominant to submissive) is generally considered to be the third relevant dimension in human emotions and in the PAD model (pleasure-arousal-dominance) by Mehrabian and Russell [60] a set of 18 semantic differentials are developed to capture these three dimensions (6 items per dimension). It is not yet clear how important the dominance dimension is for emotional product associations in foods and beverages. Some early indications point to age-related influences [61, 62].
12. Drawing on a similar approach in sensory CATA questions, Jaeger and colleagues [63] argue that providing participants with a pair of words rather than a single word helps to contextualize the meaning of the emotion they seek to convey.
13. It is beyond the scope of this protocol to consider emoji further, but we note that opinions differ on whether emoji are appropriate in emotion research [64, 65].
14. RATA questions are analyzed using analysis of variance and principal component analysis (PCA) [66].
15. Branded samples can also be given 3-digit random numbers, but it is not a must. Sample serving size can be increased if longer lists of emotion words terms are used to make sure there is enough sample for a complete evaluation.
16. When surveys can be completed on mobile devices, it is important to pilot the page layout and ensure that the stimulus stays visible when participants scroll down to see/select emotion words at the bottom of the CATA question.

17. Balanced presentation of the emotion terms included in the CATA question can be performed using Williams' Latin square design [67]. This can be easily achieved when collecting data using computer-based systems or using MS Office mail merge functionality.
18. Research from sensory characterization has shown that the percentage of participants who select a CATA term is linearly correlated to its average intensity [68, 69].
19. Recent research supports the interpretation of significant differences in the frequency of use of an emotion term between samples as differences in the perceived intensity of the emotional associations raised by those samples [68].

5 Conclusions

CATA questions are a simple and reliable alternative for uncovering emotional product associations. The present chapter summarizes recommendations for best practice about the implementation and analysis of product-focused emotion research using CATA questions. We encourage practitioners to provide detailed information about the empirical protocols used in their research.

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Chapter 7

Participant-Defined Questionnaire (EmoSemio)

Sara Spinelli and Erminio Monteleone

Abstract

Participant-led emotion lexicon has been proved to be particularly effective in measuring emotional responses to foods. Here, we present the EmoSemio approach, a protocol for the development of emotion questionnaires that are product-specific and that employs the words used by the consumer.

Key words Emotions, Repertory Grid Method, Interviews, Self-report, Language

1 Introduction

Questionnaires are by far the most used, and at present the most effective [1], methodology to measure consumer experience of food products. Several types of questionnaires have been employed [2], and some efforts have been put in the development of standardized questionnaires that can be used with different food products [3]. However, several studies indicated that product-specific questionnaires based on a consumer-defined lexicon have a better performance, including in terms of discriminant ability [4, 5]. Questionnaires are particularly sensitive to this problem of ambiguity in wording [6], because they tend to use isolated terms presented out of the context. However, words may have multiple meanings (polysemy) that are "negotiated" in a specific context [7]. This has many implications for the interpretation of the items included in a questionnaire. If this is a general characteristic of verbal language, this is even more true in the case of the emotion lexicons. In fact, many words used to refer to emotions have multiple and thus ambiguous meanings, depending on the contexts and on the individual experience of each speaker [8, 9]. When deciding to use verbal language to investigate consumer emotional responses to foods, it is important to keep in mind three issues that have been discussed in the scientific literature (see [1]):

Moustafa Bensafi (ed.), *Basic Protocols on Emotions, Senses, and Foods*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_7,

- You cannot assume that a given feeling, e.g., happiness, in one context is similar to the feeling of happiness in another one;
- You cannot assume that two people mean necessarily the same thing by a given feeling (e.g., happiness) as people differ in emotion granularity; for some people, the word "happiness" refers to a specific feeling state, whereas for others, it refers to a general pleasant feeling (see [10]);
- You cannot assume that an emotion word means the same thing in different cultures (see [9]).

These issues have important implications in the development of questionnaires to measure emotional responses to products [11, 12]. EmoSemio approach is a procedure to develop a product category-specific questionnaire able to solve some limitations of the current approaches aimed at investigating emotional experience of foods, where the ambiguity of emotional words is reduced as much as possible by using a language closer to the one used by the consumers to describe their experience [5, 13]. EmoSemio in fact allows to develop a questionnaire that is specific to a product category (e.g., coffee, or chocolate and hazelnut spreads, canned tomato, red wines, and vegetables) using the words most appropriate for the consumers in the context of these products.

The EmoSemio procedure consists in two phases [5]. The first phase comprises individual interviews conducted with a limited number of consumers using a modified version of the Repertory Grid Method. Based on these interviews, a questionnaire is developed and is used in a second phase for a quantitative measurement of emotional responses to the products of interest with a larger number of consumers.

2 Materials

2.1 The Stimuli

- Phase 1: To guarantee the effectiveness of the method, in planning the study it is important to select the samples most representative of the largest sensory variation within the category. At least three samples are needed, but also multiple of three can be considered. A preliminary descriptive analysis with a trained panel (or sensory characterization of products through Check-All-That-Apply with consumers) is recommended to select the most different and representative samples (*see* **Note 1**).
- Phase 2: In this phase, there is no restriction on the number of samples that can be included. Usually, the number of samples is not more than nine to avoid fatigue effects. However, depending on the type of products, even a lower number can be challenging, and appropriate breaks should be included to control carry-over and fatigue effects. The common guidelines for sensory evaluations of products should be followed [14].

2.2 The Subjects

- Phase 1: Twenty subjects are sufficient to develop a questionnaire. However, the number should be increased when the design of the study is particularly complex; e.g., if the number of products is higher than 10, or if the interest is to compare blind and branded condition, the interviews should be conducted in the two conditions (not necessarily with the same subjects) and the number of interviewees should be slightly increased (e.g., 12 + 12). When high product knowledge and specific expertise with the product of interest have to be considered in developing the questionnaire, the number of respondents can be limited to 10/15 (e.g., in the case of chefs).

  The participants should be selected keeping in mind the characteristics (e.g., in terms of age and gender) of the sample of consumers that will be involved in phase 2. This means that if the aim is to develop a questionnaire for children, participants belonging to this age group should be interviewed in phase 1.

  Frequent users of the product category of interest are generally selected, because usually they are more involved with the products, they have a higher product knowledge, and thus, they have a positive emotional experience of the product with specific nuances. Frequent users tend to be well disposed in describing their emotional responses to the product category (especially toward their favorite product), but they are also attentive in underlining negative aspects. Non-users should be selected only when the study is interested to exploring the perception of these subjects (e.g., in case of disruptive innovation studies [15]).
- Phase 2: As in all the quantitative sensory tests that include also acceptability measures, the sample should not be lower than 100 participants [14, 16–18]. This number can increase in case there is an interest in segmentation and clusterization.

3 Methods

3.1 Phase 1: Interviews

3.1.1 Conducting the Interviews

The interviews (one-on-one) are conducted following a modified version of Repertory Grid Method [5], *see* **Note 2**. The respondent is asked to taste/smell the samples and rank them according to decreasing preference. Then, the interviewer divides the samples into triads according to the ranking if the number of samples is higher than three (e.g., samples ranked as 1st–3rd and samples ranked 4th–6th in the case of six samples). For each triad, the respondent is asked to concentrate on the emotions felt during tasting and to describe how each product makes him/her feel, compared with the other two (*see* **Note 3**). When the respondents are found to be reticent or unclear, they are asked to explain what

they meant exactly (*see* **Note 4**). The procedure is repeated for each product. The samples can be tasted again in case of need when the product allows it.

3.1.2 Analyzing the Interviews

A semiotic analysis of interview to identify the relevant semantic categories related to the emotional experience is conducted by two researchers trained in semiotic analysis (*see* **Note 5**). The interview analysis is one of the most delicate stages of the procedure. The analysis is purely qualitative and made to interpret the meaning of the answers given by the subjects. The semiotic approach "decomposes" the texts (in this case, the interviews) in order to deeply investigate their meaning by identifying the semantic units in the text [19–21]. Semiotic analysis is based on the identification of redundancy in meaning so that it is possible to group under the same label (that is called isotopy) several different words that refer to a same semantic field in this context. That analysis goes well beyond the identification of synonyms that usually is the level at which the traditional analyses made with the objective to develop a questionnaire stop (*see* **Note 6**). Figure 1 represents the semantic organization of the categories resulted from text analysis in a study on chocolate and hazelnut spread.

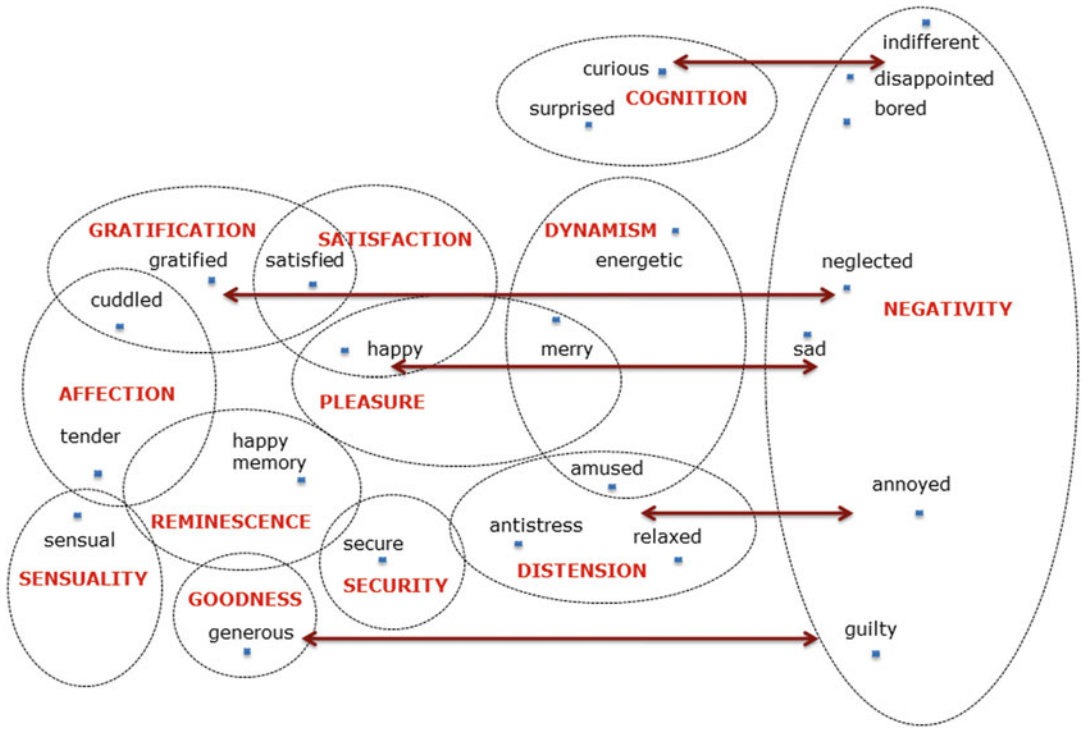

Fig. 1 Map of the semantic relationships between emotions in the case of cocoa and hazelnut spreads (EmoSemio). (Reproduced from Spinelli et al. 2014 with permission from Elsevier [5])

Table 1
The EmoSemio questionnaire for cocoa and hazelnut spreads

English translation	Original Italian
It is an antistress: it calms me, it soothes me, it reassures me	È un antistress: mi calma, mi tranquillizza, mi rassicura
I associate it with amusement and fun	Lo associo allo svago e al divertimento
I associate it to happy memories of childhood	Lo collego a dei bei ricordi dell'infanzia
It bores me	Mi annoia
It communicates sensuality, it charms me	Mi comunica sensualità, mi ammalia
It communicates security	Mi comunica sicurezza
It makes me feel sad	Mi comunica tristezza
It makes me feel indifferent	Mi è indifferente
It makes me feel good and generous	Mi fa sentire buono
It makes me feel cuddled and loved	Mi fa sentire coccolato e amato
It disappoints me	Mi fa sentire deluso
It makes me feel guilty	Mi fa sentire in colpa
It makes me feel full of energy and reinvigorated	Mi fa sentire pieno di energia e rinvigorito
It relaxes me and make me feel carefree	Mi fa sentire rilassato e spensierato
It makes me feel tender and affectionate	Mi fa sentire tenero e affettuoso
It makes me neglected, without any care for me	Mi fa sentire trascurato, senza nessuna cura per me
It gratifies me, rewards me	Mi gratifica, mi fa sentire premiato
It makes me curious	Mi incuriosisce
It annoys me, it makes me nervous	Mi infastidisce, mi innervosisce
It makes me cheerful	Mi rende allegro
It makes me happy	Mi rende felice
It satisfies me	Mi soddisfa, mi fa sentire appagato
It surprises me	Mi sorprende

Reproduced from Spinelli et al. 2014 with permission from Elsevier [5]

3.1.3 Questionnaire Development

In order to reduce ambiguity within the questionnaire as much as possible, the selected semantic categories have to be "translated" into full sentences that could answer the question "How does it make you feel?" (see an example in Table 1). This allowed to add a second level of contextualization: that of the sentence (co-text). We expect that the adoption of a discursive format in the questionnaires could be effective in many languages, but further studies on the application of this format in different cultures and language are needed.

Because we should ensure as much as possible that respondents understand what we ask them to evaluate, we recommend using the phrases that were used by the respondents in the interview stage. This will guarantee that the language will be considered appropriate in the context of the product category and will be understood. When during the interview a possibility of ambiguity is detected, this word/phrase should not be used, and a synonym/reformulation should be preferred. A pilot study is suggested to verify that the sentences formulated are not ambiguous (*see* **Note 7**).

3.2 Phase 2: Data Collection Using the EmoSemio Questionnaire

3.2.1 Data Collection

In the second phase, the questionnaire that has been developed is used with a larger number of products belonging to the same category. After the evaluation of liking using an hedonic 9-point scale [22], the respondent is asked to fill in the questionnaire. Usually, a rating scale is used, ranging from 1 (=Not at all) to 7 (=Very much) [23]. Check-All-That-Apply formats can also be used but are not recommended when the differences between the samples are small, for the lower discriminant ability of this approach (in case the intensity of an emotion is low, it may happen that the respondents are discouraged to select the corresponding word).

3.2.2 Data Analysis

The standard procedure for data analysis of quantitative hedonic data is applied. T-test and ANOVA models (followed by post hoc tests) can be used to test the differences between samples. Emotional data can be submitted to Principal Component Analysis to explore the differences among samples and to Multiple Factor Analysis or Principal Component Regression to study their relationship with other data collected on the same samples/subjects (e.g., to identify sensory drivers of emotions and to study the relationship between emotions and liking, [23–25]). We refer to the protocols on questionnaires (e.g. 6, 8) in this book for further details.

4 Notes

1. If the study is conducted in branded conditions, it is important to select the samples that also represent the largest brand variation within the category. This means that the number of samples to be considered for the interview may increase or that a double round of interviews should be conducted: one with the samples in blind and one in branded conditions (e.g., with packaging). The same is true if another dimension is considered instead of brand (e.g., sustainbility information).
2. Phase 1 can be conducted in person or online in remote conditions. In this latter case, the interview is organized through videocall and the product is sent at home [23].

3. It may happen in some cases that the number of samples that represent the largest sensory variation in the product category is not a multiple of 3. In these cases, a different number of samples can be considered, and the method should be adjusted. In case of four samples, for example, after the ranking the interview can be conducted on the first, second, and fourth sample as described. Then, the differences among all the other samples and the third should be asked.
4. When something is ambiguous or could be interpreted in different ways, the interviewer should always ask for further information, for example asking "You said *x*. In which sense?" or "What do you mean exactly?".
5. Semiotic analysis is a well-established methodology that overcomes many limitations of qualitative analysis (content analysis) allowing a good level of repeatability. However, it is good practice that two persons trained in semiotic analysis collaborate in the analysis to ensure that the analysis is comprehensive and not biased. One way to do this is that each researcher analyzes 50% of the interviews and then both discuss together when merging of the results; another way is that one researcher performs the analysis while the other (who have listened to the interviews) integrates/amends the analysis if needed.
6. The rationale behind this approach includes interpreting the meaning of the texts (i.e., the interviews), thus going beyond the words specifically used by the subjects to describe their experience. This is very important, because commonly it is considered that people communicate to be happy if they use the word "happy" or a word with a similar meaning (e.g., "merry," "glad," etc.). However, people very commonly (and this is even more true for emotions) use periphrasis (a roundabout way of expressing something) to express what they feel and do not use the most appropriate word to indicate an emotion, even if it exists and it is frequently used in everyday language. For example, they can answer to the question "how does this product makes you feel" in that way: "oh, it reminds when I was celebrating after my promotion, with friends…". Or "it brings to my mind when I was on holiday, and I was seated on my hammock in front of the sea just listening to the gentle sound of the waves." They do not say that they feel "happy" or "relaxed" but they meant it. It is clear that the interpretation of what the subjects are saying requires a contextualization of their answers. Usually, also the tone in which they speak is important and this can help the interviewer to interpret the emotion they refer to. It is apparent from what we are saying that a purely quantitative analysis of the texts (e.g., counting words) cannot bring to the same results because much of the meaning conveyed will be lost. Thus, it is very

important that the person that is analyzing the interviews (and possibly conducting them) is trained with the basic tools of text analysis. This allows to go more in depth in the analysis, obtaining consistent results and going above and beyond the count of words and the grouping of synonyms.

Caution should be suggested when using a thesaurus to identify synonyms of emotion words; these tools in fact usually register decontextualized meaning and because we know that, particularly with the emotion words, we have different meaning in different contexts, it is very important to keep in mind a contextualized concept of meaning. For this reason, it is important to refer to the uses of spoken language in the context of the product of interest.

7. The questionnaire could be piloted with a small number of subjects (e.g., 5) with a remembered product (e.g., "Imagine you are testing the product x...") asking them to report any difficulties in the understanding of the sentences; if they were appropriate to describe the emotional experience of this product; if any emotion was missing. These questions should be asked orally right after the test, preferentially. In particular, the interviewer should ask how the potentially ambiguous sentence (if any, based on the semiotic analysis of phase 1) has been interpreted.

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Chapter 8

Emotion-Picture Questionnaires (Emoji)

Sara Spinelli, Erminio Monteleone, and Sara R. Jaeger

Abstract

Recently, alternatives to self-report questionnaire that uses words to measure emotional responses to foods have appeared, with a number of published studies that use emoji. Here, we describe how to develop and implement these emoji questionnaires, taking into account specific adjustments needed in case the questionnaires are to be used with specific age groups (e.g., children, adolescents, older people).

Key words Emoji, Questionnaires, Emotions, Adults, Children, CATA, RATA

1 Introduction

In the last 5 years, self-report questionnaire to measure product emotional experience alternatives to those using words has appeared, with a number of published studies using emoji [1, 2]. Emoji[1] are small digital images that are used to express emotions or ideas in electronic communications such as emails or messages exchanged using social media platforms [3, 4]. Emoji are designed to mimic facial expressions, objects or subjects that communicate specific emotions or other situational meanings [5]. In total, there are 3633 emojis in the Unicode Standard, as of September 2021 (https://emojipedia.org/), and the majority represent activities, animals, plants, gestures, body parts, symbols, and objects. Only a small number expresses emotions and feelings mainly through facial expressions. Emojis are used more and more frequently in everyday digital communication to integrate or replace verbal communication, and this explains their application to measure emotional responses to products with consumers.

Jaeger, Ares, and colleagues did extensive research on emojis in adults, finding that they can distinguish between food stimuli, including both tasted stimuli (presented in blind conditions) such

[1] Unless otherwise noted, the emoji used in this chapter is shown in Apple iOS rendition 14.6.

Moustafa Bensafi (ed.), *Basic Protocols on Emotions, Senses, and Foods*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_8,

as varied types of muesli bars and popcorn and written stimuli such as milk, water, red wine, and chocolate presented using words [6, 7]. Furthermore, a smaller but growing body of research has assessed food-elicited emotions with children ranging from 7 to 13 years, recognizing the advantage of using emoji to measure emotions elicited by foods with this age group, because they are intuitive, easy-to-use, and child-friendly [8–11].

This protocol provides guidance in the use of emoji questionnaires, with a focus on applications for consumer goods, and in particular relating to foods and beverages. It describes emoji selection and meaning, participant considerations, ballot development, question variants, data collection, and analysis. It is accompanied by generous notes that share the authors' experiences and tips for conducting emoji research on food products.

2 Materials

2.1 Emoji Meaning

1. The intended meaning of emoji is available online on emoji reference website such as Emojipedia (https://emojipedia.org/), but the meanings that users seek to express using emoji may differ from their official definitions, resulting in several different interpretations of the same emoji [12]. Some emojis have been found to be more ambiguous than others because they can express multiple meanings depending on the context (i.e., they are polysemic) and thus are not recommended for questionnaires interested in measuring the emotional responses to products (*see* **Note 1**). Specific emoji meanings and uses could also differ across cultural groups (related to geographical areas, socioeconomical status, age, gender, etc.).
2. Many of the considerations about the meaning of emoji are similar to those made for emotion words (*see* Protocol 7 on EmoSemio in this volume [13]). However, compared to emotion word questionnaire, we may expect a less relevant role of the products in generating the context, indicating that product-specific questionnaires might have only limited benefits. In fact, while the variations in verbal language are wide and susceptible to express different nuances of meanings, the emojis are still a limited number and cannot be modified by people (they are selected at the same levels of the letters on the keyboards of the smartphones, tablets, or computers).
3. It is very important for the researcher to have clear the meaning of the emoji shared by the participants taking part in the study and researchers should consult the most recent literature available, differentiating between adults (*see* **Note 2**) and children (*see* **Note 3**). Resources providing insight on emoji meaning from outside sensory-consumer science and from diverse participants can be relevant (*see* **Note 4**).

4. Despite their similarities to facial expressions, technical variances and their own visual qualities (depending on platform used: Apple, Google, Samsung, and Facebook) is known to influence how emojis are interpreted [14].
5. The cross-cultural suitability of emoji is rooted on the hypothesized universality of facial expressions of emotions theorized by the basic emotion theory [14], but recent empirical studies do not support this [15, 16]. However, it is true that emojis are stereotypical augmented representations of facial expressions, and this may be an argument to support their universalism (*see* **Note 5**).

2.2 Emoji Selection

1. The principles for emoji selection directly draw on guidelines for term selection in emotion word questionnaires [e.g., Protocols 6 and 7 in this book]. The essence of the selection process is to ensure that the emoji suits the study aims/objectives, tested stimuli, and participants.
2. When the research is focused on emotional product associations, facial emojis are the most obvious candidates for the research questionnaire (*see* **Note 6**).
3. To guide the selection of emoji, two approaches are possible: drawing on published research or conducting preliminary tests. The second approach, despite being more time-consuming, is, however, recommended whenever the questionnaire is used with participants of specific age groups or cultures for whom a different meaning of the emoji can be hypothesized, and previous data are not available. Instances where this may apply are infrequent users of emoji, older people, people from very different cultural backgrounds. Prelimimary work on the selection of emoji appropriate for pre-adolescents is available [17].
4. It is particularly recommended to select a variety of emoji that cover different meanings and that represent a variation not only in the valence dimension (e.g., positive vs negative) but also in the dimension of arousal, related to physiological activation (e.g., calm vs. agitated) (*see* **Note 7**).
5. Recommendations for the number of emoji to include in a questionnaire follow those for number of terms in food-related consumer research, which often seems to be between ~10 and ~30 (*see* **Note 8**).

2.3 Participants

1. Regardless of whether participants are adults or children, appropriate procedures need to be followed to ensure that the research is conducted in accordance with the principles of the Declaration of Helsinki [18] and to the national/international data protection regulations about where the study takes place. Participant anonymity and informed consent are imperative.

2. Participant recruitment criteria should be developed to fit study aims and objectives. In product-focused research, it is typical that the research participants are regular product users, but infrequent users may also be considered or non-users in case of specific objectives. Other criteria such as gender, age, income, and household composition may also be used to quota-define samples and obtain a more representative sample. It is relatively uncommon that samples are nationally representative.
3. Emoji questionnaires can be used with children. Studies on pre-adolescents identified small but significant differences by age and gender, with girls and older pre-adolescents (12–13 years old (y.o.) that discriminated among positive emoji slightly better than boys and younger pre-adolescents (9–11 y.o.), respectively. This suggests that girls and older pre-adolescents may be higher in emotional granularity (the ability to experience and discriminate emotions), particularly of positive emotions [19].
4. Since emoji exposure is strongly linked to computer-mediated communication, the use of emoji questionnaires may not be appropriate for all groups of participants. Those that lack skills and experience with computers, social media, and/or smartphones may lack knowledge of emoji meaning and may not be the most suitable participants (*see* **Note 9**).
5. Emoji questionnaires are often used in conjunction with acceptability testing, and therefore, it is common to follow the guidelines for sample sizes in such testing [20–24]. Less than 100 people is no longer recommended. If the stimuli are quite similar, then larger N is expected to be needed for significant discrimination. If consumer segmentation is desired, then sample size must be several times higher, such that N per segments remains acceptable. If data are collected in several countries, N per country should also be 100 or more and possibly higher if there are major known regional or cultural within-country differences, or if rural and urban populations are very distinct.

2.4 Stimuli

1. Besides foods and beverage, stimuli such as personal and household care products can be used. These are typically physical stimuli (e.g., the real products), but it is also possible to use images or written stimuli (e.g., words) to represent the products.
2. With physical stimuli, sensory fatigue needs to be considered (*see* **Note 10**). The samples can be presented blind or branded (*see* **Note 11**). Sample serving size depends on the tests type and location, and in central location test (CLT) settings, small

servings (2–3 bites/zips) per sample are often used. In home use tests (HUTs), larger sample volumes may be used.

3. There are no firm guidelines as to the number of stimuli that can be used in a test, but published research often includes more than one and less than ten stimuli. We recommend that pilot testing be used to decide the balance between the desire to maximize data collection against the risk of sensory fatigue and participant boredom/disengagement (*see* **Note 12**).
4. To mitigate bias from sample presentation order and first-order carry-over, stimuli should be presented according to appropriate experimental designs based on Latin square designs (*see* **Note 13**). Warm-up samples can be used to further minimize first-sample presentation bias (e.g., [25]).
5. Since the principles underpinning stimuli selection for emoji questionnaires are no different from those that guide other product-focused consumer research, readers are referred to standard textbooks in sensory and consumer research for further detail (e.g., [21]).

3 Methods

3.1 Ballots and Questionnaire Variants

1. In product research, Check-All-That-Apply (CATA) questionnaires are widely the most used type of emoji questionnaire. In these questionnaires, participants are asked to select all the emoji that are appropriate to describe how the product makes them feel (Fig. 1). The question asked could be "How this product (e.g., this chocolate) makes you feel? Select all the emoji that apply." When written stimuli are used, it is appropriate to refer to an imagined consumption situation. As illustrated in Fig. 1a, b, layout of the CATA question can differ.
2. CATA questionnaires with emoji are popular and share many applied and methodological characteristics with CATA questionnaires using emotion words. Further to the information in this protocol, we refer readers to the protocol in this volume by Jaeger and Ares on CATA questions with emotion words [27].
3. Variants of CATA questions exist and have been extensively studied for sensory product characterization by consumers and, to some extent, also in relation to CATA questions with emotion words [27, 28]. The two most likely CATA question variants to be used with emoji are Rate-All-That-Apply (RATA) questions (Fig. 1c) and forced-choice yes/no questions (Fig. 1d) (*see* **Note 14**). In RATA questions, participants consider all the available emoji, and for those that apply, they rate their intensity typically using a 3-point or 5-point scale [25, 29] (*see* **Note 15**).

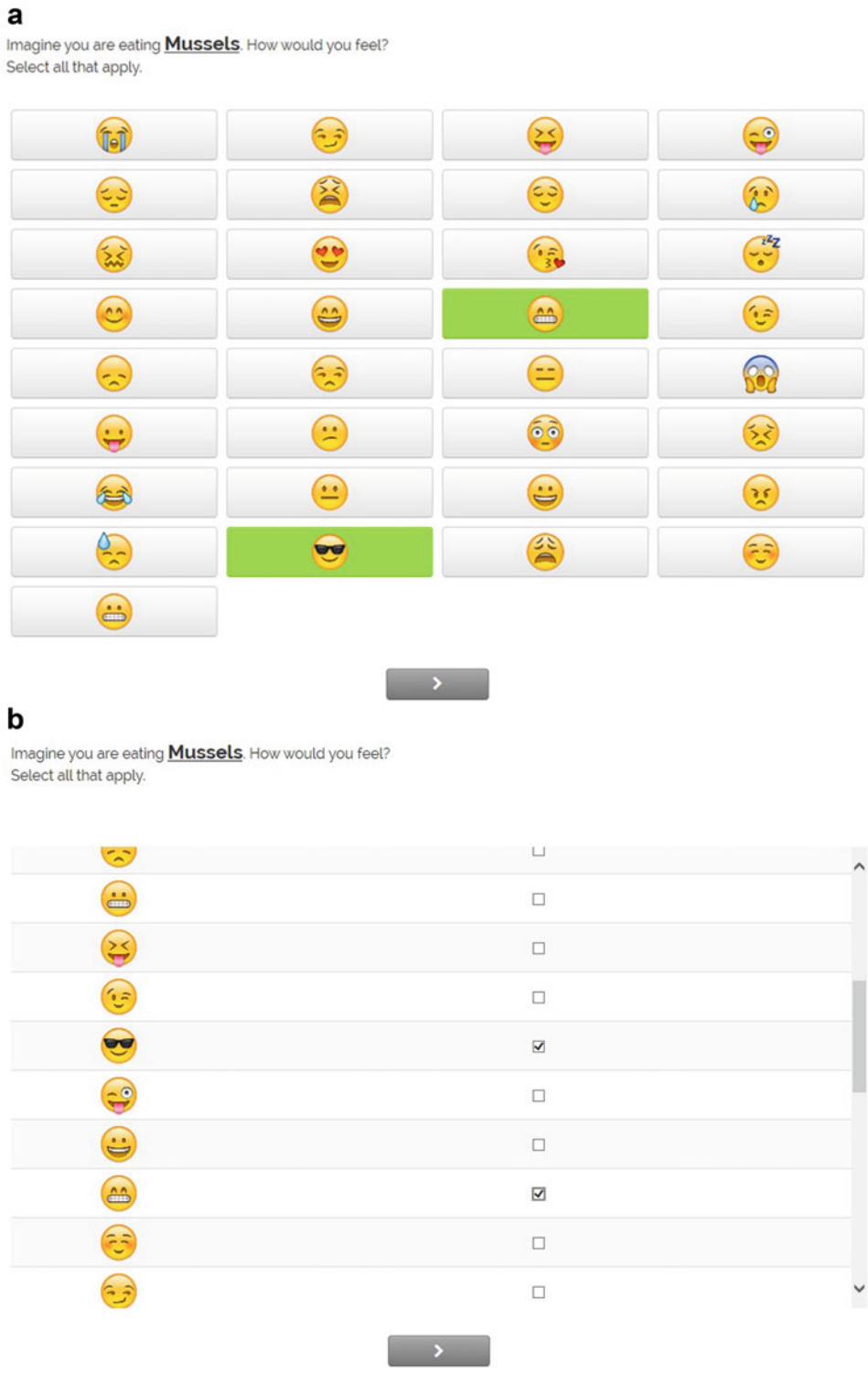

Fig. 1 Examples of emoji tests using different layout: 1a, classic Check-All-That-Apply; 1b, Check-All-That-Apply with boxes; 1c, Rate-All-That-Apply; 1d, Check-All-That-Apply with yes/no format [26]

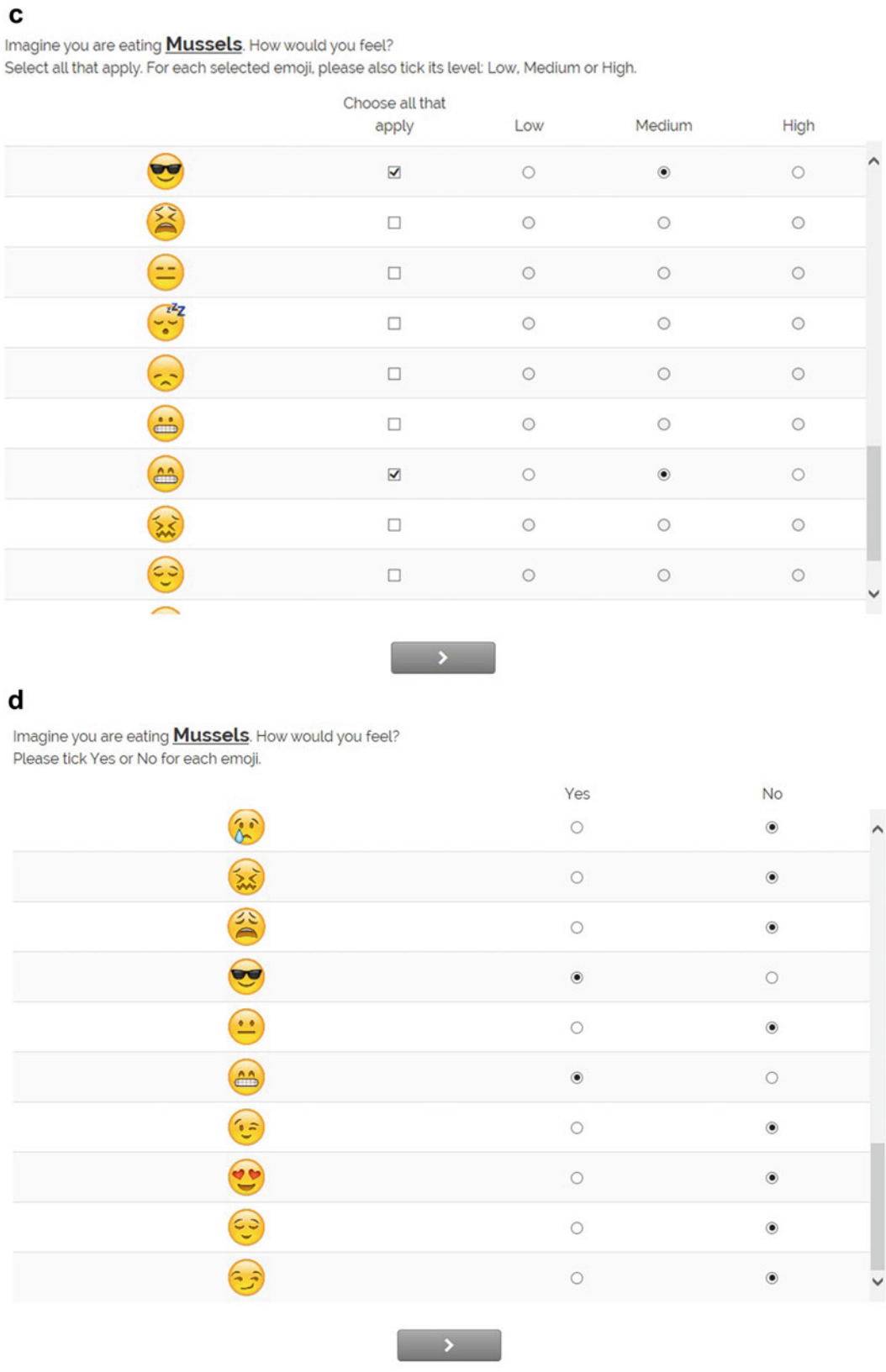

Fig. 1 (continued)

4. A rating scale, similar to the one used for emotion word, questionnaires may also be used in emoji questionnaires. An example can be seen in Fig. 2 with the Emoji Pair Questionnaire for pre-adolescents [17]. Children are instructed to taste one sample at a time and answer to the emoji pair rating scale for each sample tasted: "How does this food make you feel? Look at the emoji pairs. For each pair, indicate how much the emoji (one of the two or both) match the feeling you get from tasting the food sample." (*see* **Note 16**).
5. For emotion word questionnaires, a valence×arousal circumplex-inspired emotion questionnaire (CEQ) exists where 12 pairs of emotion words are placed in a fixed circular layout [30]. This questionnaire purposefully spans the two core dimensions of human affect—emotional valence and emotional arousal—which can increase sample discrimination compared to questionnaires that primarily use emotion words linked to degree of valence. An emoji variant now exists [31], and the Emoji Pair Questionnaire for pre-adolescents [17] also goes in this direction.

3.2 Emoji Presentation

1. Because emoji are visual representations, it is important that their nuances can be clearly seen by participants when completing the research task (*see* **Note 17**).
2. Since some emoji do look similar, it is further very important that reporting includes a full list of the emoji in the study and their name according to a central resource like http://emojipedia.org. However, these names are for reporting purposes only and are not shown on the ballots.

3.3 Test Location

1. In product testing, the test location typically falls into one of several categories—a central location, participants' home, or an institutionalized setting such as school, workplace, and nursing home. The former is very common and is also known as central location tests (CLTs), which can be test laboratories or rented hall spaces. Conversely, home use tests (HUTs) are tests which take place in participants' home and this terminology is generally reserved for physical product testing (*see* **Note 18**).
2. The use of emoji questionnaires is largely independent of test location, except for the dependency that is linked to sample population. Hence, schools and after school care facilities may be overrepresented compared to other product research in the case of studies involving children.
3. The increase in web-based survey administration and technology advances means this "test location" is now widespread. Typically, participants can complete such surveys at a location of their choosing, be it at home or elsewhere. The conduction of test in remote assisted conditions is also possible [32, 33] (*see* **Note 19**).

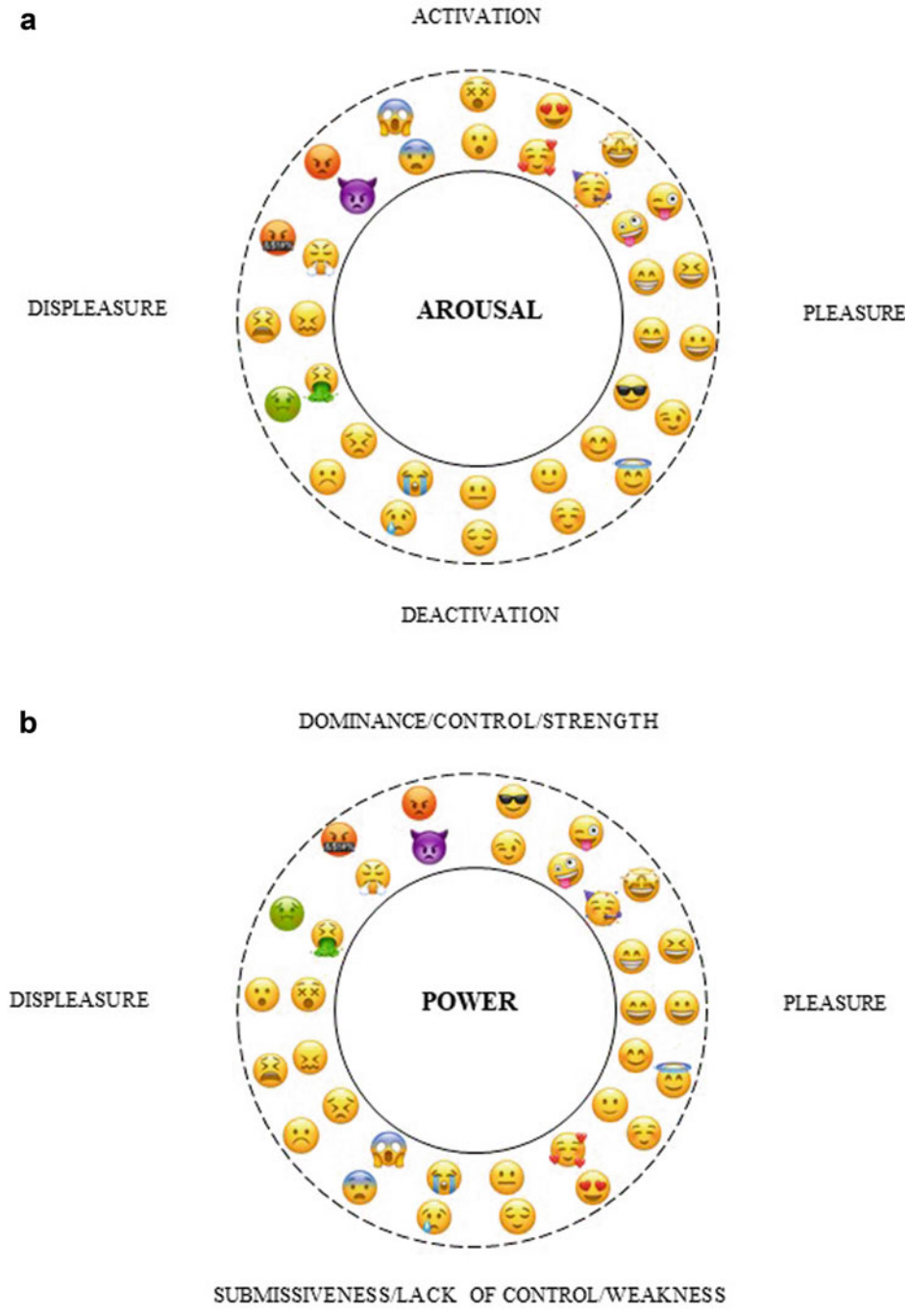

Fig. 2 Emoji circumplex showing the 17 emoji groups included in the Emoji Pair Questionnaire for pre-adolescents based on similar meanings and levels of (**a**) valence (pleasure vs. displeasure) and arousal (activation vs. deactivation) and (**b**) valence (pleasure vs. displeasure) and power (dominance/control/strength vs. submissiveness/lack of control/weakness) derived from projective mapping tasks. (Reproduced from Sick et al. 2022 with permission from Elsevier [17])

3.4 Data Analysis

1. The methods for data analysis are determined by the scaling format used in the emoji questionnaire.
2. When CATA questions are used, the data are categorical, and it is customary to code CATA term (i.e., emoji) selection as "1" and lack of selection as "0" (*see* **Note 20**). The typical steps for analysis are explained in [34]. At the level of each emoji (i.e., univariate analysis), Cochran's Q test is used to determine if the samples are significantly discriminated (*see* **Note 21**). They include data summation where a frequency table is constructed with counts of the number of times each emoji has been selected as applicable for each of the products in the study. This forms the basis for Correspondence Analysis (CA) to derive a reduced dimensionality space of the main similarities and differences between samples across the emoji included in the questionnaire.
3. If RATA questions are used, the data should be analyzed using analysis of variance and Principal Component Analysis (PCA) [35]. If rating scales are used, as per the example in Fig. 3, then it may be appropriate to treat the data as having interval properties and apply parametric methods such as ANOVA (univariate) and PCA (multivariate). It is customary to make this assumption.
4. For CA and PCA to be appropriate, the study should include five or more samples.

Fig. 3 Examples of the Emoji Pair Questionnaire using a rating scale with an extract of 3 emoji pairs with a categorical 5-point ranging from 1 = Does not fit at all to 5 = Fits very well. (Reproduced from Sick et al. 2022 with permission from Elsevier [17])

4 Notes

1. For example, polysemic meanings have been reported for the emoji money-mouth face "🤑", which was found to indicate alternatively "to be rich" but also "to be happy" and "to be cheerful" for pre-adolescents [19]. Another example is the emoji face with cold sweat "😓" which was associated with "embarrassed/shy," "depressed," and "frustrated" in adults [36].
2. For adults, studies have been conducted in China and USA to measure valence, arousal, and associated semantic meaning of 33 facial emoji [36, 37] and in UK and New Zealand to measure valence, arousal, and dominance dimensions of 24 emoji [38], *see* Tables 1a and 1b.
3. For pre-adolescents (9–12 y.o.), a group of studies have been conducted in Italy and Norway investigating the dimensional and the semantic meaning of emoji used specifically to describe food emotional experience [9, 19]. Based on these studies, an emoji-based self-report measurement tool to measure emotions in response to food products was developed, which includes 17 emoji pairs associated with specific semantic and dimensional (valence, power/dominance, and arousal) meanings [17]. The rationale behind this approach is that presenting the emoji in groups of two indicates more accurately their meaning and emphasizes that each response option corresponds to an emotion family (see also [39, 40]), thus facilitating the interpretation particularly of polysemic emoji (Table 2 and Fig. 2).
4. Some research pertaining to emoji meanings based on social media research and with diverse consumer populations exist (for example, 41–45).
5. Only small cultural differences across cultures have been highlighted in emoji research so far, but the extant research on the topic is still limited and restricted to few countries (e.g., USA vs. Chinese, New Zealand vs. UK participants [7, 37, 38, 46]).
6. We advise against including other emoji that explicitly express liking without clear emotional implications, notably thumbs up (👍) and thumbs down (👎).
7. This information can be found in the literature [17, 19, 37] or investigated using pre-tests. Using this approach to emoji selection will help to have emoji that discriminate among products that do not differ significantly in their liking ratings. In fact, it is expected that when products differ in liking they do differ also in emoji differing in valence (positive vs. negative), while positive (or negative) emoji differing in arousal may catch

Table 1a
Averages[a] for the 3 PAD dimensions in Study 1 (NZ)

Emoji name	Pleasure	Arousal	Dominance
Beating heart	2.1 j	3.4 efgh	4.4 cdef
Clapping hands	2.3 j	3.1 ghi	4.3 def
Collision	4.1 gh	2.6 hi	3.6 fgh
Exploding head	6.6 abc	3.5 efgh	5.0 abcde
Expressionless face	6.7 abc	6.3 b	5.4 abc
Face savoring food	1.9 j	3.3 fghi	4.2 efg
Face screaming in fear	6.2 bcde	3.4 efgh	5.2 abcd
Face vomiting	7.5 a	4.4 de	5.3 abc
Face with steam from nose	7.3 ab	3.5 efgh	4.3 def
Face with tongue	2.3 j	3.2 ghi	4.2 efg
Flexed biceps	3.4 hi	3.4 efgh	3.2 gh
Flushed face	6.2 bcde	4.3 def	5.9 a
Nauseated face	7.2 ab	4.9 cd	5.6 a
Oncoming fist	3.8 hi	3.1 ghi	3.1 h
Party popper	2.0 j	2.3 i	3.8 fgh
Persevering face	7.2 ab	5.0 cd	5.6 a
Person in lotus position	2.8 ij	6.2 b	4.6 bcdef
Person shrugging	5.9 cdef	5.5 bc	5.7 a
Pouting face	7.6 a	3.6 efgh	4.1 efg

(continued)

Table 1a (continued)

Emoji name	Pleasure	Arousal	Dominance
Sleeping face	5.1 fg	7.7 a	5.5 ab
Smiling face with sunglasses	2.2 j	3.7 efg	4.1 efg
Warning	5.5 def	3.6 efgh	3.7 fgh
Yawning face	6.6 abcd	7.7 a	5.7 a
Zzz	5.3 ef	7.6 a	5.4 ab

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For P dimension (*Pleasure*), the low anchor (1) is associated with pleasure and the high anchor (9) is associated with displeasure; for A dimension (*Arousal*), the low anchor (1) is associated with arousal and the high anchor (9) is associated with non-arousal; for D dimension (*Dominance*), the low anchor (1) is associated with dominance and the high anchor (9) is associated with submissiveness

Notes. [a]Tukey's HSD used for post hoc tests. Within columns, emoji that share a letter are not significantly different at the 5% level

differences between products that do not significantly differ in acceptability ratings (e.g., products that are both liked but one makes one feel calm and the other energetic). In addition, it should be considered that every year new emojis are released, and thus, current lists should be integrated.

8. Questionnaires with many emoji (>30) can be cumbersome for participants since for CATA (Check-All-That-Apply) questions they are expected to consider and select all suitable options and long lists may increase the likelihood of satisficing response behavior where only a few of the most applicable emoji are chosen with a reduction of the discriminant ability [11]. When rating scales are used, this may be exacerbated because of the need to evaluate and rate each of the emoji included in the questionnaire.
9. Older consumers (typically 60+) may more frequently fall into the category of participants who lack emoji experience, but it should not be assumed that increased age "equals" poor emoji literacy. To counter concerns about lack of emoji knowledge during data collection, one strategy can be to include a screening question during participant recruitment to exclude candidate participants who state that they lack emoji exposure/knowledge. Alternatively, participants can be excluded post hoc if they do not meet imposed criteria such as receiving and sending emoji once or more per week. If the latter approach is implemented, it is necessary to be aware that sample size may be reduced and drop below established targets.

Table 1b
Averages[a] for the 3 PAD dimensions in Study 2 (UK)

Emoji name	Pleasure	Arousal	Dominance
Beating heart	3.1 kl	4.1 ghi	4.8 cdefgh
Clapping hands	3.4 jkl	4 ghij	4.5 ghijk
Collision	4.6 hi	3.4 ij	4.2 ijkl
Exploding head	6 cde	3.7 hij	5.0 bcdefg
Expressionless face	5.8 def	6 bc	5.1 abcdef
Face savoring food	2.7 l	3.9 ghij	4.7 defghi
Face screaming in fear	6 cde	4.3 fgh	5.2 abcde
Face vomiting	6.8 ab	5.1 de	5.2 abcd
Face with steam from nose	6.6 bc	4.4 fg	4.7 defgh
Face with tongue	3.2 kl	3.8 ghij	4.6 efghij
Flexed biceps	4.1 ij	4.1 gh	3.9 l
Flushed face	5.7 def	4.8 ef	5.5 ab
Nauseated face	6.6 bc	5.5 cd	5.5 ab
Oncoming fist	4.7 ghi	4.4 fg	4.1 jkl
Party popper	2.7 l	3.4 j	4.3 hijkl
Persevering face	6.8 ab	5.4 cd	5.5 a
Person in lotus position	3.5 jk	6.2 b	4.9 cdefg
Person shrugging	5.6 ef	5.2 de	5.3 abc
Pouting face	7.4 a	4.1 gh	4.5 fghij

(continued)

Table 1b
(continued)

Emoji name	Pleasure	Arousal	Dominance
Sleeping face	5.2 fgh	7.2 a	5.5 ab
Smiling face with sunglasses	2.9 kl	4.3 fgh	3.9 kl
Warning	5.8 def	4.4 fg	4.5 fghij
Yawning face	6.4 bcd	7.1 a	5.5 ab
Zzz	5.3 efg	7.1 a	5.4 ab

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For P dimension (*Pleasure*), the low anchor (1) is associated with pleasure and the high anchor (9) is associated with displeasure; for A dimension (*Arousal*), the low anchor (1) is associated with arousal and the high anchor (9) is associated with non-arousal; for D dimension (*Dominance*), the low anchor (1) is associated with dominance and the high anchor (9) is associated with submissiveness
Notes. [a]Tukey's HSD used for post hoc tests. Within columns, emojis that share a letter are not significantly different at the 5% level

10. Risk of sensory fatigue is a particular concern with aroma stimuli. The number of stimuli evaluated by each participant may need to be lowered or sample evaluation spaced over longer time, following the general guidelines for sensory and acceptability testing with consumers.
11. Blind samples are most common in academic product-focused consumer research industry-led research interested in the emotional profile of a product for its sensory properties, while branded samples (or a study that includes blind, expected, and branded, in different sessions) may be most appropriate in industry-led research marketing-oriented. The decision should be guided by study aims/objectives.
12. Children or elderly may be able to manage fewer samples, and if the number of emoji included in the questionnaire is very long, this may add to participants' task burden.
13. Where this is not possible to use Latin square designs, randomization should be used. Fixed presentation order of stimuli across all participants should be avoided.
14. The key motivation for using yes/no questions is to increase the attention consumers give to the task. By having to indicate "yes" or "no" for each CATA term participants are forced to consider applicability for all options. However, an accompanying decrease in sample discrimination is possible [47].

Table 2
Semantic meaning of the emoji groups included in the Emoji Pair Questionnaire for pre-adolescents

Emoji group	Semantic meaning
	happy[a], cuddled[a], serene[a], cheerful[a], in love[b]
	happy[a], cheerful[a], energetic[a], enthusiastic[a], amused[a], festive[b], suitable for a party[c], content[c], suitable for a special occasion[c], "wow" (surprised/impressed)[c]
	happy[a], cheerful[a], energetic[a], amused[a], crazy[b], makes me feel good[c]
	happy[a], cheerful[a], amused[a], enthusiastic[a], serene[a], relaxed[c], quiet (indifferent in a positive way)[c], content[c], smiling[c], normal[c]
	happy[a], cheerful[a], serene[a], amused[a], indifferent
	happy[a], serene[a], cheerful[a], calm[a], quiet[a], I am good/ I feel like an angel[b], makes me feel good[c], makes me feel special/important[c], keeps myself from doing things[c], new/mysterious[c], calm[c], content[c], ashamed[c]
	happy[a], confident[a], at ease[a], satisfied[a], cheerful[a], feeling cool[b], proud[c], makes me feel good[c], beautiful[c], superior[c], lucky[c], enjoyment[c]
	happy[a], serene[a], calm[a], quiet[a], sad[c], I have to make do with it[c], forced[c], indifferent[c], calm[c]
	indifferent[a], serene[a], calm[a], makes me feel superior[c], makes me feel good[c], forced[c], proud[c], indifferent[c], satisfied[c], content[c]
	sad[a], unhappy[a], disappointed[a], suffering[c], indifferent (negative)[c], I want to cry[c]
	sad[a], unhappy[a], disappointed[a], dissatisfied[a], guilty[a], does not make me feel good[c]
	unhappy[a], sad[a], disgusted[a], melancholic[a], annoyed[a], guilty[a], forced/ I find it unfair[c], I want to complain[c], desperate[c], I want to cry[c], I am satisfied/feeling pleased[c]
	disgusted[a], urge to vomit/nauseated, feeling sick[b], makes me feel bad[c]
	angry[a], annoyed[a], forced[c]
	angry[a], disgusted[c]
	surprised[a], worried[a], scared/frightened[b], makes me feel bad[c], afraid[c]
	surprised[a], worried[a], dead/deceased[b], curious[c]

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[a]Emotion words from CATA questionnaires using as stimuli the emoji and the words
[b]Additional emotion words from open-ended responses
[c]Emotion words construct from one-on-one interviews

15. The choice of RATA questions is usually made in search of improved sample discrimination, but empirical evidence has shown that CATA questions can perform just as well as RATA questions in this regard [29, 35].
16. The emoji pair questionnaire for pre-adolescents can be used also with CATA format.
17. Care should especially be taken to ensure that they are not displayed too small and that the size the emojis are shown in

on the test ballot is the same for all emoji and across all samples. Naturally, they should also be shown in color and since emoji rendering differs across platforms, it is very important when reporting on a study that uses emoji to clearly state what rendition (and version) was used.

18. Some population groups—typically the young end very elderly—can be more conveniently reached in nurseries, schools, nursing homes, or day activity centers.
19. It is advised that they are asked to choose a location where they can complete the survey without being disturbed.
20. The analysis procedure for yes/no variants of CATA questions is the same as for standard CATA questions (assigning 1 to "Yes" and 0 to "No").
21. If the number of participants is large and sufficient responses are obtained for individual emoji, it may be acceptable to apply analysis of variance (ANOVA) models to CATA data [48].

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Chapter 9

Measuring Food Emotions Using Semi-Guided Interviews

Marylou Mantel, Jean-Michel Roy, and Moustafa Bensafi

Abstract

To understand the whole experience evoked by food stimuli, qualitative approaches can be used, including introspection and interview. Here, we present a protocol aimed at collecting the emotional experience provoked by odors using a semi-guided interview. The subjects' verbalizations can then be transcribed and organized with dedicated software to draw a portrait of food-related emotions for the olfactory modality, but also for other senses such as vision or taste by adapting the protocol. Altogether, these subjective data are a complement to standard ratings or choice within a list of emotions, and they can help better understand the variety and the context surrounding the affective responses to food.

Key words Subjectivity, Qualitative methods, Olfaction, Discourse analysis

1 Introduction

In current research on food and the emotions it evokes, one of the most common types of measurement consists in choosing emotions within a preexisting list and/or rating the strength of such experienced emotions elicited by a specific food stimulus [1]. These are not restricted to the domain of food emotions. Most protocols in sensory analysis and experimental psychology use quantitative ratings or forced-choice descriptors [2, 3]. This allows for the standardized measurement of a complex phenomenon that can be compared between the individuals and then applied to marketing [4, 5].

However, one can wonder if this kind of protocol is a complete portrayal of the emotions experienced by the subjects, and if it is enough to understand the affective dimension of food. Indeed, it provides a preexisting list of emotions to the participants, which can bias them toward certain elements that they would not have spontaneously mentioned otherwise. Moreover, such lists can reflect some expectations from the experimenter, and the subjects' answers may be influenced by those expectations [6]. Also, these measurements do not include the context surrounding the

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emotions chosen by the participants, which could help understand why they chose them and why they answered differently compared to another subject [7].

To get a more complete description of the emotions associated with food, different types of protocols are available, specifically aimed at collecting the spontaneous, unbiased subjective emotional experience of participants. Such qualitative approaches have already been applied in other areas of research, like in meditation, dreaming [8, 9], or attentional processes [10]. They allow for a full oral description of a subjective phenomenon, sometimes with the help of the experimenter. Several methodologies exist, including phenomenology [11, 12], inspired by the work of the philosopher Husserl and consisting of a detailed account of the structure of one's experience following thorough training. Modern forms of introspection are also used [13], along with semi-guided interviews (especially the Explicitation Interviews developed by Petitmengin [14–18]), which have the advantage of the presence of an experimenter helping the subjects verbalizing their experience, a task that participants often perceive as difficult.

Here, we will detail a recently published protocol [19] inspired by the Explicitation Interview and based on the verbal descriptions of people from different linguistic and cultural backgrounds (French and German). It was conducted with olfactory stimuli, smell being an important part of the emotional value of food, and a major contributor to food behavior [20–24]. However, this kind of protocol can easily be applied to other sensory modalities as well (*see* **Note 1**) and we provide here methodological elements to document subjectivity of emotional events.

2 Materials

1. An audio recorder with a microphone (e.g., Philips DVT 1201).
2. A computer to access and analyze the data (e.g., MacBook Pro, 3.1 GHz processor, 8 Go memory).
3. The stimuli used in your protocol depend on your specific aims. As an example, we present here a list used in Mantel et al. [19]. Twenty olfactory stimuli (from Sigma-Aldrich, with Compound Identification number): isovaleric acid (CID: 10430, diluted at 0.48%), butanoic acid (CID: 264, diluted at 0.11%), butyl butyrate (CID: 7983, diluted at 0.18%), propanoic acid (CID: 1032, diluted at 0.04%), ethyl salicylate (CID: 8365, diluted at 5.49%), 1-octen-3-one (CID: 61346, diluted at 0.27%), acetic acid (CID: 176, diluted at 0.01%), cis-3-hexenol (CID: 5281167, diluted at 0.24%), guaiacol (CID: 460, diluted at 2.09%), eugenol (CID: 3314, diluted

at 13.45%), geraniol (CID: 637566, diluted at 11.29%), benzaldehyde (CID: 240, diluted at 2%), cineole (CID: 2758, diluted at 1%), linalool (CID: 6549, diluted at 2.17%), beta-caryophyllene (CID: 5281515, diluted at 33.64%), L-carvone (CID: 439570, diluted at 1.93%), isoamyl acetate (CID: 31276, diluted at 0.03%), alpha-santalol (CID: 5368797, pure), myrcene (CID: 31253, diluted at 0.15%), and ethyl octanoate (CID: 7799, diluted at 1.71%). This number of stimuli was chosen as a trade-off between the need for sensory variety and the risk of tiring participants. The odorants should be presented in 15 mL opaque vials, diluted in mineral oil [19, 25, 26].

4. For the subsequent analysis, a qualitative analysis software is recommended, such as NVivo (https://www.qsrinternational.com/nvivo-qualitative-data-analysis-software/home/).
5. Participants: The study must be validated by an ethics committee: participants should be informed about the aims and procedures of the study, and if they agree to participate, they complete the consent form. The estimation of the number of participants needed must be done according to a statistical power calculation. For example, a set of 37 participants allowed us to reach a power of 93% in a recent study using this protocol [19].

3 Methods

1. Instructions: Before starting the actual experiment, participants receive an instruction sheet, with information about the protocol and what is expected of them. We recommend taking time to make sure they understand the instructions and procedure, and to allow questions, as it could impact the later verbalizations. The protocol is not aimed solely at emotions, but at the whole subjective experience associated to odors, so the instructions should be adapted (*see* **Note 2**).

   Here is the example used to explain what subjective experience means compared to identification: *"In this study, we are interested in what you experience when you smell odors. Here, you are in a position of a witness: we want to hear your own subjective experience and not an interpretation about the source of the odors that will be presented to you. There is thus no good nor bad answer. For example, if you think that a vial smells like a rose to you, the fact that this odor is typically produced by a rose is not relevant in this experiment. We are more interested in the fact that, for you, this odor is associated with what you experience when you smell a rose. We are aware that this descriptive exercise may seem difficult, but we ask you to be as attentive as you can to the odors that you*

will be presented and to put aside your external thoughts -. To familiarize yourself with the task, you will have a training vial and you will be able to ask any question that you may have to the experimenter before starting the actual experiment."

2. Protocol (Training session): First, there is a training trial, which is important to make sure that the participants clearly understand what is expected of them. The experimenter starts with an explanation of their role during the interview: *"To help you in your description, I will ask you some questions and sometimes repeat what you said to make sure I understood you. If you have trouble describing an element, don't hesitate to use your own words or generic terms like 'this thing' or 'this feeling', you can also come back to it later."*
3. Protocol (Experimental session): When the participants are ready, they can take the vial and smell it. They can close their eyes to focus on the smell and it is advisable for the experimenter to wait a few seconds before starting to talk: "*Focus on your experience. It should become clearer and clearer as you focus on it. Now, try to put some words to this experience. What comes to mind?"*. The aim of the experimenter is to let the participant speak on their own. He/she intervenes only when participants begin to struggle with their words or are getting outside of the scope of the experiment. In this case, the interviewer asks several questions to help the participant verbalize his experience: *"what do you think when you smell this odor?", "what else does it evoke for you?", "how would you describe what it is like to smell this odor to a friend who never experienced it?"*. In addition to encourage him, the experimenter can repeat certain words said by the participant or ask him what he means by a certain expression. In total, this exchange should not last more than 2 min after the first sniff. When the participant is done or if the time limit is reached, the participant puts the vial back and takes the next one when ready. In the case of olfactory stimuli especially, a break may be needed between two trials to clear the nose from the previous odorant. Participants are allowed to smell each odorant several times if they want to.
4. Protocol (Examples of interviews): Here are some examples of interviews in Table 1. As one can see, the experimenter does not intervene a lot in the interview, only when the participant seems to stop or struggle to verbalize his experience. In total, each interview should not exceed 2 min per odorant, and 1 h in total. If needed, the experiment can be divided into several sessions to allow for the testing of more odorants.
5. Transcription of the verbal data: After audiorecording each interview for each trial per subject, the first step consists in transcribing the verbal data. To this end, one can use automated voice recognition software, but it is recommended to also check the validity of the transcription manually.

Table 1
Examples of interviews' transcriptions for three food-related odors

	Experimenter	Subject
Odor 1 (L-carvone)		I like this one, it makes me think about the smell of mint chewing gums, so this is... I like it a lot...
		Yes, it is really this smell
		So, I like it a lot and it reminds me of... well, all the time in fact, because I chew a lot of gum
		And yes, I see the very long, thin, and green gum. Yes, it is a pleasant odor
	Do you think about other things when you smell this odor?	
		Yes, I saw myself buying my pack of gum at the supermarket.
		But yes, it is positive, because I buy it regularly and for a very long time so... yes, at least since elementary school.
Odor 2 (Isoamyl acetate)		This makes me think about candy ice cream. I do not like it at all, I find it nauseating.
		Yes, it seems chemical.
	Do you think of other things for this odor?	
		I do not like smelling it, but...
		Yes, it looks like candy ice cream... it smells exactly like this
	Ok, something else about how you would describe it to someone for example?	
		I would say that it is like candy ice cream that you find everywhere at the beach.
Odor 3 (Guaiacol)		This is an odor I find very unpleasant
	Ok, how is this odor?	
		It has a smoked dimension, not like smoked meat but like smoke
	Ok, do you have other things that come to mind when you smell it?	
		I have the impression it changes my taste
	How would you describe it to someone who had never smelled it?	
		Rough, rough. I cannot say if this is a chemical or natural odor. I would tell them that it is very unpleasant to smell.
		But I don't know what kind of odor it could be, or if it is created by nature or by the chemistry industry. I don't know at all, but it is almost sickening.

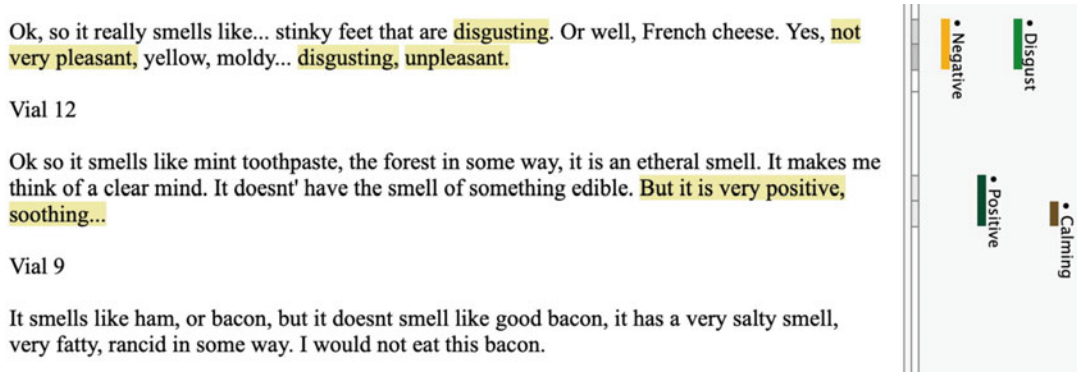

Fig. 1 Example of emotional terms in a subject's verbalizations using NVivo. For the odorants showed here, the participant mentions disgusting and soothing aspects, as well as general terms for negative and positive emotions

6. Organization of the textual data: To organize the textual data, we recommend the use of software dedicated to qualitative analysis. Here, we show an example using NVivo (Fig. 1), but it is possible to use other software, as long as it is able to tag textual data with specific categories and organize them (e.g., QDA Miner Lite, Atlas.ti).
7. Analysis of the textual data: The first step of analysis is to decide on a categorizing matrix. In the present protocol, the matrix is based on the existing literature on the various aspects of olfactory perception [27–29], which includes the source of the odor, its effect on the individual, qualitative characterization, and memories associated to the odor (*see* **Note 3**).

   In a second step, one must assign words or expressions in the textual data to the categories or subcategories of interest (named "codes" in the NVivo software) with the help of the software. Fig. 1 shows an example of verbalizations with terms pertaining to emotions highlighted and assigned to various sublevels (e.g., "disgust," "negative," "emotion").

   Once all the elements of interest have been assigned to categories, it is possible to visualize the repartition of the verbal references easily with a histogram of the number of mentions for each category, as shown in Fig. 2. With the NVivo software, it is possible to differentiate between different odorants or experimental groups, by assigning textual data to elements called "cases," corresponding either to a specific individual or to a stimulus.

   The results can also be exported to a spreadsheet file for further statistical comparisons between the individuals and the odors tested. See Fig. 3 for an example of such file. The relative weight of emotional terms can also be compared with other categories of references, to identify stimuli that are especially

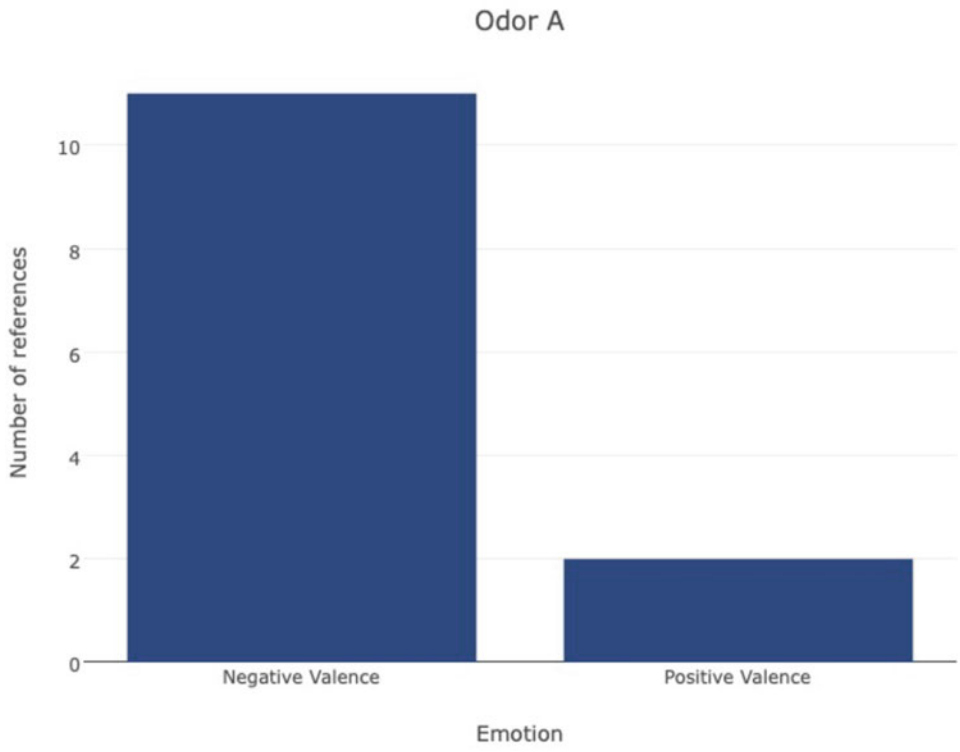

Fig. 2 Example of a simple histogram available with NVivo software for data visualization. The number of references related to negative versus positive emotions can be easily displayed for each tested odorant

emotive for example, and quantitative ratings may be collected after the verbalizations (*see* **Note 4**), to complement the emotional characterization of the stimuli.

8. Conclusions: This semi-guided interview protocol is aimed at collecting the spontaneous experience of an individual during his perception of a food stimulus, here using the olfactory modality. It allows for a more ecological portrait of the affective value of food, in which the participants do not have to choose from a preestablished list of possible emotions but rather have the possibility to express in their own words how they feel and why. The results emphasize the main emotions experienced by the individuals and their relative importance for them, as reflected by the number of references to specific emotions. This type of protocol can be a good complement to more standard questionnaires and can easily be adapted to other sensory modalities involved in food processing or to ecological food samples.

	A : Ambiguous	B : Anger	C : Disgust	D : Joy	E : Neutral	F : Stress
1 : (A) - VAL	2	0	16	0	0	0
2 : (B) - BUTB	0	0	3	0	4	0
3 : (C) - BUT	0	0	12	0	1	1
4 : (D) - PROP	0	0	6	0	1	1
5 : (E) - OCTN	0	0	3	4	0	0
6 : (F) - ESAL	0	1	2	1	1	0
7 : (G) - ACE	0	1	1	0	6	0
8 : (H) - CIS	0	0	0	8	1	0
9 : (I) - GUA	1	0	0	3	1	1
10 : (J) - EUG	1	0	3	1	2	1
11 : (K) - GER	0	0	0	5	0	0
12 : (L) - BENZ	0	0	1	4	1	0
13 : (M) - CIN	1	0	0	4	1	0
14 : (N) - LIN	0	0	0	4	1	1
15 : (O) - BCAR	0	0	0	1	4	0
16 : (P) - CAR	0	0	0	3	3	0
17 : (Q) - SAN	0	0	2	5	6	0
18 : (R) - IAA	0	0	0	4	2	1
19 : (S) - EOCT	0	0	1	4	2	0
20 : (T) - MYR	0	0	2	2	15	0

Fig. 3 Example of an output file of the number of references to different emotions for various odorants. The rows correspond the name of each odorant, and the columns represent the types of emotions that were mentioned by the participant (ambiguous if the emotion is unclear or mixed, anger, disgust, joy, stress, or neutral if the participant mentions that there is no emotion associated to the odorant). In the cells, the number of references to each emotion is indicated for each stimulus

4 Notes

1. The semi-guided interview is not specific to a sensory modality and the instructions can apply to taste, vision, and texture as well. One must, however, be aware that verbalizations can be more or less difficult for the participants depending on their access to a specific sensory vocabulary, which is richer for vision and audition than for olfaction and taste for example. In these cases, the help of the experimenter is even more important to guide and encourage participants in their verbalizations. It is recommended that the experimenter practice before the actual

semi-interview, by reading transcripts of previous interviews and by conducting mock interviews.

2. If the experiment is aimed at collecting the emotional experience only, the instructions should focus on describing how a participant feels when perceiving the stimulus, regardless of its nature and its general value in society. For example, it is possible to feel sadness or disgust when smelling candy despite its positive cultural value, as it could have a specific meaning for the individual based on his past experiences and beliefs. The absence of right and false answers should thus be emphasized as well, so that the participants feel reassured about the legitimacy of their own experience and the possibility for them to explain the context they associate to the stimulus and its emotional value.
3. It is possible to create as many sublevels in the categories as wanted, depending on the degree of precision one wants to achieve and based of the objectives of the study. For example, if one is interested in the emotions elicited by a stimulus, the first level can be the valence (positive or negative) and the second level the discrete emotion (joy, disgust, etc.).
4. It is recommended to collect quantitative ratings about the stimuli after the verbalizations, so that the proposed list of emotions and other perceptual attributes does not interfere with the spontaneous descriptions of the subject. For example, in a second phase of the experiment, the participants may be represented with all the stimuli and rate them accordingly. In a subsequent analysis, the similarity between the verbalizations and the ratings can be assessed with a Mantel test for the distance between matrices.

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Part II

Behavioral and Psychophysiological Approaches

Chapter 10

Measuring Olfaction in Children and Young People with Profound Intellectual and Multiple Disabilities

Geneviève Petitpierre and Juliane Dind

Abstract

People with profound intellectual and multiple disabilities (PIMD) present intense and complex support needs as well as major motor, communicative, and cognitive limitations. The protocols presented in this chapter describe detailed practical procedures in order to evaluate the food, sensory, and emotional spheres of people with PIMD, taking the sense of smell (discrimination, habituation) and its relations with the emotional system (preferences) as an input modality. The protocols are designed to meet three conditions: (1) to respect the basic methodological principles required for all scientific research, which constitutes the common language of researchers and guarantees the objectivity, rigor, and verifiability of the approach; (2) to form a consistent experimental setting, from study conceptualization to data collection and coding, appropriate for the participants' characteristics; and (3) build on the person's ecological environment, in particular the cues allowing the person to be comfortable, safe, and secure, which contributes to the person's successful participation in and completion of the research.

Key words Profound intellectual and multiple disabilities, Methodological challenges, Olfactory abilities, Preferences, Participation in research

1 Introduction

Individuals with profound multiple and intellectual disabilities (PIMD) have in common profound intellectual impairments and profound motor disorders which considerably limit their functional and behavioral repertoire [1]. They often have to deal with medical complications and require support 24 h a day [2–5]. It is important not to give up involving individuals with PIMD in scientific research as these persons have the same right to benefit from scientific research findings as neurotypical people in order to guide educational, medical, and/or therapeutic action [6].

Moustafa Bensafi (ed.), *Basic Protocols on Emotions, Senses, and Foods*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_10,

1.1 Methodological Challenges of Research with People with PIMD

Involving individuals with PIMD in research raises serious methodological challenges and precautions to frame their participation and ensure their welfare [7]. The planning of the experimental procedure, as well as the experimental setting, requires special accommodations for a number of important reasons:

- The presence of severe to profound intellectual disability—a consensus estimates the developmental age of people with PIMD at a maximum of 24 months—generally goes hand in hand with the impossibility of relying on their explicit collaboration as they do not understand or respond to instructions. This mode of functioning means that the researcher must prioritize situations and/or observation conditions involving a minimum of prerequisites.
- The presence of motor deficits and tone fluctuations, the second main characteristic of people with PIMD, is likely to interfere with behavioral responses. In the absence of postural and tonic stability of the head, trunk, and/or body, the behavioral response of the person with PIMD may be affected and become difficult to interpret. It is essential to determine the most favorable postural conditions to minimize the tonic and motor fluctuations that could overlap with the response to the stimulus.
- Individuals with PIMD need to use a large part of their brain capacity to sit up and control head and eye movements and have to divide their attention between these various tasks at the same time as they must concentrate on a stimulus [8]. Research with individuals with PIMD therefore involves minimizing dual-task situations and conditions that require conscious effort on their part.
- The high prevalence of visual or auditory particularities in individuals with PIMD means that the modalities of contact must be adapted, for example how to inform the person of the researcher's presence, how to signal the beginning or end of the presentation of the stimuli, etc.
- It may also be difficult for the researcher to capture the interest of people with multiple disabilities or severe cerebral palsy, due to fatigability or to alertness fluctuations related to epileptic seizures or side effects of medication [9, 10]. The researcher should be careful not to push the person beyond a certain length of time, plan for breaks, and ask about the person's general condition before beginning the session.
- Communication in people with PIMD is primarily non-verbal. Most individuals also use idiosyncratic behavior, which complicates interpretation of their reactions. The coding procedure must take this double complexity into account.

- Finally, it is not feasible for individuals with PIMD to travel away from their home or school to attend a research session. Such a move will stress them, increase their postural tone, lose their reference points, and interfere with the care and educational measures they should be able to receive on a regular basis. The researcher must therefore plan to collect data in the participant's ecological environment and respectfully negotiate conditions that best suit the needs of the study.

1.2 Protocols for Measuring Olfaction in People with PIMD

Olfactory functioning involves various processes and skills both at the neurophysiological and at the cognitive levels. The three protocols proposed in this chapter target the assessment of olfactory discrimination ability, expression of olfactory preferences, and habituation to an odorant (habituation stimulus) including discrimination of another odorant not presented before (novel stimulus). As persons with PIMD are also hindered by visual and/or auditory impairments, the olfactory function, if intact, can serve as a compensatory modality. Studying discrimination abilities may help to understand whether people with PIMD are "aware", even implicitly, of olfactive stimuli around them. Studying their ability to express preferences gives valuable information about how they function hedonically, and whether certain stimuli elicit reactions close to or far from those of neurotypical people. Finally, as habituation is considered the simplest form of learning and a huge neuroadaptive mechanism, studying short-term habituation may help us to understand whether people with PIMD can rely on memory traces in their interaction with the environment, for instance whether they can use activity or food olfactory cues to interact with it.

2 Materials

2.1 Stimuli

2.1.1 Odorant Selection

Table 1 displays an example of a panel of stimuli that were selected in one of our past studies [11]. The choice of odorants depends on the needs and objectives of the study; however, selecting the stimuli in collaboration with the direct carers (i.e., teachers, support workers, therapists, or family members) may help to choose stimuli that make sense for the study population and limit those that do not correspond to their life experience because of their impairment (*see* **Note 1**). Do not omit to calibrate each stimulus intensity and check any useful characteristics—hedonicity, familiarity, categorial membership—if necessary (*see* **Note 2**).

2.1.2 Odorant Preparation

Fill 14 cm-long and 1.3 cm-diameter Burghart stick-like devices with your odors. Fill one further stick with an odorless solvent (N) (*see* **Note 3**). Make as many protective cylindrical rings (Fig. 1a) as the total number of odorous plus odorless sticks (*see* **Note 4**). Store each stick with its ring in a 1-L airtight glass jar (*see* **Note 5**).

Table 1
Characteristics of stimuli

Stimuli	Food / Non food	Hedonicity*	Perceived Intensity**		Children's odor familiarity***		Castro's categorical membership****	Concentration %
			M (min.–max.)	*SD*	*M*	*SD*		
Orange (Burghart)	F	P	5.00 (2–6)	0.92	0.48	0.48	C1	–
Nutella®	F	P	5.82 (4–7)	0.61	0.48	0.51	C7	5
Cinnamon	F	P	6.10 (5–9)	0.93	0.29	0.46	C7	10
Strawberry	F	P	6.00 (4–7)	0.80	0.62	0.49	C5	20
Cheese	F	U	5.07 (2–8)	1.33	0.81	0.40	C8	1
Garlic (Burghart)	F	U	6.45 (5–9)	0.91	0.38	0.49	C8	–
Green vegetables	F	N	5.85 (3–9)	1.13	0.86	0.35	C6	20
Rancid butter	F	N	5.52 (3–8)	1.09	0.05	0.22	C8	10
Apple pie	F	N	5.90 (4–8)	0.90	0.48	0.51	C7	20
Basil	F	N	6.04 (4–8)	0.92	0.38	0.49	C4	5
Lily of the valley	NF	P	5.36 (4–9)	1.02	0.13	0.35	C4	20
Summer rain	NF	P	3.59 (2–5)	0.93	0.48	0.51	C6	50
Rose (Burghart)	NF	P	5.68 (4–8)	0.81	0.20	0.41	C4	–
Hand sanitizer (Sterillium®)	NF	U	5.21 (2–8)	1.17	0.52	0.51	C2	–
Sweat	NF	U	5.66 (2–8)	1.44	0.33	0.48	C8	1
Swimming pool	NF	N	2.80 (1–6)	1.21	0.70	0.47	C2	1
Pony	NF	N	5.38 (4–7)	0.98	0.14	0.35	C8	5
Grass (Burghart)	NF	N	5.62 (1–8)	1.26	0.52	0.51	C6	–
Odorless solvent	NF	–	–	–	–	–	–	–

Legend: In this example, Hedonicity* was determined by neurotypical adults on a 3-level categorical scale: U = unpleasant; N = neither pleasant nor unpleasant; P = pleasant; perceived intensity** was determined by neurotypical adults on a 9-level Likert scale; children's odor familiarity*** was determined by their parents or legal guardians on a binary scale (0 = unfamiliar or not familiar at all; 1 = somewhat familiar or very familiar) and categorical membership of the stimuli**** in Castro et al. (2013) two-dimensional embedding of the descriptor space: C1: Citrus, etc.; C2: disinfectants, alcohol, chemical, etc.; C3: mint, camphor, etc. (not presented); C4: flowers, plants, aromatics, etc.; C5: fruits except citrus, etc.; C6: leaves, wood, grass, etc.; C7: honey, nuts, bakery, etc.; C8: apple, acid, putrid, rancid, garlic, etc.

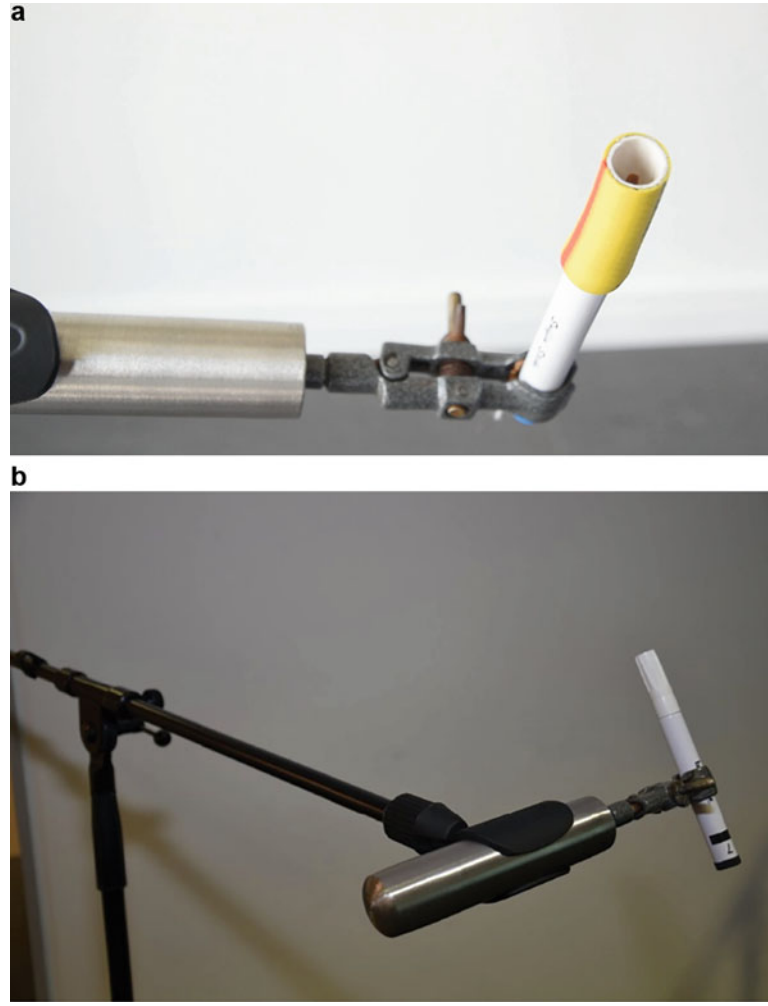

Fig. 1 (**a**) Clip holding a stick with its protective cylindrical ring. (**b**) Tripod with articulated arm and closed stick in the clip

2.2 Additional Material

- A tripod device: i.e., an adjustable microphone stand with a 70 cm-long articulated arm and a clip (Fig. 1b).
- Cotton gloves.
- One stopwatch.
- One black fabric screen with a 12.5 × 12.5 cm white grid.
- A transparent pouch containing numbered cards corresponding to the steps of the study and the odorants presented.
- A poster informing that the experiment is in progress.
- One photograph of the child and the photographs of the experimenters.
- Pictograms to present the task to the participant (*see* **Note 6**), a 45 × 60 cm whiteboard with magnets (Fig. 2).

Fig. 2 Whiteboard with the participant's and the experimenters' photographs, as well as pictograms used to present the unfolds of the session to the participant. The pictograms used in the whiteboard come from Boardmaker program (https:/goboardmaker.com/pages/picture-communication-symbols)

- A small bell to announce to the participant the beginning and end of each session.
- Two cameras, one for wide shots (the whole body of the child), one for close-ups (the face only).

3 Methods

The recommendations formulated below are drawn from our past experiences and studies with participants with PIMD [11, 12]. They aim to promote a respectful involvement of this population in research, while at the same time controlling the influence of confounding factors and increasing the rigor of an experimental design conducted in a natural context. Repeated-measures designs are generally preferred because of the challenges of constructing groups that can serve as controls for such a unique population. Following the above recommendations does not invalidate the need for a pilot study [6, 13].

3.1 Discrimination Protocol

This protocol aims to compare the participants' behavioral responses (e.g., 'head alignment on the stick', 'chewing', 'moving one's lips', and 'sniffing', etc.) in odorous versus odorless condition. It requires the presence of two researchers, one for the odor presentation, the other for filming and timing the stick presentations (i.e., beeping when the scent has to be presented or removed). The implementation takes place in a quiet room in the child's school or living space (*see* **Note 7**).

1. Before the child's arrival, prepare the odorous and the odorless sticks that you intend to present, as well as the gloves, the whiteboard and magnets, the tripod with the clamp, the photographs of the child and experimenters, the pictograms, the bell. Fix the black fabric screen on the wall in front of which the child will be seated in order to standardize the plane surface behind them, facilitate adjustment of the focus of the cameras, as well as the coding of behavioral data such as head displacements. Fix the pouch on the black screen in order to make the steps in the presentation of the stimuli visible. Insert in the pouch, in the planned order, the cards with the numbers of the odorants you intend to present. Remove potential sources of distraction in the room (*see* **Note 8**).
2. Set up and adjust the focus of the cameras. Prepare the necessary equipment to monitor the procedure (stopwatch, order of presentation of odorants and odorless stick, duration of presentation). Ventilate the room before the child's arrival.
3. Welcome the child and his or her teacher (*see* **Note 9**). Take information on the child's actual mood and health. If the child is not in their usual state of health (e.g., if he or she has a cold, is sleepy because of a seizure, etc.), postpone the session (*see* **Note 10**).
4. Make sure the child is comfortable in his/her seat (*see* **Note 11**). Briefly remind him or her of the context of the activity using the whiteboard and the magnets to fix the photographs and the pictograms, for instance "I, Juliane, have come to see you (show your photograph). Geneviève is also here (show the photograph of the other experimenter). I'm going to introduce you to some odors, you're going to have to use your nose". Ring the bell to mark the start of the data collection session (*see* **Note 12**).
5. Ensure the card with the number of the first odor to be presented is visible in the pouch, take the stick with the corresponding odor out of its jar, remove the cap, add the protective ring, fix the stick on the clip, and present the stick about 2 cm in front of the participant's nose, between their nose and chin (*see* **Note 13**) [14]. People with PIMD frequently have sudden drops in tone or, on the contrary, sudden hypertonic reactions, so be very attentive, and reactive in order to remove the stick in time, using the tripod stand. The same applies if the odorant makes the child want to put it in his or her mouth.
6. Ensure that the stick is presented in line with the child's nose. The child must be left completely free to turn his or her head away, stay aligned on the stick, or turn away and approach it again. Do not pursue the child with the stick.

7. Present the odorants successively in a randomized order (Hasard® software): N (Odorless), O_1 (Odor 1), N, O_2, N, ... O_6. Odorous sticks are presented for 15 s. In order to enable sufficient recovery, provide an interstimulus interval (ISI) of 30 s between each odorant during which the odorless stimulus is presented. If you plan to present more than six odors, conduct the experiment in spaced sessions in order to avoid a possible fatigue and olfactory saturation effect. Do not plan more than 5 min per session. Do not speak to the participant once the session starts and until it ends.
8. Use the tripod to bring the stimuli close to the participant's nose and prevent you from bringing your arm or hand too close to the child's face, which might prompt him or her to respond to your proximity, rather than to the odor. Using the tripod also prevents your hand from hiding the child's face, as well as preventing olfactory interference with your own body odors. Stand to the right or left of the child while presenting the stimuli and alternate your position from one session to another in order to avoid participant orientation bias.
9. Ring the bell to mark the end of the session of odor presentation, congratulate the child on his/her participation, and say goodbye to both the child and his or her teacher.
10. With respect to the coding step, use extra precautions to select behavioral indicators (*see* **Note 14**). Follow the available methodological recommendations to define the indicators and obtain the highest possible inter-rater agreement (*see* **Note 15**). If necessary, ask the participant's proxies to help you interpret the participants' idiosyncratic behaviors or behaviors you struggle to interpret (*see* **Note 16**). In case of doubt, consider coding a response as non-interpretable rather than over-coding it (*see* **Note 17**). See Fig. 3 for an example of indicators.

3.2 Preference Protocol

This protocol aims to compare the participants' behavioral responses to a pleasant (O_P) versus an unpleasant odorant (O_U).

1. Fill a first stick with an odor usually referred to as pleasant, a second stick with an unpleasant odor, and a third with an odorless solvent. The odorants selected should be close in intensity. Continue by following **steps 1–5** of the discrimination protocol.
2. Present the odorants successively in the following order N, O_P, N, O_U, N, O_P, N, O_U. The total assessment time for this session is 4 min per participant, the odorants and the odorless stimulus being presented successively for 30 s each. In this protocol, the time of exposure to the odor is longer than in the previous one because we wish to observe not only if the

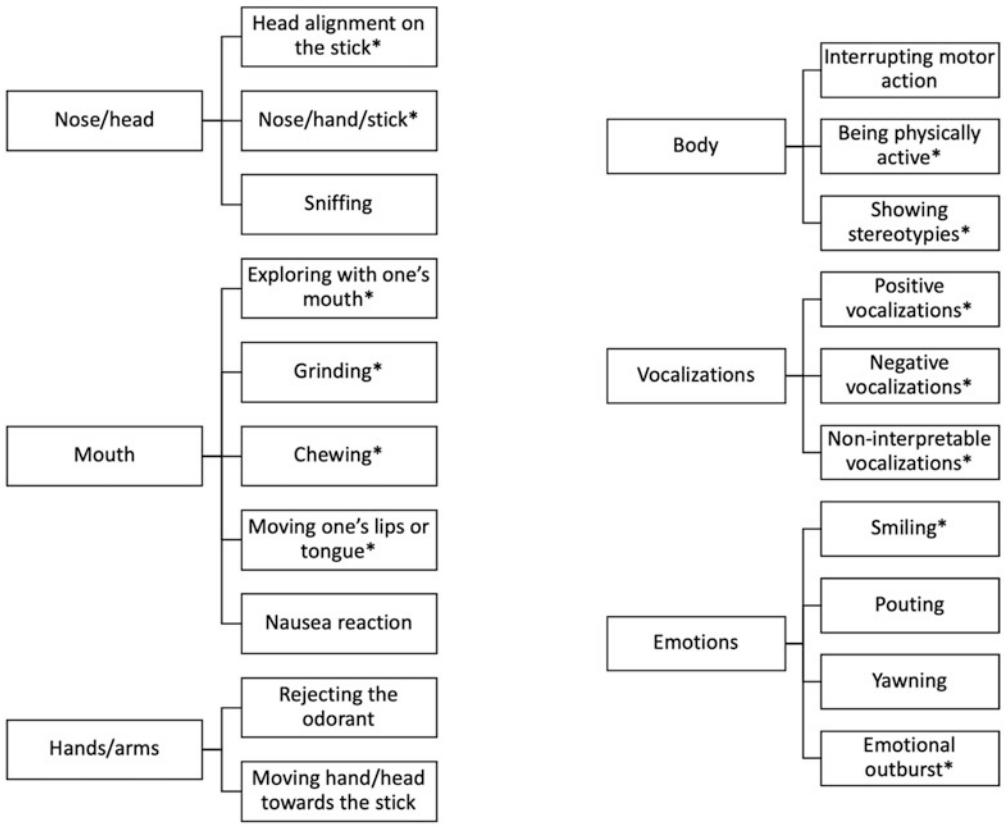

Fig. 3 Example of indicators. Their definition is available online ([11], supplement). Legend: Indicators without a star are coded on occurrences; indicators with a star (*) are coded on duration (ms)

child reacts to the odor but also how he or she copes emotionally with the odor. If you plan to present several pairs of odors, conduct the experiment in spaced sessions in order to avoid a possible fatigue and olfactory saturation effect. Do not speak to the participant once the session starts and until it ends.

3. Continue by following **steps 7–9** of the discrimination protocol.

3.3 Fixed-Trial Short-Term Habituation Protocol

This protocol aims to assess the ability to habituate to an odorant (O1) presented six times consecutively and then to discriminate a newly introduced odorant (O2). Fixed-trial short-term habituation protocol is considered the simplest habituation protocol because it is easy to automate and conduct and because the data generated are uniform for each participant and therefore easy to analyze. Two odors close in intensity are used: one is the habituation stimulus (O1), the second is the novel stimulus (O2). An odorless stimulus is presented during interstimulus intervals.

1. Fill a first stick with the habituation stimulus, a second with the novel stimulus, and a third with an odorless solvent. Continue by following **steps 1–5** of the discrimination protocol.
2. Present the odorants successively in the following order N, O_1, N, O_1, N, O_1, N, O_1, N, O_1, N, O_1, N, O_2. Odorants are presented for 30 s and the odorless solvent for 15 s. The total assessment time for this protocol is 5.25 min per participant. If you plan to collect the participants' habituation response through two different pairs of stimuli, which is methodologically recommended as it enhances the generalizability of the findings [15], conduct the experiment in spaced sessions in order to avoid a possible fatigue and olfactory saturation effect. Do not speak to the participant once the session starts and until it ends.
3. Continue by following **steps 7–9** of the discrimination protocol.

4 Notes

1. Literature found that familiarity and diversity in the children's olfactory environment are responsible for differences in the responses [16, 17]. Odorants such as Play Doh, bubblegum, or popcorn used with neurotypical children are not suitable for children with PIMD, who seldom play with Play Doh, or eat bubblegum or popcorn, because of their motor disabilities and risk of inhalation. Some odorants may also not be suitable because of differences in cultural habits (e.g., peanut butter is seldom eaten by Swiss children). Selecting the stimuli with the direct carers reduces the risk of choosing smells that are not part of the child's life. The assessment of olfactory response benefits from being carried out on a wide and diversified range of odors, choosing odorants covering the main dimensions of human odor descriptor space, for instance that identified by Castro et al. [18].
2. If you plan to create homemade odorants, be sure that all the ingredients used in the production of the stimuli meet the safety standards in terms of the regulations on cosmetic products. Do not omit to calibrate the intensity of your homemade odorants. Since the intensity calibration procedure cannot be carried out with people with PIMD who express only non-verbally and pre-symbolically, perform the calibration procedure with a population that shares certain characteristics with the study population, for example chronological age, as children usually rate odorants as being more intense and more pleasant than adults [19]. Consider including some odorants from a standardized battery in your homemade material in order to strengthen the external validity of the latter.

3. Most people with PIMD will not tolerate a cannula in their nose and they will not follow your instructions not to remove it/touch their nose, so presenting the stimuli in ambient air from a spatially localized source (i.e., a stick or a bottle) is a more realistic way of odor presentation compared to air delivered to the nose via an olfactometer. Stick devices offer the advantage of very accurate location and low ambient pollution.
4. The protective cylindrical ring is added at the head of the stick in order to prevent the child's skin or especially eyes from coming into contact with the soaked wick of the stick. The ring can be made from a garden hose cut into 5 cm sections. Each stick has its own protective ring. Both are stored in the same glass container to avoid mixing odors.
5. Stick-like devices offer the advantage of a very hermetic closing method which limits ambient olfactory "contamination" and makes them reusable. However, the sticks can nevertheless release a small amount of odor from one data collection session to another, so long-term storage in glass bottles with cork lids is recommended to prevent leakage.
6. The pictograms used in the whiteboard come from Boar dmaker.com. However, it is not the only database that offers pictograms. A large selection of pictograms is for instance available free of charge on the website of the Government of Aragon (Spain): https://arasaac.org/. Ask about the pictograms used by the participants, if they used them.
7. The chances of observing the participants' usual behavior are increased if the experiment takes place in their daily environment. However, the natural environment can present a lot of interference, which is why a dedicated room in the school or institution, enabling a minimum of control over competing stimuli, is required. Ask for a quiet, easily ventilated room, as far as possible from the kitchen and/or places that may generate odors. Post a notice on the door so as not to be interrupted during the experiment. Do not wear perfume on the day you collect the data.
8. Some children have atypical interests. Some are, for instance, attracted by the light coming from outside on sunny days. If possible, ask for a room with few sources of distraction or make the adjustments required (draw the curtains, lower the blinds, hide the door handle under a cloth, if the child's eyes are drawn to that object, etc.) in order to limit competing stimuli. Meet every participant before the data collection in order to check if the conditions of the planned setting are compatible with the way they behave (move/express). If they are not, consider adapting the setting.

9. The presence of a direct support worker who knows the participant well is necessary to help the researchers set the child up, inform them if the child is in a stable behavioral and/or health condition for the experiment, and intervene if the participant has an epileptic seizure. This person will be instructed to remain silent and motionless during the presentation of the odorants, but asked to interrupt the session if the participant shows any signs or discomfort.
10. If the child is not in their usual state of health, the session must be postponed. Consider permanently discontinuing a child's participation in the research if the child shows discomfort after two sessions of odor exposure. The discomfort can be witnessed by the teacher or support staff attending the session and/or by the researchers themselves in agreement with the teacher. The participant's idiosyncratic distress behaviors must be particularly monitored, as well as the following behaviors: crying, whining, gag reflexes, massive increase in stereotypies. Written commitment must be given prior to the research. Moreover, some people with PIMD are prone to oral-pharyngeal congestion due to poor muscle coordination, spinal abnormalities, and/or digestive problems. As causes of olfactory loss often include sinonasal disease or upper respiratory tract infections [20], the exclusion of participants with chronic airway problems is recommended**.** The care offered in their case is also often incompatible with the presentation of stimuli involving the utilization of the oro-rhino-pharyngeal sphere. With regard to allergic risks, odorants usually do not cause allergic reactions per se [21] and there is no reason to believe that children and young people with PIMD are more prone to allergies than neurotypical children; however, the precautionary principle is necessary with this very vulnerable population and our advice is to avoid recruiting children known to have this type of disorder. In the event of a nausea reflex during the presentation of an odorant, remove it immediately; present it again during a future session, and if there is a nausea reflex again, remove this odorant from the material used with this child.
11. The entire perceptual-motor response chain is influenced by postural and tonic conditions. Behavioral responses, even when held, such as slight head movement, can be maximized or, on the contrary hindered, by postural conditions. Ask about the type of personal seat used by each participant. Be sure that the seat allows freedom of movement. If necessary, establish a better postural adaptation with the children's physio- and/or occupational therapists, i.e., headrest or support behind the neck. Test these adaptations before the experiment takes place.

12. It is not always possible to know what individuals with PIMD understand about the world around them. However, introducing certain regularities into the environment may help them to relate and anticipate what is happening to them. We recommend using a ritual to notify the beginning and end of the presentation of stimuli. In our research, we began by reminding the child that we were there to see how they use their nose, who was there, and that we were going to present the first activity, then take a break and then present a second activity (Fig. 2). The sound of the bell is used to announce the beginning and the end of the session.
13. The tripod is very suitable for children with significant motor impairments in manipulating objects. For people with better manipulative skills, the difficulty is that they try to take hold of the pliers or stick.
14. The choice of behavioral indicators must rely on the specific aim of the study and on the behavioral possibilities of people with PIMD. In studies involving people with PIMD, the indicators can be based on the following criteria: (1) the indicator has been described in the scientific literature after observation of the non-verbal reactions of neurotypical babies to odors, and it belongs to the register of behaviors of people with PIMD (i.e., head alignment on the stick, sniffing, smiling, pouting, chewing, moving one's lips/tongue, nausea reaction, vocalizations); (2) the indicator is a non-verbal behavior observed in neurotypical children when they are exposed to alimentary/non alimentary, pleasant/unpleasant odors, and it belongs to the repertoire of PIMD individuals, but not to that of infants because of their motor immaturity (nose/hand coordination on the stick, rejecting the odorant); and finally (3) the indicator expresses the overall reactions of the person with PIMD to a task or stimulus: fatigue/boredom, alert/active, stressed or disengaged states (yawning, interrupting motor action, being physically active, showing stereotypies, grinding, emotional outburst).
15. With participants who have poor communication skills or are difficult to understand, it is especially important to develop an extensive scoring manual and provide coders with intensive training to improve inter-observer agreement and facilitate the coding decision in situations where the behavior is ambiguous [6]. Using a two-level grid can help, with the operational definition of each indicator as well as behaviors that should not be coded being specified in the first level and, if necessary, additional information for participants who express themselves in an idiosyncratic way specified in the second level. The operational definition benefits from being accompanied by a video illustrating both the targeted or excluded behaviors.

16. It happens that despite the precautions taken, some participants show behaviors that are difficult to interpret for a person who does not know them. In these situations, the opinion of a proxy can sometimes remove doubt.
17. Over-coding a behavior which is difficult to interpret is not a good idea, nor is under-coding, because it biases the results and over- or undervalues the person's abilities. Coding a behavior as non-interpretable when its valence is not inferable or interpretable is a better solution. This category gives more reliability/validity to the coding without ignoring the behavioral manifestation.

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Chapter 11

Measuring Hedonic Behaviors to Food Odors in Children

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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 the analysis of motor behavior in response to 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.

Key words 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 spoliation 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

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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 3–17 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 Materials

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 six food olfactory stimuli are used: two monomolecular odorants and four 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, 5 mL of odorous solutions is 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 s. Then, put a porous polypropylene absorbent paper (3 cm × 7 cm; 3 M, 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 four 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 (Fig. 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 (1280 × 720 pixels) is used to record participant exploratory behavior (24 images/s). The camera is placed at 1 m on the side of the participants, in order to see the participant's profile (*see* **Note 4**).

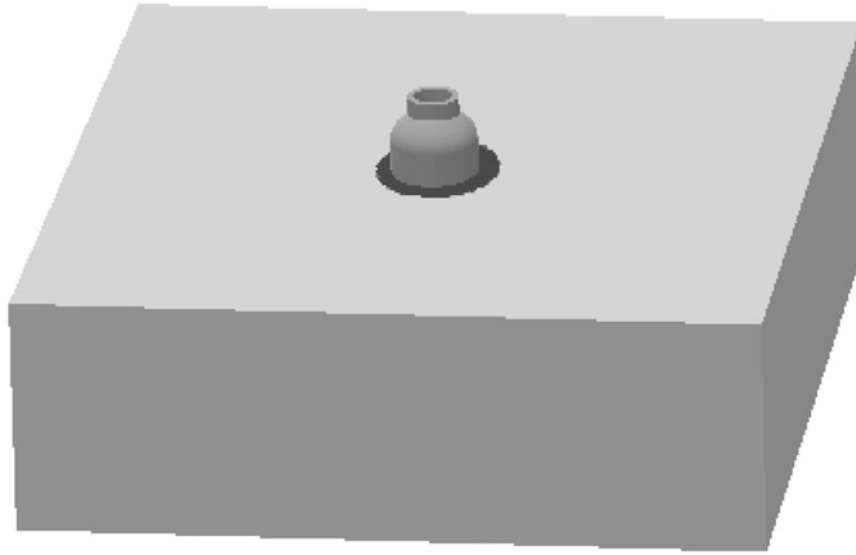

Fig. 1 Cardboard box holding the odors

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 two 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) (Fig. 2).
3. Odor identification task (optional): a series of images is presented to the children. For each odor, four images are proposed: one of the pictures represents the odor source, and three pictures represent distractors.

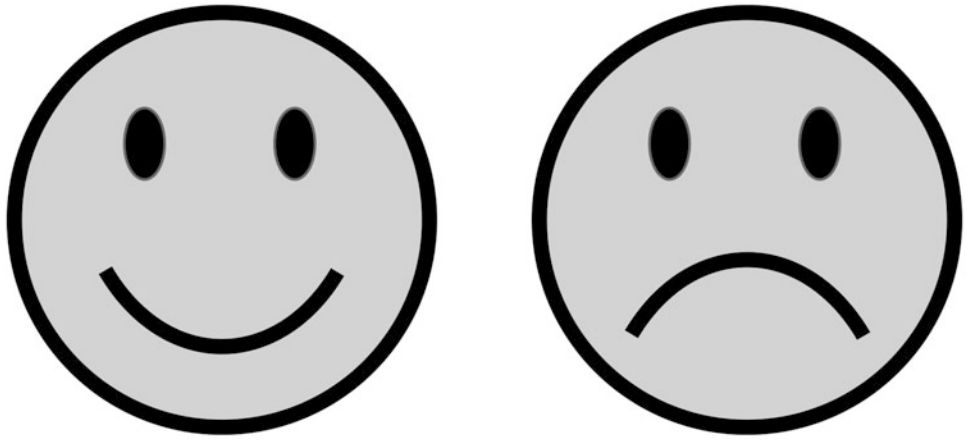

Fig. 2 Pictogram to answer the yes/no questions. The green pictogram for means a "yes" answer, and the red one means a "no" answer

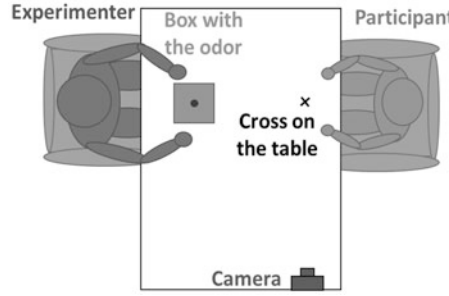

Fig. 3 Setting up the material and the participant

3 Methods

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 is 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 is 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 (Fig. 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 s to smell the odor and can smell the odor several times in the time window. After 10 s, 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 s, 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 s, 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. Afterward, or after 1 min 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 s to the child.
15. Present a new odorant, the first one of the six 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 six 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 four 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 min (*see* **Note 11**).
21. Allow 30 s 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-s 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).

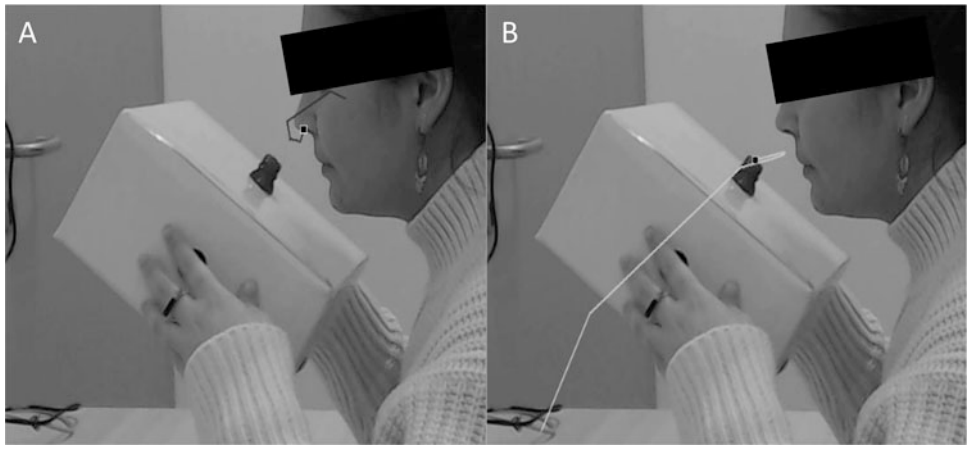

Fig. 4 Drawing of trajectories of the nose (**a**) and the flask (**b**)

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 (40 ms between each image). Click on the top of the nose again. Repeat it until the participant approaches and withdraws from the flask (Fig. 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 5 cm) 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

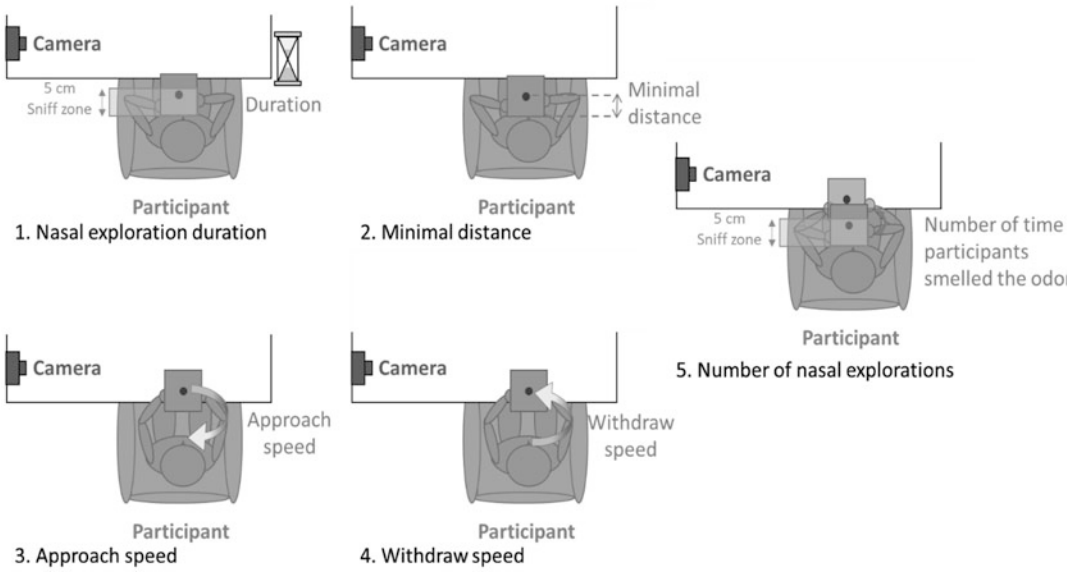

Fig. 5 Extracted behavioral variables from video analysis

distance between participant's nose and the flask, (3) average approach speed from the nose toward the flask, (4) average withdraw speed of the nose from the flask, and (5) the number of nasal explorations (i.e., number of time participants smelled the odor at less than 5 cm of the nose) (*see* Fig. 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 (Fig. 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 (Fig. 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, and naturally smelling objects), but take care to standardize the perceived

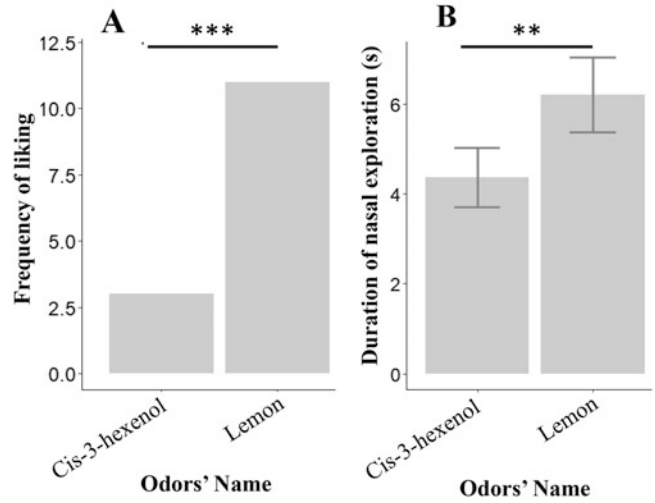

Fig. 6 Example of results from declarative data and behavioral data. Results from declarative data (**a**) show that the odor of lemon was liked by more participants ($N = 12$) than the cis-3-hexenol (liked by respectively 92% and 42% of participants, $\chi^2(1) = 11.0$, $p < 0.001$), and the results of the duration of nasal exploration (**b**) show that the lemon was also explored significantly longer that the cis-3-hexenol (respectively, $M = 6.2 \pm 2.9$ and $M = 4.4 \pm 2.3$, $t\,[11] = -3.85$, $p = 0.003$)

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 two-fold: (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 autism spectrum disorder (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 (e.g., 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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Chapter 12

Modulation of Visual Perception by Odors

Jonas Yde Junge, Surabhi Bhutani, and Valentina Parma

Abstract

Olfaction and vision are both senses that impact our pre-consumption perception and intake of food and beverages. As compared to the visual system, the olfactory system has preferential access to cortical and subcortical areas involved in affective processing, altering behavior following the emotional experience. Most of the studies linking visual, olfactory, and affective information have been conducted in the lab (e.g., affective priming), sometimes questioning the ecological validity of the findings. Olfactory affective matching is a paradigm based on which an affective stimulus (for instance, a food odor) evokes an emotion that influences responses to a stimulus presented subsequently (for instance, an object). Here, we describe an olfactory affective matching protocol originally developed to test how children match odors with social visual information to assess odor-induced behavioral choices. This protocol was devised to be deployed in a citizen science setting, yet it can be used to explore affective food experiences with small adaptations in the laboratory and for other age groups.

Key words Smell, Food odors, Visuo-olfactory matching, Affective matching, Behavioral choice, Citizen science

1 Introduction

Several studies have shown that odors alert us to food in our environment and orient our appetite [1, 2]. Like vision, olfaction is considered a *distant sense* in that the processing of food (or other stimuli) may occur before direct contact with such stimulus, including visual [3, 4], tactile [4], auditory [5], and gustatory perception [6], and influence action [7] and decision making, including food choice [8]. As an example, adults experiencing a fruity odor (e.g., melon) non-consciously process congruent words (e.g., "melon") faster and choose congruent food items (e.g., fruity desserts) more often [9]. Odors, by virtue of their preferential neural connections

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with the limbic system [10], also elicit strong affective responses [11]. In other words, the valence of an odor, i.e., whether it is judged to be pleasant or unpleasant, can strongly modulate the processing speed of behavioral responses [12]. For example, Hermans and colleagues showed that presenting adults with pleasant or unpleasant odors can regulate the speed at which subsequent words are identified based on their affective value [13]. Specifically, participants identified 'positive' words more quickly when an odor prime was perceived as pleasant vs. unpleasant [13]. Additionally, shoppers spent more money when the smell inside the store was perceived as pleasant than when the air was unscented [14]. In the realm of social information processing, facial expressions—a basic dimension of social communication relevant throughout development [15]—are strongly influenced by the presence of contextual olfactory information [16–21]. In a study by Demattè and colleagues, adults judged male faces presented with an unpleasant odor as less attractive than when the same faces were presented with a pleasant odor or clean air [16]. Similar effects are observed in studies where a linear modulation in the valence of the odor results in linear changes in facial attractiveness ratings [22]. Also, unpleasant ambient smells lead to negative judgments and dislike toward members of minority groups [23].

Overall, the findings summarized above imply that both pleasant and unpleasant odors modulate the processing of visual information, and induce affective reactions to influence people's decisions and regulate behavior toward various stimuli. In particular, face processing appears to be sensitive to olfactory contexts, both when experienced overtly and covertly [17–21]. Critically, this body of evidence has primarily been collected in adults, and how olfaction and its emotional influences develop during childhood and affect choices is under researched. To fill this gap, below we describe a protocol that gets inspiration from the olfactory priming literature to investigate visuo-olfactory affective matching in a large sample of children aged 3–11 years [24]. This affective olfactory matching paradigm uses one of three odors categorized as either pleasant (non-food odor, rose), unpleasant (food odor, fish), or neutral (no odor), to bias the choice of one of two facial expressions (i.e., happy or disgusted). Importantly, this protocol implements citizen science methods, such as conducting research studies in public areas (*see* **Note 1**). In the notes, we highlight ways in which the protocol can be adapted to meet other experimental needs including to explore affective food experiences in a more ecological fashion (i.e., testing adults, using odor delivery devices that allow a covert odor stimulation, choosing different dependent variables).

2 Materials

Below, we describe the materials and procedures used by Cavazzana et al. [24] as an example of overt visuo-olfactory affective matching in children. The protocol has been developed to be deployed at a children's museum as part of a citizen science effort (*see* **Note 2**) [25].

2.1 Computer and Software

For practical use in the context of our citizen science experimental setting (i.e., children's museum), the visual stimuli are presented full screen on an iPad, which run an automatically-timed PowerPoint presentation, allowing for the control of the presentation of the visual stimulation. To facilitate data collection in this context, the experimenter records throughout the session the responses of the participant via a paper and pencil method (*see* **Note 3**).

2.2 Olfactory Affective Matching Task

Below, we report on the sensory stimuli included in this paradigm.

1. *Olfactory Stimuli*: Several are the odor delivery options available to implement an olfactory affective matching task (*see* **Note 4**). To prioritize use in a citizen science-friendly experimental setup, we rely on commercially-available odor delivery devices, the Sniffin' Sticks (Burghart Instruments, Wedel, Germany). The Sniffin' Sticks are odorous pens commonly used to test olfactory function in adults [26] and children [27, 28]. These pens include odors of common household items, such as rose and fish for odor identification test. The two odors are chosen as typical pleasant and unpleasant odor, respectively, following a pilot study including 31 participants. A blank pen acquired from the same producer is included and it was consistently rated as neutral to slightly unpleasant (*see* **Note 5**). The three pens look alike and cannot be visually identified by the participants. Each participant completes 18 experimental trials with each odor pen presented a total of 6 times in a random order.
2. *Visual Stimuli*: The odors in this procedure are presented before the appearance of the visual stimuli, which are human faces expressing happiness or disgust (*see* Fig. 1). Twenty Caucasian models (10 women and 10 men) from the Karolinska Directed Emotional Faces standardized database are used [29] (*see* **Note 6**). The happiness and disgust expressions are portrayed in a straight viewing angle and are chosen from the same individual. Across trials, 20 faces are used (10 male, 10 female, two are part of the training phase). To help participants focus on the emotional expressions, and not on distracting features (i.e., hair, neck, background), we crop the pictures with Adobe 10.1.12 [29]. For consistency across visual stimuli, each face is

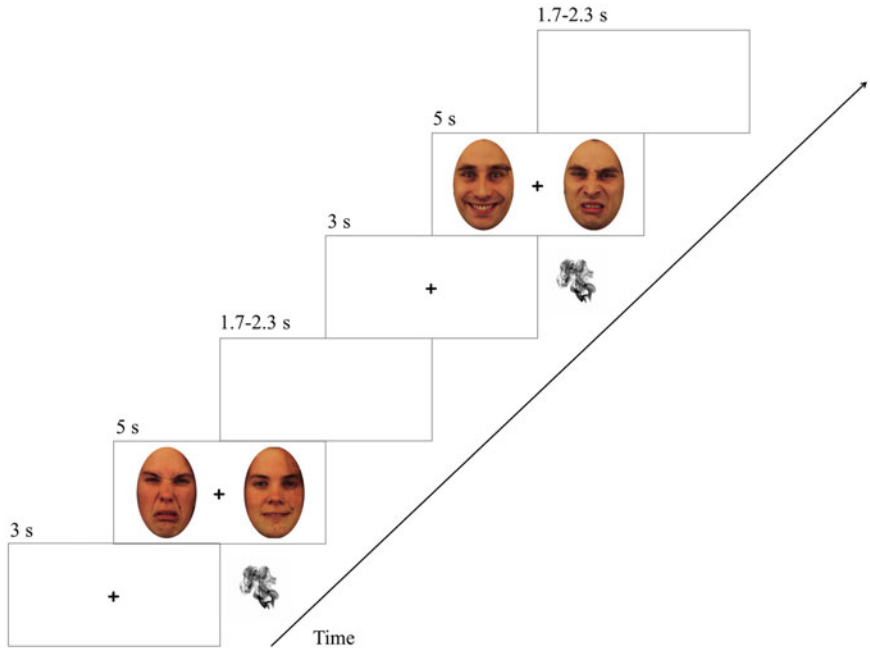

Fig. 1 Schematic example of the experimental procedure. An odor was presented for 3 s, while a fixation cross appeared at the center of the screen. Subsequently, two, side-by-side facial stimuli were presented for 5 s and followed by a blank screen

framed by an oval-shaped window (7.3 cm wide, 10.16 cm tall) through which only the forehead, eyes, nose, cheeks, mouth, and chin were visible (*see* Fig. 1). A pair of facial expressions (one cropped happy face and one cropped disgusted face) belonging to the same individual are displayed simultaneously, side by side on the screen. A black cross is shown in the middle of the screen, at equal distance from each face.

2.3 Olfactory Pleasantness

An olfactory pleasantness test is conducted to assess the pleasantness of the odors presented via a 3 facial hedonic scale measuring 10 cm in length when printed on paper, or shown on the iPad. The left anchor of the scale shows an emoticon with a disgusted expression; the right anchor of the scale shows an emoticon with a happy expression. At the center of the scale (5 cm mark), a neutral emoticon is shown (*see* **Note 7**). After presenting each odor, participants are prompted to rate the pleasantness of the odor on the given scale by marking their rating on the iPad screen with their finger.

2.4 Olfactory Skill Assessment

To evaluate olfactory function, participants are asked to complete a Sniffin' Sticks-based 4-alternative forced-choice odor identification test specifically designed for children [30]. The odors presented via the Sniffin' Sticks pens are as follows: orange, leather, cinnamon, peppermint, banana, lemon, liquorice, garlic, coffee, cloves, pineapple, aniseed, rose, and fish [30] (*see* **Note 7**).

2.5 Personal Information

With the help of their parents, participants provide on a paper and pencil/iPad questionnaire information on their date of birth, sex (i.e., male, female), ethnicity (i.e., Hispanic or not), race (i.e., American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, Other, Decline to answer), handedness (i.e., right, left, ambidextrous, decline to answer), health status (i.e., Indicate ongoing medical diagnosis), and self-proclaimed integrity of their sense of smell (i.e., "Can you (your child) smell?". Answers: yes, no, other).

2.6 Token of Appreciation

Stickers with cartoon characters, animals, or natural elements are presented to the child for them to choose one as a token of appreciation for their participation. We sourced them from local stores to ensure that participants would appreciate them.

3 Methods

Below we report a step-by-step account of the procedures in Cavazzana et al. [24].

3.1 Experimental Session

(a) *Consent*: The investigators obtain the consent from the parent and the assent from the child. When a child has agreed to participate in the study, parents are asked to read and sign the written informed consent form before any other experimental activities take place.

(b) *Instructions to the Participants*: Participants are told that they will be asked to play a few odor games and will answer some questions with the help of their parents. They are informed to keep their eyes focused on the screen for the duration of the experiment. The experiment starts with a white blank screen with a black cross in the middle, followed by the appearance of a green cross. When the cross turns green, the experimenter will present an odor via an odor pen positioned approximately 2 cm under their nostrils. They are asked to breathe normally to the odor, and not move their head to avoid the tip of the odorous pen touching their face (*see* **Note 8**). Then, the participants are instructed to "*choose one of the two faces on the screen"* by pointing at it and the experimenter records the response. No instruction regarding the relation between the odors and the faces, nor the valence of either stimulus is offered. Additionally, no feedback is offered about response accuracy (*see* **Note 9**).

(c) *Trial Structure*: The experimenter controls the start of the experiment to ensure the participant's readiness to initiate the task, as well as the distance from the screen (~30 cm) and alignment with the participant's body midline. Once

ready, the following trial structure is repeatedly executed: baseline period, odor exposure, visual exposure, response recording (see **Note 10**).

(d) *Training Task*: Our study included a training session of two trials for the participant to familiarize with the task and for the experimenter to make sure that the participant understood the task. The order of events is as follows:

I. The experimenter begins the training session, and a white slide with a black cross at the center of it appears on the iPad screen.

II. When the black cross turns into a green cross, the experimenter brings an odor pen under the participant's nose for 3 s (*see* **Note 11**).

III. A slide with two faces, one on the right and one on the left side, equidistant to the black cross, appears and stays on the screen for 5 s. The participant has to point within that time frame at the face they chose. The choice is noted by the experimenter on the recording sheet (*see* **Note 12**).

IV. The screen turns white again, which is the signal for the experimenter to remove the odor pen and cap it.

V. Following a variable time of 1.7–2.3 s, the slide automatically advances and presents a new trial.

VI. Steps *I* to *V* are repeated for the next and last trial.

VII. The experimenter asks the participants whether they have any questions. If no questions arise, the experimenter asks the participant to repeat the instructions of the task. When the instructions are correctly repeated, the experimental phase starts.

Experiment

I. *Task*: For the experiment task, **steps I–V** described for the training protocol are performed 18 times, corresponding to 18 experimental trials (see Fig. 1). As such, each odor pen (pleasant odor, unpleasant odor, neutral odor) is presented six times in a pseudo-randomized sequence, followed by presentation of human faces expressing happiness or disgust side by side on the iPad screen. The participant has to point at the face they chose without having any knowledge of the context of the experiment.

II. *Olfactory Pleasantness Ratings*: Participants are asked to smell one of the three odors one at a time in a randomized order and to rate on a visual analog facial hedonic scale how much they like each odor. The scale is presented on the iPad screen and participants mark their rating on the screen with their finger. When the scale is presented on paper,

participants mark their rating with a pen/pencil on the paper sheet.

III. *Olfactory Skill Assessment*: Participants completed 4-alternative forced-choice odor identification test specifically designed for children [30] which uses similar odorous pens as the ones used for the task at hand (*see* **Note 13**). The visual/verbal alternatives are printed in color on A4 sheets which are plasticized to preserve the integrity of the stimuli.

IV. *Personal Information*: Participants, helped by their parents, are asked to report their demographics and health status (*see* **Note 14**).

V. *Token of Appreciation*: At the completion of the whole experimental session, the experimenter thanks the participants for their participation and provides stickers as a token of appreciation for their effort.

3.2 Index Calculation

(a) *Primary Dependent Measure*: The number of times that participants choose happy vs. disgusted faces under each odor condition (rose, fish, and blank odor) is the dependent measure (*see* **Note 15**). The valence of the odor presented directly before the face selection task biases children to choose the affectively congruent face. The likelihood of choosing a disgusted face increases incrementally as a function of exposure to a disgusting odor (aka fish odor for most, but not exclusively).

4 Notes

1. Citizen science is a research trend that has been flourishing suggestion: in recent years, along with the evolution of science communication models from the classical top-down deficit model (e.g., the scientist teaches) to the dialogue and participatory models (e.g., scientists and citizens co-participate in research; [31]). On the one hand, the benefits of this type of research for scientists are the creation of large-scale databases, increased sampling from the general population, and, as a result, better scientific predictions [32]. On the other hand, direct engagement in science also benefits citizens, who are exposed to a specific research question, engage in a scientific method, and can track the results of their participation in the experimental process. These results are achieved in an environment which showcases the enthusiasm of scientists for their research topic and promotes a rewarding experience for citizens, thereby increasing the possibility of participating (repeatedly) in scientific experiences, as they are perceived as positive events. The National Science Foundation promotes the

interaction between research institutions and museums to bring research experiences to the community (https://www.nsf.gov/news/special_reports/science_nation/livinglab.jsp) and the Cavazzana et al. [24] inspiring this protocol has been supported through that mechanism (*see* **Note 2**). Including these aspects of citizen science, however, is also aligned with some of the benefits of field experiments, which primarily revolve around the improvement of external validity, as field experiments allow the phenomenon under investigation to operate under more realistic conditions.

2. This experiment was run in a collaboration with the Please Touch Museum of Philadelphia, thanks to a grant awarded by the National Living Lab Initiative developed by the Boston Museum of Science [33]. In contrast to a traditional laboratory experience, this project was run in a real-life context with a user-centric approach. Given that the duration and degree of participation are open for definition by its participants, the investigators have simplified the task, only included equipment familiar to children of diverse backgrounds, as those in regular attendance at the Please Touch Museum, and adapted the setting to perform the experiment in a quiet corner of a well-ventilated exhibition room. iPads and odorous pens were positioned on children-size tables and chairs, which served as testing stations. The testing happened in a reserved and shielded area within a large room where the researchers table was the main activity showcased.
3. The choice of the dependent measures was done with two principles in mind: resistance to noise and reliability. Although technology (e.g., eye-tracking glasses and smartphone apps for gaze localization [34]) has now evolved to allow for the deployment of an experimental setup that allows for the collection of more fine-grained measures (i.e., fixations measured via eye-tracking devices), such setup was not available at the time of our data collection unless bulky and/or delicate equipment would be used. In alignment with manually coded, reliable behavioral data permeating the developmental literature [35–37], we used an overt alternative forced choice (i.e., choice by indication of the face or of one of the visual/verbal options proposed in an odor identification task) and ratings on visual analog scales as dependent variables.
4. Olfactory affective paradigms can rely on either implicit or explicit odor presentation. Implicit odor delivery requires the odor to be presented covertly, without the participant noticing the olfactory stimulation. This is most often done through ambient odor delivery, where the odor is diffused in a room where the participant conducts the behavioral task [38, 39]. When choosing explicit odor delivery methods, like

the one used here, participants are aware that an odor will be presented. Overt odor delivery often capitalizes on standardized odor stimuli and devices used to assess olfactory function [40], such as the Sniffin' Sticks [27], chosen for this study, or can use self-prepared stimuli presented in jars or bottles. For customized olfactory stimuli, the suprathreshold concentrations should be defined with a pilot study with participants from the same population. To improve the timing of odor delivery and synchronize it with the presentation of other stimuli, a computer-controlled odor delivery device, such as an olfactometer [41], is the most appropriate choice. Portable [42] and now highly portable devices [43] will be critical to bring millisecond-precision in odor delivery within citizen science settings.

5. Many odors, and particularly food odors, vary in familiarity and valence across demographic groups [27, 44, 45]. Therefore, it is necessary not to assume the default pleasantness of an odor, but to pilot the odor valence of the chosen stimuli in an independent group of pilot participants who are selected from the targeted population and who will not participate in the main experiment. To account for individual differences within the sample under study, we advise to use individual odor valence as a covariate in the analysis as in Cavazzana et al. [24].
6. The study of facial expressions is widespread. As a result, multiple databases are available with facial stimuli portraying emotional expressions [46]. Considerations on the choice of the best database for a specific study should include the following: (i) the type of expressions portrayed, (ii) the demographic features of the actors posing and the relationship with the participants' demographic features [47], (iii) the 2D or 3D nature of the images [48], (iv) the resolution of the images, (v) spontaneous [47, 48] vs. posed expressions, and (vi) for posed expressions, number of actors across the needed facial expressions to allow for trial counterbalancing.
7. Most assessment methods for olfactory function in children are directly derived from olfactory tests for adults. Here, we have used psychophysical methods that have been developed and validated in children, specifically from age 3 and that likely reflect changes in the sensory properties of the experience rather than odor learning [49–52]. The use of a 3-point facial hedonic scale [53–55] has been chosen for its wide use in investigating chemosensory judgments in the age population tested. Given that the olfactory stimuli proposed were hypothesized to be pleasant, unpleasant, and neutral, the 3-point hedonic scale matched the possibility to depict accurate odor valence judgments, while maintaining ease and speed of use [56].

8. In the testing script, we incorporate age-appropriate instructions to remark this concept. We ask children to "stay as still as statues" when the odor is presented and "breathe like you normally do, without taking big breaths." We ask children to practice before initiating the experimental task and ensure that they comply with the instructions.
9. Experimenters should provide neutral instructions (i.e., "pick one [face]!") and should not provide feedback on the correctness of any responses, even if asked. Instructions ought to be neutral and clear to avoid social desirability effects [55]. Participants are rewarded with acknowledging their central participation in this scientific experiment and by receiving a token of appreciation.
10. The length of the experimental session is defined based on the ability of the youngest group (children of age 3) to engage and complete the task. Based on a previous pilot study, our task included 18 randomized trials, equally divided among pleasant, unpleasant, and no-odor ("blank") primes (6 times each). The trials are presented in a pseudo-randomized sequence, which preserves participants from habituating to the same odor [57]. Piloting the full experiment and debriefing participants at the end allows investigators to determine the rate of habituation to the odors at the concentration presented.
11. Odor delivery via the Sniffin' Sticks pens follows the instructions of administration of the Universal Sniff Test [30]. Each odor was presented one at a time. The cap of a pen is removed just before presentation and the uncapped pen is held under the nose of the participant for 3 s. The pen should be close enough to the participant's nose that it can be smelled and at the same time at a sufficient distance to be sure not to hit the nose or lip, and contaminate the pen (~2 cm). Importantly, the pen can be moved three times from one nostril to the other to allow for the odorous volatiles to reach the olfactory epithelium more easily, and to time an odor presentation lasting 3 s. To reduce odor contamination in the experimental area, the cap of the open pen should be repositioned on the pen quickly at the end of the 3-s odor stimulation.
12. Participants who fail to provide a response and select a face in 5 s, or fail to comply with other aspects of the training, are asked to repeat the training session one additional time. It is important to note that in a citizen science setting all participants who are interested in the science experience have access to it, even if they fail to comply to complete the training session multiple times. Collection of partial or unreliable data is noted by the experimenter in the recording sheet and excluded post hoc.

13. A prerequisite for assessing the accuracy of a task including odors is to verify that participants have a functional sense of smell. In children, the majority of the assessments includes an odor identification test done with a test using odorous pens (derived from the Sniffin' Sticks [58]), such as in this case the Universal Sniff Test [28], or using microencapsulated "scratch-and-sniff" odors (derived from the UPSIT [59]), such as the Smell Wheel [60] or the NIH Toolbox Odor Identification test [61]. More recently, the SCENTinel test [62] has been piloted in children age 7 and above [63]. By leveraging Lift'n' Smell patches placed on a card, the SCENTinel test represents the most rapid and inexpensive way to assess multiple olfactory skills via self-administration.
14. Personal information is collected to serve as a control in the data analysis. To allow the data collection to occur in a citizen science setting (i.e., at a high-volume science museum for children), we collected data using a paper and pencil questionnaire, which allowed for testing multiple participants simultaneously. However, an online survey accessed through a QR code should be preferred, if it is available.
15. To quantify possible biases that confound the analysis of the dependent variable, we controlled for position bias and gender bias. To control for position bias, we compare the number of times that each face is chosen in one position as compared to the handedness of the participant. In other words, we anticipate that if a position bias is present, right-handed participants would point more often to the face on the right side of the screen, irrespective of the valence of the facial expression. To control for gender bias, we compare the number of times that the face matching a participant's gender is chosen. In other words, we anticipate that if a gender bias is present, participants would point more often to the face sharing their gender, irrespective of the valence of the facial expression.

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Chapter 13

Characterizing Emotional Response to Olfactory, Auditory, and Visual Stimulations in a Virtual Reality Environment

Lucile Rey, Clément Désoche, Marc Thévenet, Samuel Garcia, Barbara Tillmann, and Jane Plailly

Abstract

Food stimuli are multisensorial. Eating a particular type of food is associated with a specific sound, odor, taste, color, form, texture, etc., that allows people to enjoy the diversity of the meals. The understanding of consumers' emotional experiences requires the study of each sensory component, isolated from each other's or combined, in different contexts and conditions. Virtual reality recent development offers a unique tool allowing the complete and highly controlled study of human sensory experiences. It bridges the gap between real-life and laboratory investigations and provides rich and adjustable environments that can be combined with the increasing number of methods measuring emotional processes. The protocol presented here was developed to investigate implicit behavioral and physiological emotional responses to emotional olfactory, auditory, and visual stimuli delivered in a rich virtual environment. It was developed without any reference to food consumption behavior but can be easily transferable to all kinds of stimuli.

Key words Breathing, Electrodermal response, Emotion, Cardiac activity, Multisensory, Odor, Virtual Reality

1 Introduction

The method developed in this chapter aims to investigate emotional responses to olfactory, auditory, and visual stimuli in an ecological and fully controlled virtual reality (VR) environment. It consists in an association between a VR software, a device allowing rigorous olfactory stimulation called "an olfactometer," and a physiological recording belt giving access to physiological emotional processing. Albeit this method was conceived for fundamental research, it could inspire industry players or laboratory working in the scope of food science, olfactory design, or cosmetology to test the emotional response to environmental or object-specific odor (*see* **Note 1**).

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All stimuli we meet in everyday life are embedded in a rich, multisensory environment that influences the perception and emotions evoked by these stimuli (i.e., [1]). To control for these biases, the majority of research works are done in poor and limited environments. But, when characterizing cognitive or emotional processes, whether for fundamental or applied research, it is important to consider the multisensory environment in which these processes take part: it allows for the experimental conditions to be closer to real life and the conclusions to be more plausible than in impoverished experimental contexts [2–4]. However, real-life ecological situations are difficult to control and to manipulate. For instance, the exact content of the context and the timing of its evolution are impossible to control. VR offers the opportunity to explore a cognitive process in a multisensory environment without these disadvantages. Importantly, it also enables the determination of the interactions between the main process of interest and its environment.

While VR commonly offers auditory stimulations in addition to the visual context [5–7], it has been rarely coupled to olfactory stimulations [8]. In some studies, environmental smells [9] or food odors [10] have been simply added to the ambient air, being diffused through commercial electronic scent diffusers in the experimental room while participants explored a virtual environment. In these cases, the development is easy but the possibilities are limited to environmental odors diffusion, which is independent of the participants' behavior. In other studies, specific and more complex installations have been developed to enable various scents to be diffused during different virtual scenarios, at a precise time and space, with the possibility to synchronize odorant delivery with pictures, videos, or sounds delivery [11] and even in combination to haptic stimulation [12]. Here, the development is complex and necessitates a combination of specific technological developments, but the possibilities are much larger. Odors may be delivered specifically to the nose of the participants and their delivery can be triggered by the participants themselves—voluntarily or not—according to a location or to biological feedback for example. The method we developed is intermediate in complexity. It necessitates limited developments in comparison with dedicated installations, since it combines an existing technique of olfactory stimulation [13] with a VR software, but offers similar possibilities of multisensory stimulations and interactivity, in addition to being readily transportable.

The method described here aims to study the implicit and objective emotional responses to olfactory, auditory, and visual stimuli in two different ways, which are developed in the method section: (1) by recording the number of times the participants voluntarily activated the presentation of each stimulus during the exploration of the virtual environment, a behavior reflecting their

attraction or repulsion toward them and (2) by recording participants' physiological parameters, reflecting the response to emotional stimuli (respiratory and heart signals, and electrodermal responses [14–16]) during virtual environment exploration. These measures are also linked to explicit and subjective sensory evaluations of the stimulations, recorded afterward. In this chapter, for illustration, we refer to the experiments we performed [17]. They aimed to investigate odor-evoked memory specificities, and especially its link with emotional processes, by directly comparing memories evoked by odors with the same memories evoked by other potent memory cues with strong ecological validity, music, and faces [18, 19].

2 Materials

2.1 Participants

- Participants: They must report a normal sense of smell (*see* **Note 2**) and no visual or auditory impairments.

2.2 Computer Equipment

- A computer: Its characteristics must be sufficient enough to support the RV software. For example, the computer used in our experiment has the following characteristics: a RAM of 12 Go, a processor of 64 bits, and a graphical card Nvidia Quadro K420.
- Two screens: One on the participant's side and the other on the experimenter's side (Fig. 1a, b).

2.3 Virtual Reality Software

- A 3D development software: Unity (Unity Technologies, USA) or Virtools Dev 3.0 (3DS, Aix-en-Provence, France) for instance.
- An 3D environment: Its composition and its degree of immersivity (first-person view non-immersive VR or immersive) depend on the process investigated (*see* **Note 3**). The environment we used, developed by the local technical platform NeuroImmersion (CRNL), is a virtual house, since we needed an environment where people usually perceive smells, music, and pictures of face. The house is composed of three rooms, a bedroom, an office, and a living room connected to a corridor by closable doors (Fig. 1c–e). Each of these rooms is singularized by its furniture, its decorative elements, the nature of the floor, and its wallpaper. For example, the bedroom includes a queen-size bed, a chest of drawers, two night tables, three matching carpets, and four paintings. In addition, three clickable elements are placed in each room at specific locations, allowing to send specific sensory stimulations: a fragrance diffuser (glass bottle with capillary stems) delivers an olfactory stimulus, a radio

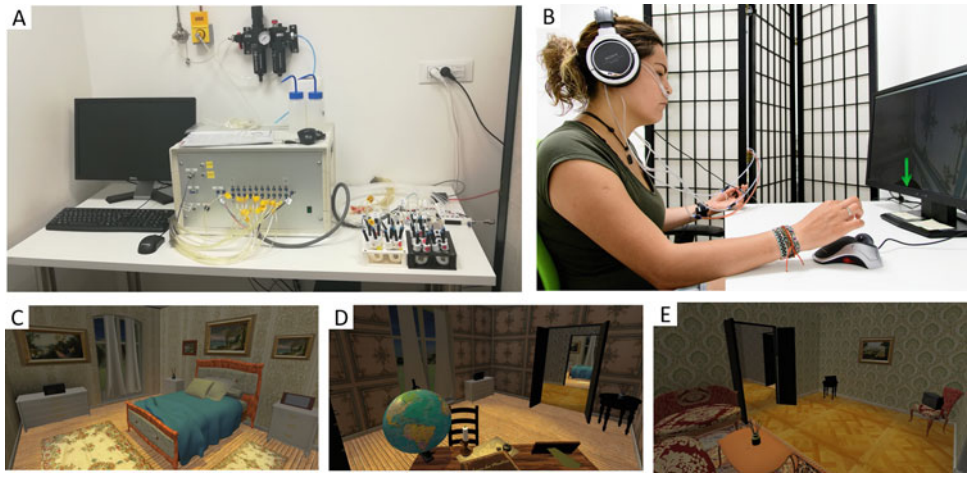

Fig. 1 Experimental setup. The experimental setup was split by a screen into two parts: (**a**) On the experimenter side, on the desk: a computer, the olfactometer, and the odorants were positioned from left to right; and (**b**) on the participant side, a participant wearing nasal cannulas and earphones were investigating the virtual environment. The virtual reality software represents a house made of three rooms: (**c**) a bedroom, (**d**) an office, and (**e**) a living room

receiver delivers an auditory stimulus, and a small portable picture frame delivers a visual stimulus (*see* **Note 4**). These elements are highlighted by an arrow that appears when participants are nearby, helping participants to find them. As is widely the case in VR, the software involves auditory stimulations of synthetic footsteps paced on the rhythm of participants' speed to increase their immersivity [20].

- A device to interact with the environment: A trackball, a joystick, a mouse, etc. (*see* **Note 5**).
- A control panel: To be adaptable and usable for different protocols, the software may include a configuration window with the following tabs/pages:
- Graphic tab: It includes the screen resolution, graphics quality, and identity of the monitor for display.
- Input tab: It includes the coding of the movement (either key of the keyboard or mouse/joystick/trackball).
- Home page: It includes the name of the session, the participant's identifier, the date, and the hour of the experiment (*see* **Note 6**) and allows to load the input file presenting the parameters for the sensory stimulations. It also contains the start and exit buttons to launch the experiment and close the software, respectively.
- Input/Output files: They must be constructed to be easily readable by the software and the data analyst. At least, the input file must define which stimulation is associated with each

clickable element (*see* **Note 7**), and the duration of each stimulation. The output file must report the significant timings of the experiments (*see* **Note 8**).

2.4 Sensory Stimulations

Different types of stimulations can be used in our VR software, depending on the topic of the experiment. In our environment, we choose to present stimulations that vary with sensory modalities (odors, musical pieces, and pictures of faces) and emotional categories (negative, neutral, and positive) (*see* **Note 9**). These emotional categories are only used for stimulus selection, as the subjective emotion evoked by each stimulus in each participant is recorded in a separate experimental session. Other criteria could be, for instance, the identifiability of the stimulations to control for any effect of language or semantic knowledge on perception, or the edibility of a smell when the experiment concerns the food universe.

2.4.1 Olfactory Stimulations

- Odorants: Essential oils, single or mixtures of monomolecular chemical compounds, and fragrances could be used.
- U-shaped Pyrex® tubes (VS Technologies, Saint-Priest, France).
- Microporous beads of polyether block amide (PEBAX®, Arkema, Puteaux, France; ref. 33 SA 01 MED).
- O-rings (Radiospare; ref. 129-050).
- Customized drilled TEFLON caps.
- Homemade rack printed with a 3D printer (Fig. 1a).
- An olfactometer: The odor diffusion system complexity depends on the aim of the experiment. In our experiment, the odorants were presented with a 20-channel computer-controlled olfactometer designed by our team (Fig. 1a, for more details, please see [13]). It was developed to allow rigorous computer-controlled olfactory stimulations and to adjust odorant presentation with nasal respiration (*see* **Note 10**).
- Olfactometer controlling software: The olfactometer is controlled by a software developed with LabView (National Instruments, Austin, TX, USA). It interacts with the VR software to synchronize odor stimulation with the participants' click on the interactive object, via a TCI-IP connection.

2.4.2 Auditory Stimulations

- Musical pieces: The sounds are presented at a comfortable loudness.
- Headphones.

2.4.3 Visual Stimulations

- Pictures of faces: The 2863 × 1718-pixel pictures are presented in JPEG format at the center of the screen.

2.5 Physiological Responses Recording: Sensor Belt

Participants' physiological response is acquired using the Equivital EQ02 LifeMonitor (AD Instruments, Paris, France; ref. HIDA3330-IFU-26-1 0A) consisting in:

- Sensor Belt: It is composed of a module placed on the body, named the Equivital Sensor Electronic Module, able to communicate with a computer through wireless communication. The belt also contains electrocardiogram (ECG) electrodes and respiratory sensors, i.e., a chest expansion-based respiratory belt transducer.
- Equivital Galvanic skin resistance (GSR) Add-On (ref EQ-ACC-034): It connects to the belt to enable the recording of GSR signals (or electrodermal activity) with two MLA1010 disposable electrodes placed on the participants' ring and middle fingers.
- Data acquisition device (PowerLab 16/30, ADInstruments) and analysis software (LabChart 7, AD Instruments, Paris, France; ref. HIDA3330-IFU-26-1 0A AD Instruments): They are used for data acquisition, display, and analyses.

2.6 Emotional Evaluations of the Sensory Stimulations

- Non-graduated scales printed on paper (*see* **Note 11**): In our experiment, the evaluations concern three complementary facets of the emotional response (1) pleasantness (unpleasant–neutral–pleasant; the pleasantness scale is divided into two equal parts by a "neutral" value separating the ratings of unpleasantness and pleasantness); (2) emotional intensity (very weak–very intense); and (3) motivation (also called wanting) (very weak–very intense).

3 Methods

3.1 Preparation of the Stimulations

1. Olfactory stimulations: The undiluted odorants are placed in U-shaped tubes filled with microporous beads and are closed with O-rings and caps. In preliminary tests, absorption of the odorous substance by the microporous beads is performed in Petri boxes. While filled with beads, a 1 cm empty space is preserved in each arm of the U-tube. The U-tubes are ordered and placed vertically in a rack (Fig. 1a). When not in use, odorant tubes are closed with a flexible tube joining the two drilled caps, hermetically sealing them. They are then kept in a ventilated panel.
2. Auditory stimulations: Musical pieces might be modified to reduce or enhance potentially evoked emotional features by changing the tempo, the mode (major/minor), and/or by the deletion or addition of a few notes (*see* **Note 9**).

3. Visual stimulations: Pictures of faces might be modified to be less easily recognizable and to only differs on facial attributes (for example, we used the same background and the same non-gendered hairstyle and outfit for each face) and on emotion with photo editing software (*see* **Note 9**).

3.2 Exploration of the Virtual Environment

1. Participants are equipped with the physiological responses recording belt and the two finger electrodes measuring the electrodermal response, and are placed in front of a screen.
2. The following instructions are given (*see* **Note 12**):

> *You are going to visit a house with three rooms. We want you to feel as if you were in this house, for real. We want you to immerse yourself in this house. You are free to explore this house at your own pace. You can move around the house with the trackball, and observe the rooms of the house, its furniture, the ambient light, and the landscape through the windows. In each room, you will find particular objects you can interact with by clicking on them. They all look the same in any room. They are [*show pictures*]: a perfume diffuser that allows you to smell a scent, a radio that allows you to listen to music, and a photo frame that allows you to see a picture of a face. The smells, music, and pictures of faces will be different for each room. You will have a minimum of 10 minutes to discover the house. You can click as many times as you want on the interactive objects. For the odors, here are some precautions. When you click on the diffuser, you will hear a 'click': do not pay attention to it. It is due to the sending of the smell. It takes time for the scent to reach your nose, so it's normal for this click to occur as you are exhaling. Don't hesitate to tell me if you don't smell anything since each diffuser should be associated with a smell. We also advise you to take a break (about 20 seconds) between two smells, so that your nose is not saturated and you can smell anything. Finally, keep in mind that you must continue to breathe normally through your nose when a smell is sent as well as throughout the experience. Do you have any questions? Is everything clear to you?*

3. The participants put on the nasal cannula and the headphones.
4. The quality of the breathing signal is checked and the comfort level of the sound is determined.
5. The self-paced free exploration begins.

3.3 Attraction/ Repulsion Behavior

Emotional responses are investigated by the number of times the participant clicked on each clickable object to perceive the stimulus during the exploration of the virtual environment. This behavior reflected an attraction/repulsion behavior toward each stimulus. In our experiment, the number of clicks was shown to depend on the subjective emotional evaluation, as the example shown in Fig. 2.

3.4 Emotional Physiological Responses

Breathing, ECG, and GSR signals are recorded to access covert, physiological manifestations of the emotional response.

1. These signals are displayed and extracted in text files with LabChart software, as presented in Fig. 3.
2. Breathing signals are considered in their inspiratory part, their expiratory part, and both parts together (forming a breathing cycle). The amplitude of the signal (maximum), its duration, and its volume are measured.

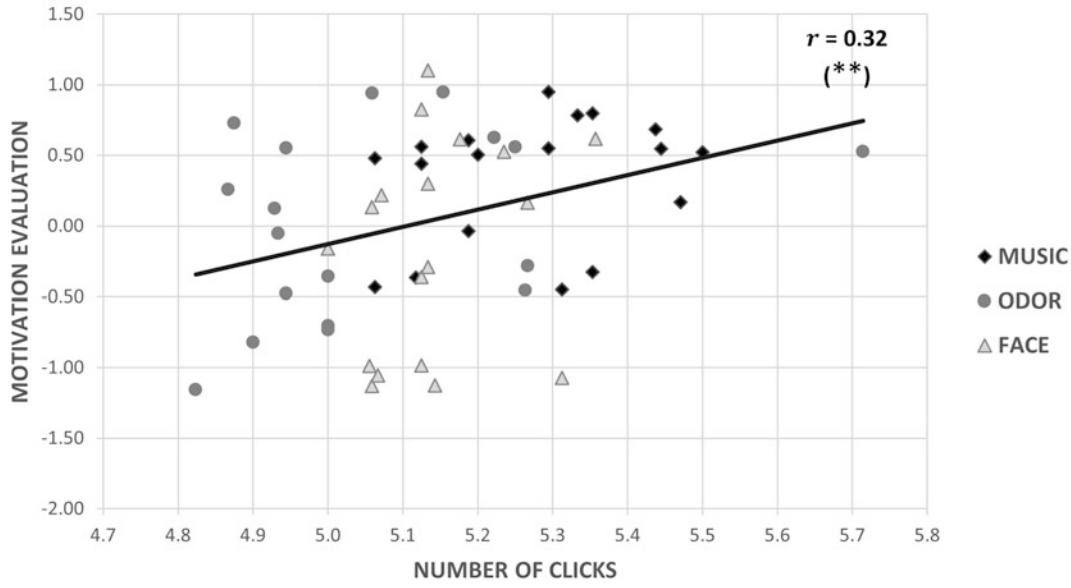

Fig. 2 Attraction/repulsion behavior. The mean number of click for each odor, music, and face as a function of mean motivation evaluations (z-score, arbitrary units). Here, the higher the motivation, the more often the participants tended to click on the objects to perceive the stimuli. The parametric Pearson *r* computed with JASP was added.** $p < 0.01$

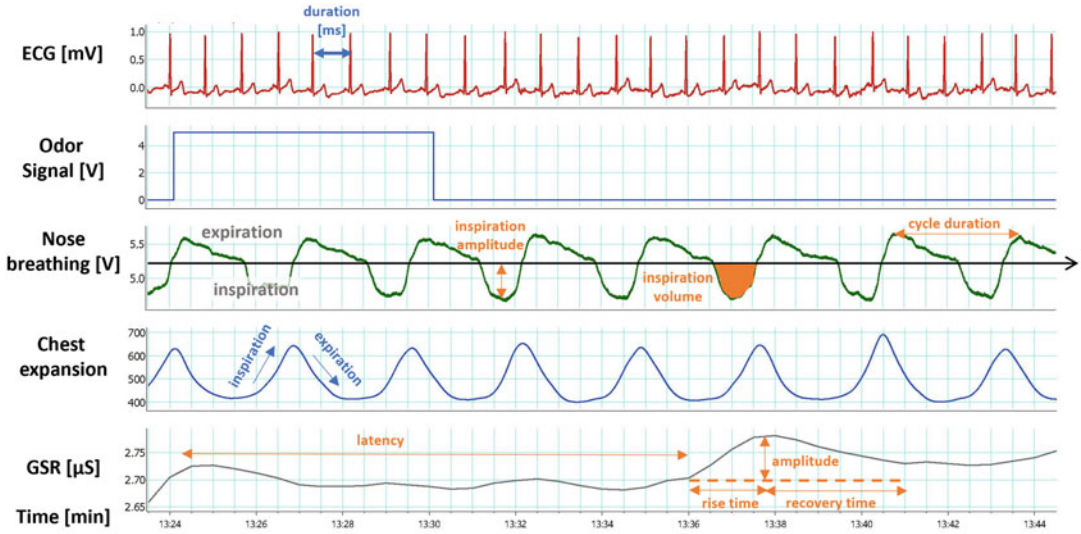

Fig. 3 Physiological signals from LabChart. An example of a 20 s time window where an odor (second raw) was sent. The ECG signal from the sensor belt (first raw), the respiratory signal from the olfactometer (third raw), the respiratory signal from the sensor belt (fourth raw), and the GSR from the sensor belt (fifth raw) are displayed

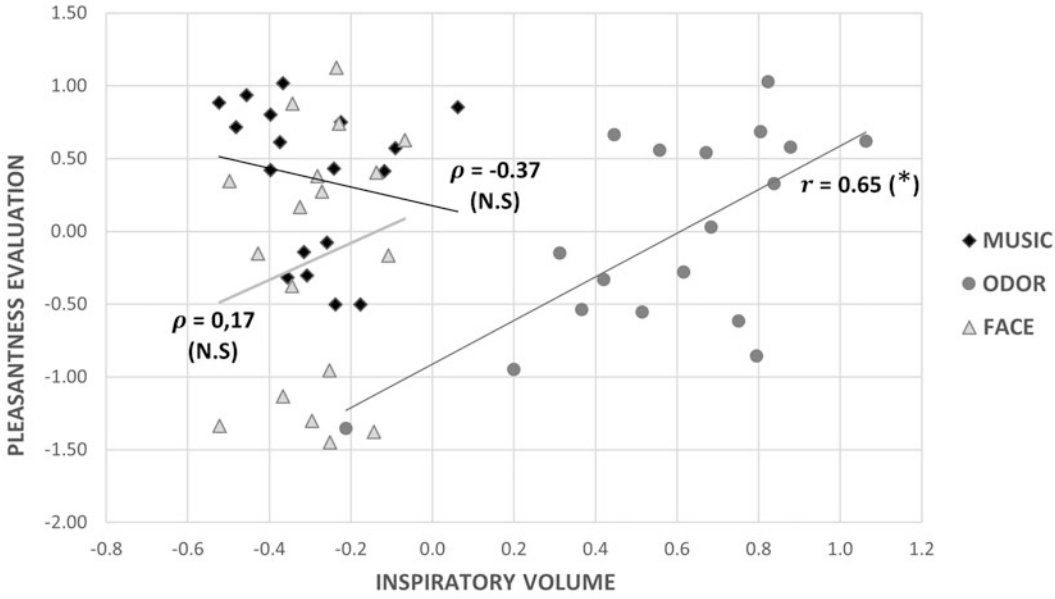

Fig. 4 Breathing modulations with pleasantness evaluations. Mean inspiratory volume as a function of mean evaluation of pleasantness (z-scored, arbitrary units) for odors, music clips, and face pictures. In this example, the more pleasant the odors, the bigger the inspiratory volume. The parametric Pearson r and the non-parametric spearman rho (ρ) computed with JASP were added. N.S.: non-significant; * $p < 0.05$

3. The ECG signals reflect the electrical activity of the heart. From this signal are extracted the heart frequency and the duration between two beats to calculate heart rate variability (HRV).
4. The GSR signals reflect the skin conductance (or electrodermal) response. The peaks of this signal and their parameters (e.g., onsets and peak amplitude) are extracted.

When external or internal stimulus that is physiologically arousing occurs, breathing, HRV, and GSR signals might show variations [21] that can be analyzed through diverse techniques and toolboxes (*see* **Note 13**). Figure 4 represents the covariation of breathing with the pleasantness evaluations that we observed with odor but not with music nor picture of face stimulations.

3.5 Emotional Evaluations of the Sensory Stimulations

After the exploration of the virtual environment, participants are told to evaluate their emotional responses to each sensory stimulation encountered in the virtual house.

1. The instructions are the followings:

This is the last part of the experiment. We want you to characterize the odor, music, and face stimuli you discovered in the experiment. For each stimulus you will be asked three questions: 1) 'How much do you like or dislike this stimulus?'; this is the pleasantness; it might go from very unpleasant to very pleasant, the

middle value being neutral. 2) 'How strong is the emotion evoked by this stimulus?'; this is the emotional intensity; it might go from very weak to very intense. 3) 'How intense would be your motivation to be sent this stimulus again?'; this is the motivation; it might go from very weak to very intense. After each stimulus presentation, you will answer the questions making a precise mark on the scales presented to you on the sheet of paper. There are no good or bad answers, this is your own feeling.

2. The participants are given the response sheets and each unimodal stimulus is presented. As an example, Fig. 5 presents pleasantness evaluations of the stimuli used in the experiment. These emotional evaluations may be further correlated or regressed with behavioral and physiological measures, with for example the JASP (https://jasp-stats.org/) or R (https://www.R-project.org/) software.

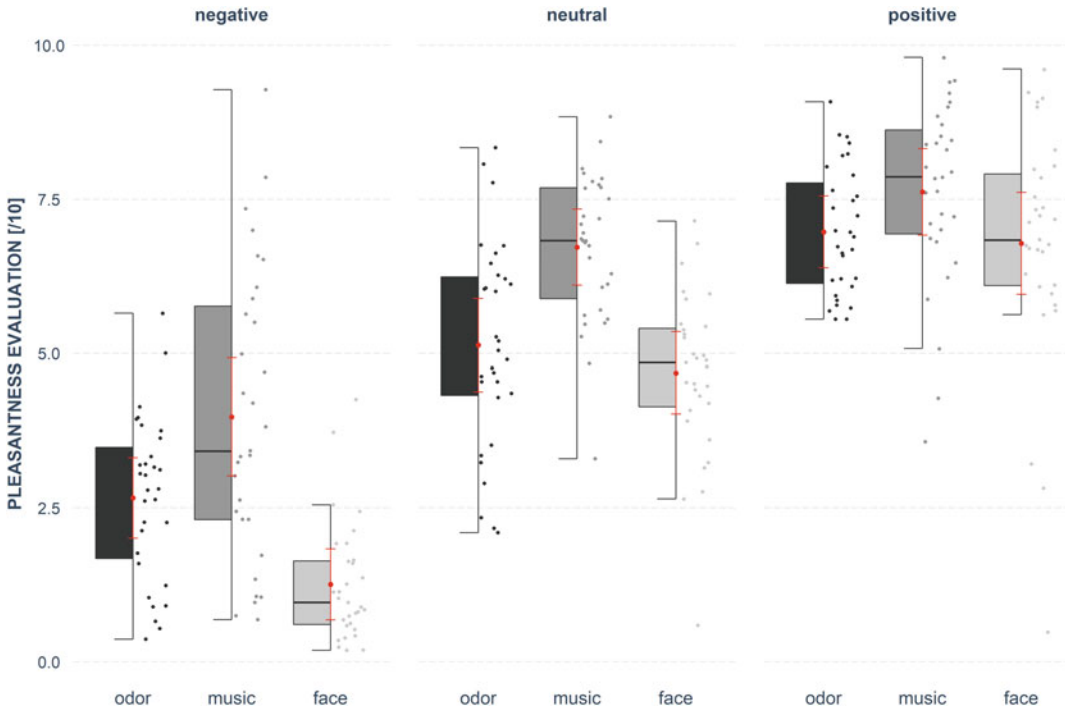

Fig. 5 Emotional sensory evaluations. Mean ratings of pleasantness of odor, music, and face stimuli a priori selected as being part of negative, neutral, and positive emotional categories. Individual raw data are represented with dots. The distribution of data is displayed with boxplots (minimum, first quartile, median, third quartile, maximum). Estimated means and their dispersion (standard error of the mean) are represented on the right side of each boxplot

4 Notes

Note 1 Examples of Potential Applications
Methods used in this chapter may be applied to address a vast number of questions using a fully controlled environment, with stimulation that can be precisely controlled (timing, amount of stimulation, number of explorations). For example, it may be useful to study which odor or sound of a food product may be more important in emotionally guided decision behavior or on subsequent product memory. It also offers the possibility to measure several variables that can be used to study behaviors more precisely, such as the number of explorations or the navigation in the environment. These methods may also be used to study the effect of many different contexts on the emotion evoked by the product. For the cosmetic industry, this protocol can be used to choose which packaging matches the best with a fragrance, for example by studying which packaging-fragrance association is more emotional or more memorized as a function of the social or environmental context (see [22] for other applications).

Note 2 Evaluation of the Sense of Smell
The evaluation of smelling capacities might be either subjective or objective. When subjective, participants might self-evaluate the quality of their sense of smell using rating scales for instance. However, a recent work recommended rating the vividness of olfactory mental images instead [23] with the vividness of olfactory imagery questionnaire (VOIQ; [24]). The olfactory abilities might also be objectively tested using validated tests such as ETOC [25] or Sniffin' Sticks [26].

Note 3 Non-Immersive vs Immersive VR SetUP
Immersivity defines the degree to which the user is isolated from the real-world environment and is reached by introducing a sense of presence with rich sensory-motor perception and interactivity in the VR environment. In non-immersive VR, the users interact with a computer-generated environment but do not have the feeling of being immersed in this virtual environment, as in usual video games of game console. In immersive VR, the users also interact with a computer-generated environment, but with the feeling of being immersed in this new world, as if they were part of the environment, with the impossibility to know what is going on around us in the real world. The degree of immersivity can change the perception, response latency [10, 22], engagement [27], and consuming decision [28]. However, the choice of an immersive environment depends on other variables:

- Financial cost: Immersive VR setup requires purchasing specific materials (room, goggles...), while non-immersive VR needs only common computer devices (screen, mouse...).
- Feeling of discomfort: Immersive VR might generate feelings of sickness, while it is not an issue with non-immersive VR. Accordingly, the presence of virtual characters may provoke the famous uncanny valley effect, explaining the feeling of strangeness felt when an object, a robot, or a character resembles a real human while keeping non-human attributes [29, 30].
- Matching with the current real-life environment: An issue with non-immersive VR can be the mismatch between reality and VR conditions. For example, the immersivity feeling can be reduced if the house is presented with a night environment while the experiment is run in the middle of the day. This problem does not exist with immersive VR setup since only the virtual environment is perceived.
- Physiological recordings: Movement influences physiological recordings such as HRV and electrodermal responses. Results are thus less accurate and precise when VR setup implies movements [8].

Note 4 Emotional Contagion
Emotional contagion might happen in the virtual environment (emotional spread of one stimulus on another), and the spatial repartition of the stimuli in the environment might be considered. This is of great importance since odors seem to be particularly prone to be influenced by other stimuli [1, 31]. For example, in our experiment, we choose to place in the same room stimuli with similar emotions.

Note 5 Trackball vs Other Devices
In a semi-immersive VR, participants can move and explore an environment with many different devices, such as a mouse, a trackball, a joystick, or a wheel. This interactivity with the environment is crucial, as it distinguishes VR from watching a video. The choice of this device is important as it depends on the experiment. For example, when running an fMRI study the trackball is a better choice than a mouse. Its use limits the movements of the participants in the scanner in comparison with a computer mouse since only fingers move but the hand is stable.

Note 6 Timestamping
Timestamping must be automated so that files always have different names and are easily identified, which limits the possibility of errors.

Note 7 Simultaneous Multisensory Stimulation
In the current version of the software, only one stimulus was sent per clickable element, but the input file configuration allowed the simultaneous delivery of an olfactory stimulus, an auditory

stimulus, and a visual stimulus. While it might be interesting to deliver simultaneous stimulations from different sensory modalities, it raised several questions among which is the congruence between these sensory stimulations and attentional competition.

Note 8 Significant Timings
We advise time 0 to be the click on the start button and to record the total duration of the exploration and the time when each sensory stimulation is sent.

Note 9 Stimulus Selection
The stimuli we used in our experiments are given as examples. They were selected to be as similar as possible between sensory modalities (odors, music, faces) and to be of different emotional categories (negative, neutral, positive).

Odorants: They were selected from previous works [32, 33]. Negative odorants were composed of carrot (Givaudan-Roure®, Vernier, Suisse), musk (Givaudan-Roure), pastry (Perlarom 93, Grasse, France), stemone (Créations aromatiques, Neuilly-Sur-Seine, France), vetiver (Davenne, Montfavet, France), and yeast (Givaudan®, Vernier, Suisse). Neutral odorants were composed of basil (Créations aromatiques), black olive (Meilleur du Chef®, Bassussarry, France), cis-3-hexenyl salicylate (Créations aromatiques), dill (Pharmacie Croix Blanche, Dijon, France), honey (Givaudan-Roure), and ylang (Givaudan). Positive odorants were composed of Bien-Être (EmoSens®, Lyon, France), blackberry (Givaudan), cosy (EmoSens), lovely ion (EmoSens), osmose (EmoSens), and tomato (Givaudan-Roure).

Musical pieces: They were selected and modified from the materials used in Vieillard et al. [14] (Copyright, Bernard Bouchard, 1998) (http://www.peretzlab.ca/knowledge_transfer/). Negative excerpts were P01, P05, P06, P10, P11, and P12. Neutral excerpts were M03, M06, M09, M10, M11, and M16. Positive excerpts were G03, G06, G07, G10, G11, and G13. Music was modified in MIDI using the Digital performer software (Digital Performer®, MOTU, Cambridge, USA) aiming to reduce or enhance potentially evoked emotional features. The musical clips were then played with an acoustic piano timbre using another software, Cubase (Cubase®, Steinberg Media Technologies, Hamburg Germany).

Pictures of faces: They were selected from the Compound Facial Expressions of Emotions Database [34] (http://cbcsl.ece.ohio-state.edu/dbform_compound.html). They were composed of nine women and nine men Caucasian faces. To be less recognizable, faces were virtualized with CrazyTalk®8 software (Reallusion Inc., California, U.S.A.). They were then turned into black and white, presented on a white background with similar relative positions and dimensions using the Photoshop software (Photoshop®, Adobe, Dublin, Ireland).

Note 10 The Olfactometer
This odor diffusion system was developed to allow computer-controlled rigorous olfactory stimulations and to adjust odorant presentation with nasal respiration. The participants' nasal respiratory signals are acquired using a nasal cannula positioned in both nostrils (Teleflex, Le Faget, France; ref. 1103) and connected to an airflow sensor. Respiratory signal is used to trigger the odor stimulation. During the odor stimulation, the olfactometer waits for the participants' expiration to deliver the odor, allowing it to be perceived at the beginning of the subsequent inspiration. When an expiration is detected, an unodorized airflow is sent to one of the U-shaped odorous tubes. The unodorized vector air directly comes from the laboratory distribution network or a portable air compressor. The odorized airflow is sent to and mixed with air carrier in a homemade drilled mixing head made of PTFE (polytetrafluoroethylene) through low-absorption tubes (Teflon® Fluorinated Ethylene Propylene). The concentration of the odorant and of air carrier was established through pilot experiments for each odorant, such as the final physical intensity seems comparable between stimuli. From the mixing head output, the odorized airflow is sent to the nostrils through two flexible tubes (Soft Polyurethane Tubing; SMC France, Marne La Vallée, France; ref. TUS0425N-20), fixed to the nasal cannula with a tape, opening out under the nostrils (Fig. 1b). Odorized airflow is not sent directly into the nostrils to limit discomfort and mechanical stimulation by the airflow.

Note 11 Sensory Evaluation Scales
In this protocol, scales were printed on paper and ratings were a posteriori transformed into scores from 0 to 10. Scales might also be presented using other methods such as the self-assessment manikin [35]. The use of software to assess subjective emotion instead of a printed paper, after the experiment, might be also of interest. It allows restricting the response time and measuring physiological responses evoked at rating time.

Note 12 Instructions
To not bias the participants' behavior, the exact aim of the experiment was hidden and the participants were told that the study aimed at investigating the perception of various environments involving odors, music, and pictures.

Note 13 Physiological Signal Analysis
Many metrics can be used to study breathing, ECG, and GSR signals. For example, the breathing signal can be studied using respiratory frequency, inspiration volume, minute ventilation, etc. In the same manner, HRV can be analyzed in the time or the frequency domains using several metrics such as the standard deviation between intervals (NN or RR intervals [36]). These metrics

can be extracted with homemade scripts or with toolboxes such as NeuroKit2 [37], a toolbox written in Python. For information about the relationship between emotion and physiological measures, please see [21, 38].

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Chapter 14

Characterization of Facial Emotions to Food Odors in Children with Autism

Anne-Claude Luisier, Geneviève Petitpierre, Annick Clerc Bérod, and Moustafa Bensafi

Abstract

Most children with autism spectrum disorder (ASD) have difficulty expressing their emotions verbally. In addition, subjective measures of emotional states can be complicated to use in children with ASD and it is therefore necessary to use more objective approaches. The present protocol is based on the use of an automatic emotion recognition method, particularly in relation to food odors. This noninvasive method allows a more standardized, faster, and less subjective approach to the measurement of facial expressions. It also opens the door to longitudinal studies to evaluate the emotional changes induced by interventionist protocols related to food in children with ASD.

Key words Autism, Food, Senses, Odors, Emotions

1 Introduction

According to the American Psychiatric Association's Diagnostic and Statistical Manual, Fifth Edition (DSM-5), autism spectrum disorder (ASD) is characterized by both (i) deficits in social communication and social interaction and (ii) stereotyped, restricted, repetitive patterns of behavior, interest, or activity (including atypical speech and movement, resistance to change, and atypical sensory behavior) [1]. These symptoms are present in early childhood and combine to limit and impair everyday functioning at both behavioral and emotional levels.

Emotional responses to sensory stimuli can be atypical and could be among the symptoms at the core of ASD [2, 3]. These sensory peculiarities complicate the lives of children and their families [4]. In particular, the eating domain is impacted [5–8] with eating disorders significantly more frequent than in typically developing children [9–12]. More generally, the emotional competencies of individuals with ASD differ from those of typically

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developing children [13–15]. In particular, they show difficulties in consciously identifying their own emotions (cf. e.g. [16]). A better understanding of the emotional feelings of children with ASD when faced with sensory stimuli is therefore an important scientific issue. This chapter aims to present a protocol for measuring the emotional responses of children with ASD exposed to food odors.

Most children with ASD exhibit difficulties in verbally expressing their emotional feelings [13, 15, 17–24]. It could be observed for example that some children had difficulty answering questions verbally notably because of a lack of attention, a misunderstanding of the question or the lack of oral language skills. Sometimes their nonverbal attitude can be different from their verbal expression. In addition, it can be difficult to use a measurement scale that allows for nuance because children may not understand it as typically developing children do. More objective approaches such as measuring facial expression are judicious to use.

Facial expressions are supported by both conscious and nonconscious processing, and their use in populations with autism is conditional on the control of certain parameters such as the physical and social environment (*see* **Note 1**). Although they should not be considered an exact external reflection of any emotion (emotions are accompanied by other behavioral and physiological responses), facial expressions represent an observable sign from which inferences can be made about the valence and/or type of emotion induced.

This protocol is based on the use of an emotion recognition software that allows a more standardized, faster and less subjective approach to the measurement of facial expressions than if a manual and human analysis were used (*see* **Note 2**). We propose to use food and nonfood odors as an example of stimulation, to present the stimuli according to a calibrated protocol performed by our team [22] while facial expressions are recording continuously throughout the experiment.

2 Materials

1. Ethics: The study must be validated by an ethics committee.
2. Sample size: The sample size of the participants must be calculated according to your scientific objectives a priori.
3. Diagnostic for autism: The autism spectrum disorder (ASD) diagnosis should be reported for each participant, e.g., by reporting the score on diagnostic tests such as the Autism Diagnostic Observation Schedule (ADOS) [25–27]. Intelligence level can also be measured (e.g., Raven's Colored Progressive Matrices; [28]). The general language abilities of the test subjects are also needed. This information can be collected

by the researcher from people who know the child well (parents, teachers, etc.).

4. Sample characterization: Since the protocol is related to sensory processing and food, it may be interesting to have parents complete a food neophobia questionnaire [29] and a standardized tool providing information about the child's sensory processing patterns in ecological contexts of home, school, and community-based activities (e.g., Short Sensory Profile, [30]) in order to characterize the participant's sample. *See* **Note 3** for issues dealing with sample heterogeneity.
5. Selection of stimuli: The odorants used in your protocol depend on your scientific objectives (*see* **Note 4**). As an example, we present here a list used in Luisier et al. [22]. Six food odorants (Firmenich SA, Geneva, Switzerland) varying in quality and pleasantness are used: ghee (cheese-like), fish, orange, pineapple, strawberry, and banana. In addition, a flask containing only 4 mL propylene glycol ("non-odorized" flask) is also used (seven stimuli in total) (*see* **Note 5**).
6. Preparation of stimuli: All odorants are diluted in propylene glycol. The absorption of the solvent by the polypropylene avoids accidental leaks and prevents direct contact of the child with the stimulus. Concentrations should be determined from a pilot study in order to (1) allow the odorant to be detected, (2) not produce an intensity that is too strong, and (3) achieve a relatively comparable level of perceived intensity between all stimuli; 2).
7. Odorants are presented in 30 mL (nominal volume) flasks (opening diameter: 3.05 cm; height: 4.5 cm) filled with 4 mL dilution absorbed on scentless polypropylene fabric (3 cm Å ~ 8 cm; 3 M, Valley, NE, USA) to optimize evaporation and air/oil partitioning.
8. Odor presenting device: The device (Fig. 1) includes support (in the form of a small tube) to carry the flask (rotative arm). This rotative arm can slide on an axis in order to raise or lower the flask and also to orient the flask to the left or to the right. This axis is fixed on a base which allows to maintain it at 90°.
9. Digital camera: one digital video camera (ex. Sony Alpha 6000 Hybrid).
10. Light diffuser: two light diffusers (Philips cool White LED 1521 lm, 4000 K).
11. Background: one light gray background.
12. Automatic emotion recognition software: FACET™ SDK software (iMotions Inc., Cambridge Innovation Center, US) or another equivalent software [31].

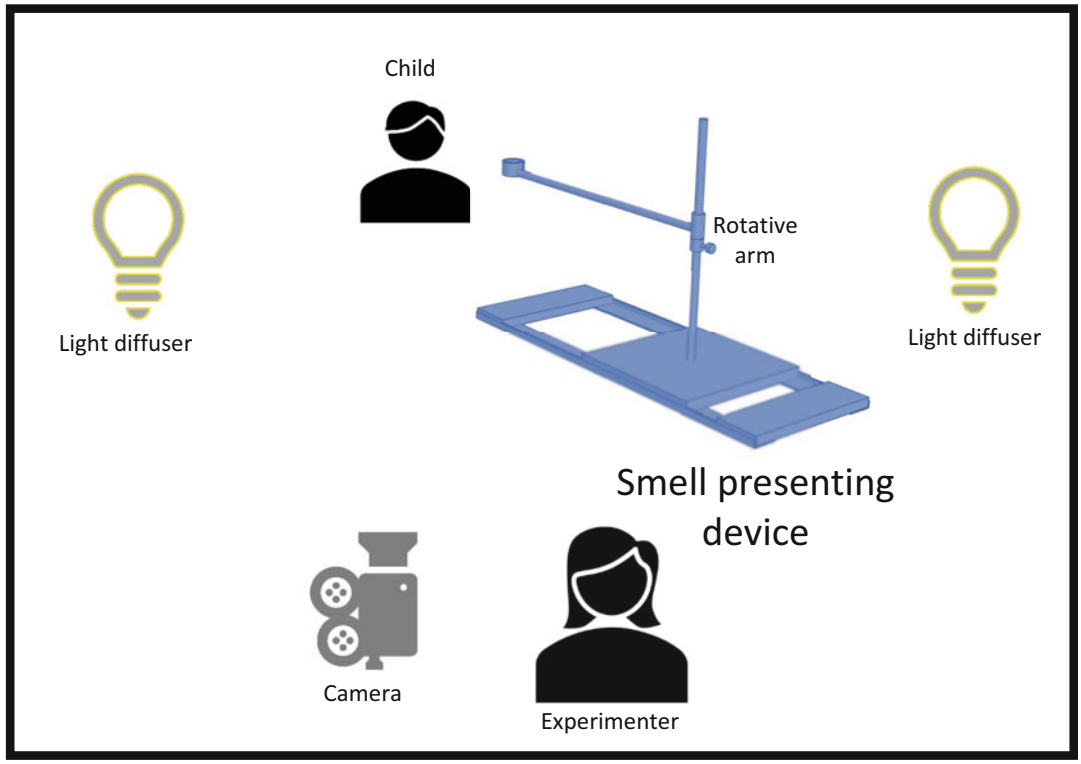

Fig. 1 The odor presenting device and the whole experimental setup. The device includes a smell presenting device (with a rotative arm) controlled by the experimenter enabling presenting the odor in front of the nose of the participant, a camera to record facial expressions, and two light diffusers to illuminate of the participant's face. Note that a light gray background is also used for optimization of shadows and contrasts (not shown in the figure)

3 Methods

1. The experiment starts with a detailed explanation of the procedure to the child.
2. The participant is asked to sit down on a chair in front of the experimenter (*see* **Note 6** for familiarization of the child with the experimental setting).
3. All experimental trials are videotaped with the digital video camera located in front of the child's face (Fig. 1). The camera is run in autofocus mode (phase detection AF/contrast-detection AF) to achieve optimum focus, exposure, and white balance.
4. The face of the child is framed on a regular basis and the illumination of the participant's face is optimized using the two light diffusers on each side of the child. Moreover, a light gray background is used. These controls allow optimization of

shadows and contrasts and facilitate the automatic analysis by the facial recognition software.

5. Regarding odor presentation, the odor-presenting device is placed on the table next to the experimenter (Fig. 1). The seven stimuli (six odors and the non-odorized flask) are presented successively in random order.
6. To habituate the participant to the experimental setting, the empty flask is first presented to the child before starting the experiment (*see* **Note 7**).
7. For all trials, including the first empty trial, open the flask and place it on the rotating arm of the odor presentation device (*see* Fig. 1). The timer is started (*see* **Note 8**) when the vial is under the participant's face. Please note that the vial must be placed under the chin so that the whole face of the child is visible and can be analyzed. After 10 s of presentation, the bottle is removed by turning the rotating arm towards the examiner. The examiner then closes the vial. After 30 s, the experimenter asks the child whether he/she liked the odor or not. A new bottle is then presented to the child after about 20 s. In sum, the total duration of a trial is about 60 s, which is a sufficient time window to prevent olfactory adaptation.
8. For each trial, a video file is recorded using the digital camera (covering the whole trial: before odor presentation and after subjective ratings of liking; but this may change as a function of your design).
9. Each video file is submitted for automatic analysis of the emotional facial patterns by adapting the procedure of Garcia-Burgos and colleagues [32, 33]. Here, the video files are run through FACET™ SDK software. The automatic facial expression recognition software tracks and analyzes frame-by-frame (1/25 s) the intensity of positive, neutral, and negative valence in the 3 s after perceiving the odor stimuli (*see* **Note 9**). Some software also offer a measurement of very specific facial emotions such as joy, sadness, anger, fear, disgust, or even surprise.
10. Data analysis. The data extracted by the analysis software for each child, each odor, and each type of valence can then be analyzed with standard parametric or nonparametric analysis tools depending on the characteristics and nature of the data.
11. Example of results. Figure 2 depicts an example of data collected in a past study from our group (Luisier et al. [22]). In Fig. 2a, one can see that the intensity of positive facial emotions to a pleasant odor (orange) remains at high levels during the 3 s following the olfactory stimulation. The neutral facial emotion has a high intensity at the beginning and then decreases very quickly. Finally, this orange odor almost did not induce negative facial emotions. Figure 2b illustrates a statistical analysis

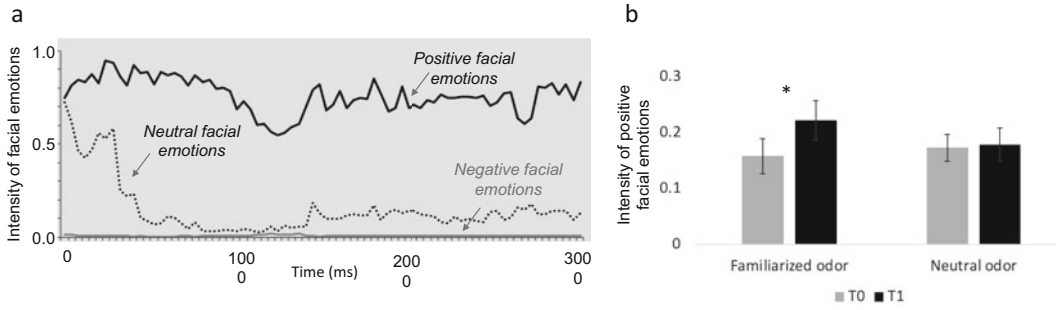

Fig. 2 Example of results. (**a**) Example of intensity data of positive (black solid line), neutral (black dashed line), and negative (gray line) emotional facial responses as a function of time after the perception of an orange smell. (**b**) Example of data from the publication of Luisier et al. [22]. * means $p < 0.05$

performed in the study of Luisier et al. [22]. The purpose of this study was to evaluate whether olfactory familiarization can make food odors more pleasant, in a population of children with autism spectrum disorders (ASD). Participants were first confronted with a series of food odors (session 1, "T0" in Fig. 2b). Then, they were familiarized four times (time interval: 5 weeks) with the most neutral of these 6 odors (session 2). Another neutral odor that was not presented during session 2 served as a control during session 3. During this session 3 (T1 in Fig. 2b), participants smelled the full set of 6 odors again. Facial responses were compared between session 1 (T0) and session 3 (T1). Results are illustrated in Fig. 2b as mean (+/− SEM) for facial expression of positive emotions at T0 and T1 for both the familiarized odor and the control odor. The results revealed a specific increase from T0 to T1 in positive facial emotions for the familiar odor (Wilcoxon test: $W = 95$, $p = 0.035$), whereas these positive facial emotions did not vary for the neutral control odor (not presented during the familiarization phase) (Wilcoxon test: $W = 161$, $p = 0.489$). These data therefore suggest that it is possible to modulate olfactory emotions of children with ASD via familiarization.

4 Notes

1. Caution should be exercised when using facial expression measures to compare a population with ASD to a control population, as studies have shown that the facial emotional expression of individuals with ASD differs from that of typically developing individuals [34, 35]. These differences appear to be related to, among other factors, the context, environment, psychological state, and/or cognitive profile of individuals such as

alexithymia, depression, and sensitivity to change. However, these differences appear to be attenuated in a nonsocial context.

Note that in the area of olfaction, Legiša et al. [20] studied hedonic responses to olfactory stimuli given by children with ASD matched to children with TD (ages 8–14 years). These responses were given verbally and were measured by facial expressions and physiological responses (heart rate and skin conductance). Children in both groups showed similar facial and physiological responses to olfactory stimuli. They differed, however, in their verbal responses, which were less consistent with facial expressions in children with ASD than in children with TD.

2. The automatic emotion recognition approach is noninvasive and more sensitive to small changes and recurrent idiosyncratic expressions. It is also interesting if one wishes to measure and compare emotions induced by repeated sensory exposure [31]. This feature opens the door to longitudinal studies in the same individual with ASD as has been done to assess emotions and eating behavior before and after an olfactory familiarization-type intervention [22].
3. Sampling procedure could be complicated in populations known to present a certain heterogeneity and/or consist of subgroups, which is the case for people with ASD [33–39]. The sampling would benefit from being done finely in order to constitute homogeneous groups. Depending on the study objectives, standardized assessments may be used to characterize verbal skills, intellectual functioning, sensory features, or ASD severity. More, factors such as alexithymia, depression, and sensitivity to change are known to influence emotional facial expression [33]. Controlling them could facilitate or provide clues for the interpretation of the results.
4. The odorants should be as close as possible to natural odors and it would be wise to avoid using too many odors that could provoke strong emotional responses such as odors known to be aversive or unknown odors.
5. The choice of the number of odors to present to the child is important. Indeed, the attention span of the children, their emotional reactivity to odors, and the anxiety they feel about the task must be taken into account. A pre-test conducted with the target population would be useful to determine the ideal number of odors to present to children during a testing session. Between 6 and 15 odorants (+ 1 stimulus without smell) can presented per session, depending on the setting [21, 22].
6. Children with ASD are very sensitive to anything they perceive as new. In an attempt to reduce the stress of participating in a research project and therefore a new task, certain

accommodations are recommended, notably: (1) Before starting the measurements, the experimenter should get to know each child by, for example, sharing activities that he or she enjoys such as a game, reading, and sensory activity in order to create a bond with the child [22, 40]; (2) The location of the experiment should be well known to the child. It is difficult to bring a child with ASD into a laboratory. It is recommended to move to a place known to the children, such as their school.; (3) Our past research has shown that the task of smelling a scent is not self-evident for all children with ASD. It may be worthwhile to conduct one or more familiarization sessions with smells before beginning the research sessions; (4) Facing the camera without speaking and moving the head is particularly difficult for children with ASD. It might be wise to familiarize the children with the experimental device. Despite all the recommendations mentioned, there is a risk that children will not be able to perform the full test on the day of the experiment, for example because they are having a bad day, because they have a cold or because one of the odors induces a strong disgust. This should be considered by including some flexibility in the planning of experimental sessions.

7. The empty stimulation flask can also be used to set up a baseline for facial emotion measurement.
8. The measurement of the odor presentation time can be done with a stopwatch or dedicated software. In both cases, it will be possible to determine the presentation time of each flask by analyzing each video afterward. The researcher must decide whether to present the stimulus only once or several times. Each option has its advantages and disadvantages. On the one hand, repetition may provide an average response, which limits the risk of interference from factors that are difficult to control, such as the child's mood and attention, at the time of the single data capture. On the other hand, when the child has already been exposed to the stimulus once, they often keep a trace of it, and their reaction at time 2 will therefore not be that of a first contact.
9. Our past studies showed that at least 70% of the video frames are analyzable for each child and each odor. Depending on the study design, the analyzed facial expressions during the presentation of the empty flask may also serve as baseline or control. In this case, for each odor, the intensity of positive, neutral, or negative valence is calculated by subtracting the intensity of the empty flask. The intensity of positive, neutral, and negative valence may be then smoothed by computing the mean of the obtained values every 1/10th of a second. For each child and each odor, missing intensity data values can be replaced by the mean of the two nearest nonmissing preceding values and the two subsequent nonmissing values.

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Chapter 15

Facial Electromyography in Food Research in a Behavioral and MR Setting

Emilio Chiappini, Giorgia Silani, Johan N. Lundström, and Sebastian Korb

Abstract

Facial electromyography (EMG) allows to detect and quantify overt as well as subtle covert contractions of striatal facial muscles. Subjective and partly implicit affective experiences, such as the hedonic pleasure felt when consuming an exquisite meal, can thus be revealed and objectively quantified. Further, facial EMG is a convenient tool for translational research, as it can be combined with other research techniques, and therefore help to unravel brain mechanisms underlying the processing of different types of rewards. In this chapter we aim to provide step-by-step guidelines for the acquisition of facial EMG in food research in humans, using noninvasive surface electrodes. Implementations of facial EMG in behavioral settings and in combination with functional magnetic resonance imaging are discussed.

Key words Facial EMG, Facial expression, Hedonic reaction, Food anticipation, Food consumption

1 Introduction

The human face contains more than 40 muscles, which receive an exceptionally high density of neuronal innervation [1]. This characteristic allows us to produce fine-scaled movement and nuanced facial expressions of both voluntary and emotional nature [2]. The face has been described as a window to the soul as changes in facial muscle activation can reveal a person's thoughts and feelings, but also brain states resulting from the integration of external stimuli, autonomic signals, and cognitive processes [3]. The great richness of information the face can convey has stimulated extensive research, starting with Darwin who pioneered the systematic study of emotional facial expressions and demonstrated their universality by examining children, people suffering from congenital blindness and mental disorders, as well as nonhuman animals [4].

Food consumption, exposure to flavors, and the mere view of images of foods can all elicit affective facial reactions, which reflect the subjective hedonic value of the substance experienced, and are

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partly shared between humans and other mammals [5–7]. For example, in monkeys and rodents (as well as in human infants) typical liking reactions to food involve tongue protrusion, facial muscle relaxation, and licking of the lips, while disliking is expressed in the form of gaping, tightening of facial muscles, and nose wrinkling [8].

To better understand the behavioral and psychological mechanisms underlying food processing, it is of great importance to develop new and improve existing instruments that allow for accurate measurement, and preferably are conducive to translational research. However, the operationalization of constructs derived from animal or theoretical models for quantitative research in humans often seems suboptimal, or hinges on subjective reports, which lack reliability [9] due to implicit or explicit biases [10]. In contrast, the tracking of facial expressions driven by food exposure is relatively easy to implement and may provide an objective measure of participants' inner affective states.

Facial movements and expressions are often assessed visually, by trained human coders or automatic computer algorithms, using for example the "Facial Action Coding System" [11]. An alternative approach takes advantage of surface electromyography (EMG) to measure subjects' physiological facial muscle activity. Emotional states can be inferred by the pattern and degree of activation and relaxation of specific facial muscles. Facial EMG is a safe and noninvasive technique that combines great sensitivity and temporal resolution, and allows to quantify the onset, magnitude, and duration of affective muscle contractions, including those of such small amplitude that they are not visible to the human eye [12, 13].

Contractions of two muscles, in particular the *zygomaticus major* (ZM) and *corrugator supercilii* (CS), are regarded as key indicators to differentiate between positive and negative emotional states [14]. The ZM is the main muscle responsible for elevating the mouth corners and display a smile – it activates primarily in response to positive stimuli. Activation of the CS knits the eyebrows as in frowning. The ZM and CS muscles relax and contract in response to, respectively, pleasant and unpleasant stimuli, along a bipolar valence continuum [14]. Past research on facial expressions to food stimuli has shown that CS activity reliably indexes aversive experience [9], while the link between ZM activation and positive experiences (hedonic pleasure) is weaker and not always confirmed [9, 15–18]. Other muscles of interest, especially when researching affective responses to food stimuli, are the masseter and suprahyoid muscles, which are associated with mastication of solid food [19, 20], as well as the levator labii superioris/alaeque nasi region, which is activated during negative hedonic reactions, such as in disgust [21].

In this protocol, we describe in detail the procedures researchers can use to investigate facial responses to food rewards using facial EMG in human participants. Specifically, we discuss the EMG-hardware and software, electrode placement, types of food stimuli, and how to combine EMG and fMRI in food research.

2 Materials

In the following, we describe the materials used by Korb et al. [16, 17]. We also provide the specifications for materials required for facial EMG signal acquisition (*see* **Note 1**). These are intended to be general guidelines in accordance with the relevant literature [22–28], and materials may partly vary according to specific experimental needs and to availability, e.g. for another recent example of EMG responses to food [19].

2.1 EMG Equipment

2.1.1 Amplifier

The EMG amplifier system acts as a differential amplifier endowed with serial components that may amplify, filter, and convert the electric signal from analog to digital. We used a TMSi REFA-8-amplifier (TMSi, The Netherlands) (*see* **Note 2**).

2.1.2 Electrodes

A pair of electrodes per targeted muscle (e.g. CS and ZM) and one electrode functioning as ground are needed. Some systems additionally require a reference electrode. We used ring-shaped Multitrode or ED6 hat floating electrodes (BrainProducts GmbH, Germany) – the latter are also suitable for recordings in combination with functional magnetic resonance imaging (fMRI) (*see* **Note 3**).

2.1.3 Cabling

We recommend using shielded electrode-to-amplifier leads to minimize signal disturbances. Cabling to connect the related hardware to the amplifier typically comes with the amplifier system (*see* **Note 4**).

2.1.4 Computer and Software

The EMG recording software acts as interface for signal acquisition and is often provided by the producer of the amplifier – we have used Portilab2 (TMSi, The Netherlands) or BrainVision Recorder (BrainProducts GmbH, Germany) running on a Windows computer (*see* **Note 5**).

2.2 Consumables

2.2.1 Scrubbing Paste/ Alcohol Solution

Cleansing of the skin to minimize electrode–skin impedance is achieved through gentle wiping of the skin with ethyl or isopropyl alcohol pads, which helps removing naturally occurring oily substances, and with mildly abrasive pastes that help to further remove dead skin cells. We have used the abrasive Nuprep Skin Prep Gel (Weaver and Company, U.S.A.).

2.2.2 Adhesive Pads

Electrodes are attached to the subject's skin through adhesive pads or double-sided adhesive rings (also called *washers*).

2.2.3 Conductive Media

An electrolytic medium to bridge the skin-electrode distance and minimize electrode–skin impedance is typically required (*see* **Note 6**). For this purpose, we have used the Signa gel (Parker Laboratories Inc., U.S.A.) or Abralyt HiCl Electrolyte-Gel (Easy-Cap GmbH, Germany).

2.3 Task

2.3.1 Stimuli (See Note 7)

In recent studies from our laboratory [16, 17; see also 29] we delivered cocoa-flavored milk, sugared milk, or a mix of flavored and unflavored milk. Liquids were preferred to solid foods to avoid mastication, which results in major recruitment of numerous facial muscles, including the ZM, and might produce (mandibular) movements affecting signal quality. In contrast, the swallowing of liquids also generates movement artifacts, but they are of lower magnitude and fade away more rapidly (Fig. 2). Using Matlab (MathWorks Inc., U.S.A.) we controlled electronic pistons pushing syringe plungers for the desired quantity of liquid that was brought to the subject's mouth via food-safe tubing (Figs. 1a, c).

2.3.2 Trials

We built a Matlab-controlled task *(for details see* [29]) designed to track affective responses to food from the anticipatory phase until a few seconds after the consumption phase. The trial structure consisted of a baseline period (*see* **Note 8**), followed by stimulus announcement, delivery preparation, delivery, and a relaxation period. Importantly, concurrently to each experimental event, markers were sent through a parallel port to the computer recording the EMG. These markers are essential to time-lock the EMG trace to an event during offline analyses (Fig. 3).

3 Methods

Follows a step-by-step description of the EMG procedures as applied in recent studies conducted by our group [16, 17].

3.1. General considerations. To prevent signal interference, it is generally recommended to conduct EMG experiments in laboratories with minimal amounts of electrical interferences ("noise"). Therefore, if possible, avoid using florescent lighting (use LED instead), use shielded electrical equipment (as well as outlets and cabling), and place the participants as far as possible from sources of electric noise. Consider performing EMG in a quiet room, possibly separating the spaces dedicated to the experimenters (with technical instrumentation and food delivery system) and participants.

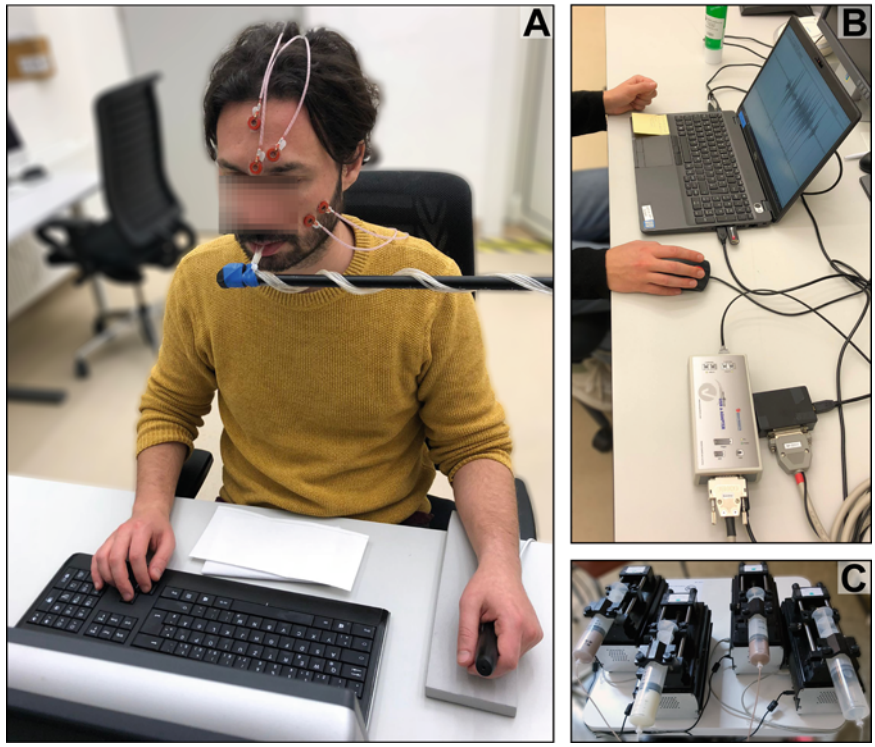

Fig. 1 Experimental setup. Panel **a** shows participant setup for a food-reward task as in Korb et al. [1, 2]. The EMG electrodes are placed with a bipolar montage longitudinally to the muscle and attached to an amplifier (not visible) to record the activity of the ZM (cheek area) and the CS (eyebrow area) muscles. The ground electrode is positioned on the forehead. The food in liquid form is administered to the participant's mouth via tubes attached to a mechanical arm. The participant receives instructions on the monitor and provides behavioral feedback using the keyboard (right hand) and the hand dynamometer (left hand). Panel **b** shows the experimenter recording the EMG activity. The EMG data are acquired and digitized via the amplifier and the USB-adapter interface (gray box) and stored in the computer. The task computer delivers markers to the EMG track essential to time-lock EMG data and task events for offline analysis using a TTL module (black box). Panel **c** shows the pumps that are controlled by the task to deliver the liquid food contained in the syringes (i.e. milk, flavored milk, a mix of the two milks, and water)

Further, we advise to have easy access to a sink for washing off the electrode gel and prepare the food stimuli.

3.2. Prior to the day of the experiment, a brief outline of the procedures allows participants to know what to expect. Specifically, participants should be informed that a certain number of small surface electrodes will be attached to their face. The true purpose of these electrodes (i.e. EMG recording) is often only revealed after the experiment, to not increase participants' self-awareness about their facial movements (*see* **Note 9**). Regarding skin treatment, verify if a history of skin allergies and sensitivity to cosmetics or lotions exists, in which case the participant might have to be excluded.

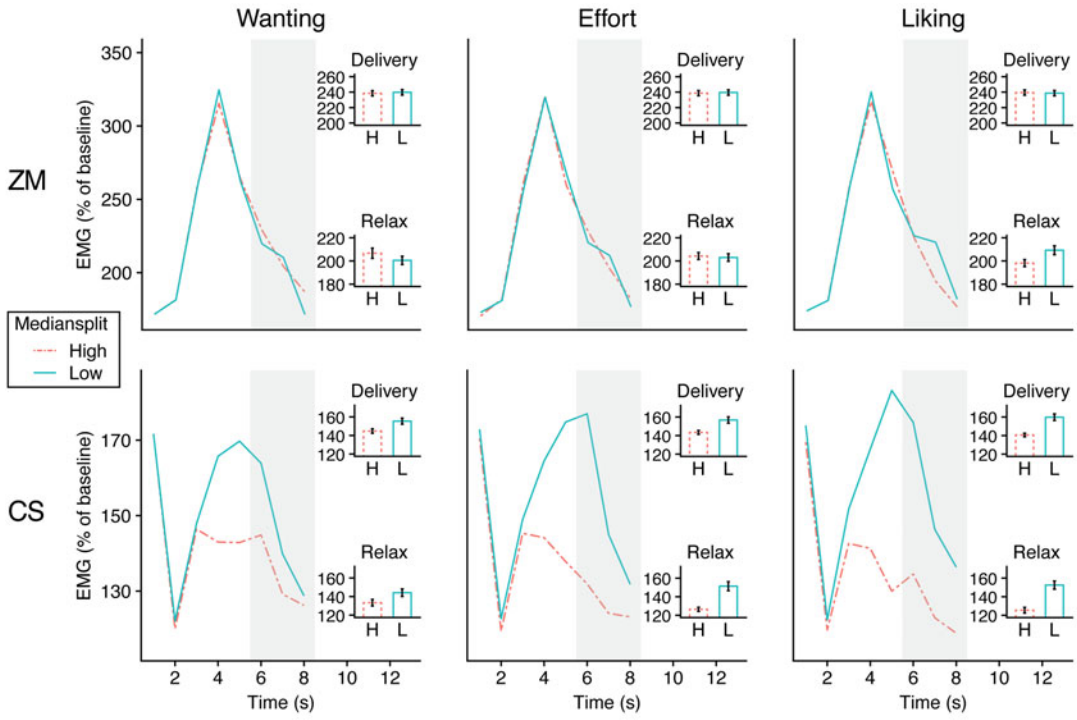

Fig. 2 Mean ZM (top) and CS (bottom) activity in response to the delivery of high versus low (according to the effort exerted or wanting and liking ratings) food rewards. Note the large activation linked to the swallowing around 4 s, which tends to decrease in the relax phase from 5.5 s (grey area). This artifact can mask your effects of interest. (Modified from Korb et al. [1] with permission from Elsevier)

Moreover, for paradigms involving the recording of lower face muscles, inform male participants that they should shave the morning of the experiment day (*see* **Note 10**).

3.3. Set up the software workspace for facial EMG acquisition and display. This procedure allows the EMG signal to be amplified and converted from analog to digital by the amplifier system, to be displayed, analyzed online, and stored for offline preprocessing and analyses (Fig. 3). Generally, workspace setup is performed once, when experimental parameters are established; settings can be saved and then loaded in the workspace for future acquisitions. Central parameters include definition of channels, electrode resistance, sampling rate, amplitude resolution (and range), and data filtering. Specifically, channel definition depends on the number of muscles to monitor; each channel is assigned to each muscle of interest. In a bipolar montage the difference of potential as detected by the pair of active electrodes on the target muscle (and the ground electrode) is reported, therefore the resistance of the electrodes must be specified as well (usually it is reported on the lead and other devices in series between the

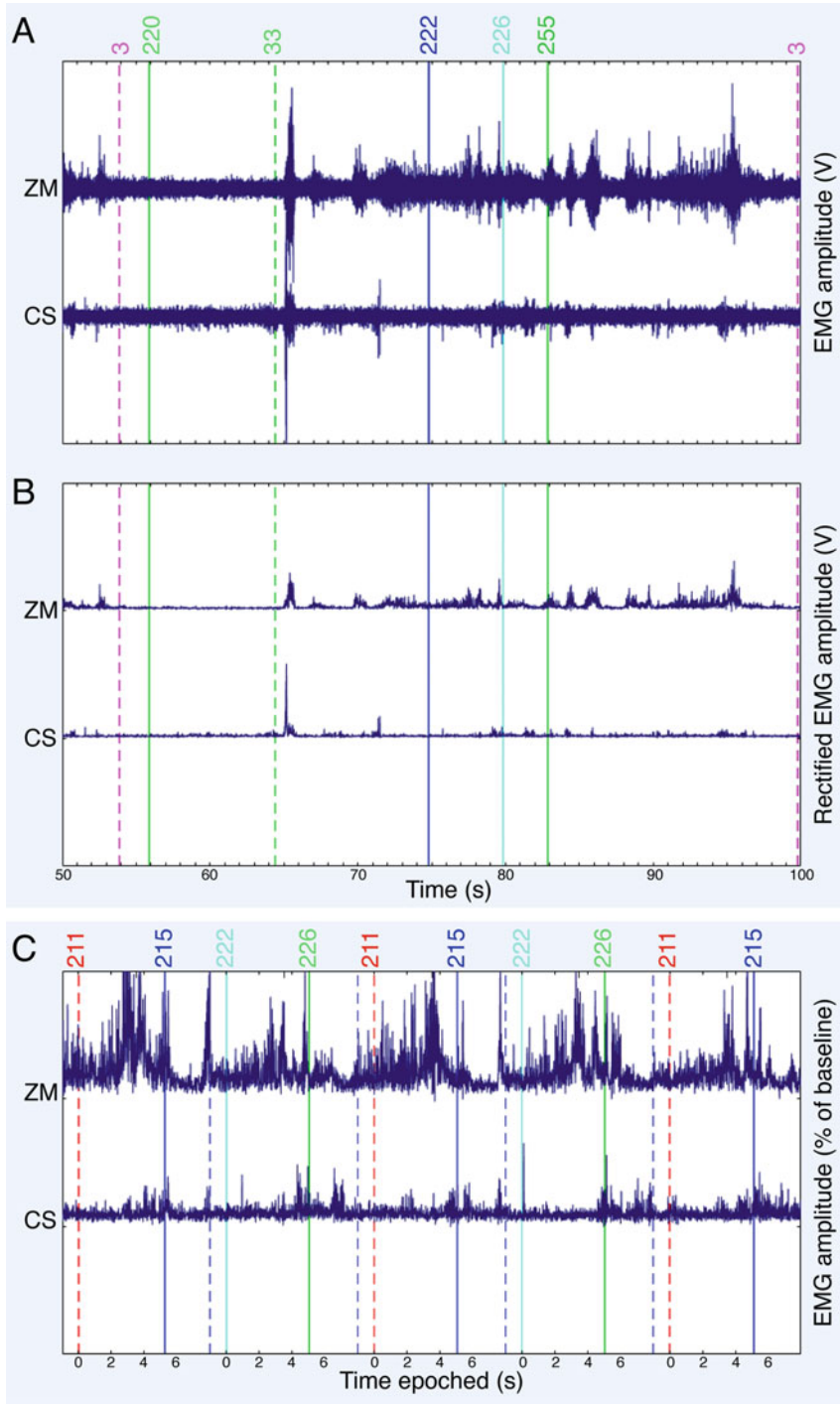

Fig. 3 Effects of food delivery on the EMG signal are evident on a single trial level, as shown on the EMG traces. Panel **a** shows the raw facial EMG signal of one trial (approx. 50 s). Vertical lines represent markers that label the various events (e.g. 3 flags the beginning of a new trial and 222 the beginning of the food delivery period). The ZM and the CS muscles are shown in separated channels. Panel **b** displays the signal of the same trial, digitally filtered (high-pass filter: 20 Hz; low-pass filter: 400 Hz; notch filter: 50 Hz), smoothed (40 Hz lowpass filter), and rectified. Panel **c** shows the EMG signal of five different trials expressed as percentage of the relative trial baseline (period during fixation cross). Markers 211 and 222 indicate the delivery of high and low reward food trials; marker 215 and 226 indicates the start of the relaxation period. Notice the large activation of the ZM muscle during food delivery, while the CS muscle shows little to no deflection

electrodes and the amplifier, or operating manuals). The sampling rate defines the rate of digital acquisition of the analog signal; if it is too low, aliasing effects may occur, resulting in loss of information. Therefore, when sufficient storage capacity is available, a high sampling rate is recommended while down-sampling can be performed offline. According to the Nyquist sampling theorem, sampling rate should be at least twice the highest frequency of interest in the signal. Given that the facial EMG signal is typically measured between 20 to 400 Hz, the sampling rate should be 800 Hz or higher, with some guidelines suggesting up to 2048 Hz [27]. If high frequency noise is expected (e.g., concurrent EMG-fMRI acquisition) the highest available sampling rate (e.g., 5–20 kHz) is recommended to allow proper offline signal processing and artifact removal. For raw acquisition, filtering should be kept to a minimum to allow wideband monitoring (*see* **Note 11**); set the lower edge cut-off between DC brain potential and 1 Hz (e.g. 0.4 Hz), the upper edge cut-off at approximately 500 Hz (e.g. 512 Hz) [30]. For display and offline analysis, common parameters involve high-pass filter at 20 Hz, low-pass filter at 400 Hz, and a sharp notch filter around the power line frequency (e.g., 49–51 Hz in Europe, or 59–61 Hz in North America). Amplitude resolution is recommended to be ≤0.5 μV; however, note that this parameter affects the voltage range (*see* **Note 12**). The voltage range in normal conditions would be of approximately 5–10 mV; pilot experiments can assist the decision on this parameter.

3.4. Localize the target muscles on the resting subject. For this purpose, see Fig. 4, but also consider consulting guidelines [22, 23] and atlases of main body muscles [25] for electrode placements. Although facial expressions are fairly symmetrical on both sides of the face [31], most typically record from the left side only to simplify the set up (thus requiring only half the electrodes that would be needed for bilateral recording), and because the left side of the face is considered to be more expressive than the right [32–34]. In line with this, we have recorded from the left half of the face. The belly of the muscle can be found visually using anatomical landmarks. Palpation and tactile localization of the muscle can be performed by experienced staff whilst the participant performs movements that result in contractions of the targeted muscle. To maximize selectivity and sensitivity, thus avoiding crosstalk from nontargeted muscles, active electrodes should be placed longitudinally with respect to the muscle fibers, next to the center of the muscle belly [25] (*see* **Note 13**). Avoid electrode sites that hinder vision or movement (think of the

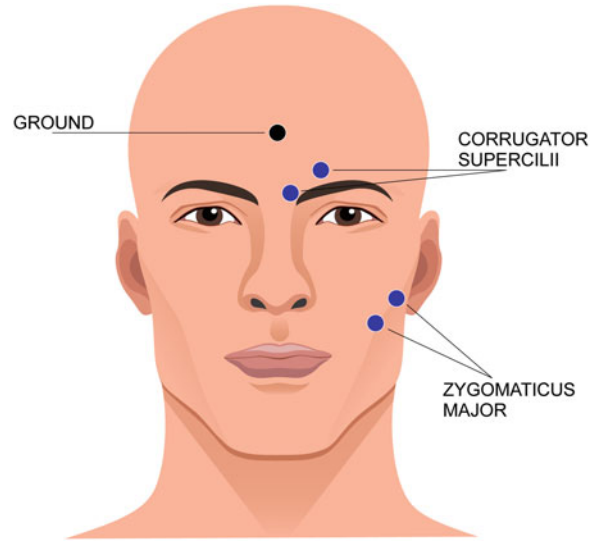

Fig. 4 EMG electrode locations. Schematic representation of the EMG electrode locations for the recordings of the left CS and left ZM muscles

electrode leads placement as well). The ground electrode, also functioning as reference, will be placed elsewhere on the participant's body to keep the amplifier potential in range, usually in a convenient location on electrically inactive tissue and away from recording electrodes, e.g. upper central forehead.

3.5. Carefully clean and wipe the selected sites using an abrasive paste with swabs or tissues (*see* **Note 14**). Remove abrasive paste residuals with a wet cloth. Accurate cleansing of the outermost layer of the skin is essential to reduce electrode–skin impedance. Dead cells rich in keratin form an insulating sheath over the skin surface which creates an unwanted low pass filter [26].

3.6. Gel the electrodes avoiding excessive quantities since this may cause gel spreading beyond the desired surface preventing proper electrode–skin adhesion and possibly inducing bridging or cross-talk effects. Apply the electrodes over the selected and cleansed areas, connect the leads to the amplifier hardware. Fixating the electrode leads to the subjects' body (e.g. by taping them to their upper back) reduces their sway and should be considered as long as it does not cause discomfort or alters natural behavior.

3.7. Activate the EMG system. Initialize the computer and the software for data acquisition (Fig. 1b). Firstly, test whether the trace is modulated by voluntary contraction. Then, check the electrode–skin impedance on the EMG recording software; optimal values depend on the input impedance of the

amplifier, so that input would be 10–100 times greater; for example, amplifiers with input impedance of 1 MΩ tolerates up to 10 kΩ of electrode–skin impedance [25]. As a general rule of thumb, it must be <15–20 kΩ; however in noisy environments where offline signal processing will be abundant, impedance <5 kΩ is recommended. Importantly, impedance differences between the active electrodes recording a same muscle should be minimized (<20%). Note that impedance may decrease further with time [26].

3.8. Instruct and prepare participants for the task. Invite them to keep facial muscles relaxed during the session and avoid large head movements. A few practice trials prior to the experimental task allow participants to familiarize with the procedures and the experimenters to ascertain the correct functioning of the instrumentation. Specifically, inspect the EMG signal being recorded (e.g., low noise, no saturation effects, reliable signal), check the presence of task markers on the trace since they are crucial to be able to extract periods of interest in the acquired data, and make sure that recordings have been saved properly to the hard drive.

3.9. Begin the experimental session. Start the EMG recording at least a few seconds before the task is launched, and do not stop it before the task is completed in its entirety. For longer experiments we suggest splitting EMG recording in several files in order to have one file for each experimental session/block. There is no agreed-upon minimum number of trials per participant and condition, but given that EMG can be contaminated by electromagnetic noise and other artifacts, and that facial responses to rewards in healthy adults often consist of small and variable changes in muscle activation, we recommend including several repetitions per condition. For example, we have recorded 16–20 trials per food condition (i.e. high and low food rewards) [16, 17]; notably, Sato and colleagues [19] have found a significant association between increased subjective liking and decreased CS activation during the consumption of different foods with just 18 trials in total, per participant.

3.10. As the session ends, switch off the amplifier, disconnect the cabling, and remove the electrodes. Provide the participant with a tissue to remove gel residuals. Clean reusable electrodes with water (unless otherwise stated in operating manual), accurately remove gel residuals, dab them with a towel, and let them air-dry.

3.11. Preprocessing of the EMG data can be performed in Matlab using scripting and the EEGLAB toolbox. The exact procedure may vary depending on the precise paradigm, but will

generally involve bandpass filtering, rectification, smoothing, epoching, and baseline correction (or express as percentage of the baseline). Adaptive filter procedures can be used to remove electrocardiogram noise [35]. Nevertheless, this noise is minor in facial EMG, as compared to trunk EMG for example, and the main electrocardiogram components are in the low-frequency bands [36, 37], which will be removed by applying a standard 20 Hz high-pass filter (*see* **Note 11**). For a standard EMG preprocessing Matlab script, which you can adapt to your needs, see this link: https://sebkorb.files.wordpress.com/2016/10/standard_emg_analyses_skorb_2015.pdf

3.12. Statistical analyses can be carried out by averaging the EMG over time – either over the entire duration of the trial or over smaller time windows of one or several seconds (keep in mind that having too many time windows and analyzing them as a categorical factor in a linear regression or repeated measures ANOVA will impact the power of your statistical tests). You can conduct statistics either in Matlab or after exporting the data as, for example, tab-delimited text file, in R, SPSS, or other statistical software of your choice.

3.13. Facial EMG can also be combined with fMRI for concurrent recording resulting in a powerful tool to investigate the spatial dynamics of brain activity and the correlated facial muscular responses [38–41]. Here we provide a few recommendations for EMG-fMRI paradigms in food research. During fMRI there are two major concerns: materials must be MRI compatible and MR-induced artifacts on the EMG data must be minimized during acquisition and removed offline. Therefore, make sure that the hardware entering the scanner room is certified for fMRI environment use, while noncompatible items remain outside in the control room (Fig. 5). In our group (respective publications are in preparation) we use a Brain Products (Brain Products GmbH, Germany) amplifier system; the amplifier and battery lie inside the scanner at the head end of the bore, other hardware modules are in the control room or in the technical room. The pumps to deliver food stimuli are kept in the control room while only the plastic tubes running through the waveguide reach the participant, who lies inside the scanner. The main cabling in the scanner room consists of fiber optic wires. A major concern for participant safety is the possibility that the metal parts of the electrodes and the electrode-amplifier leads heat up during MR acquisition. To prevent this, the leads of MR-compatible electrodes are covered by a spiral tube sheath to avoid direct contact with the

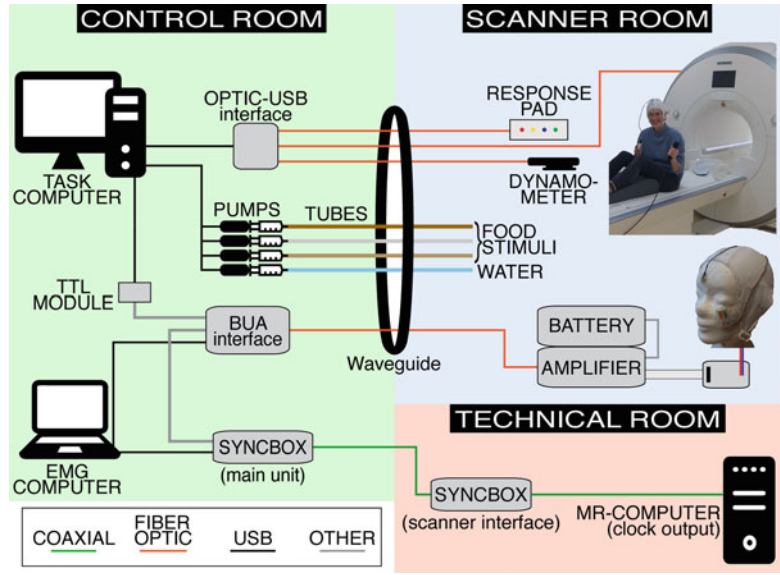

Fig. 5 EMG-fMRI setup. Schematic representation of the hardware connections for the setup of the Korb et al. [16, 17] task in MR environment combining facial EMG and fMRI

skin, and moreover they are as short as possible to prevent the formation of cable loops, which can overheat and may burn the participant or damage the equipment [42]. For the same reason, the electrodes are fixed to a cap worn by the participant – this helps to keep cabling neatly in place. Lastly, to minimize the risks of electrodes overheating, adopt only specifically tested MR acquisition sequences that are allowed by the manufacturers of the EMG amplifiers/electrodes. The EMG signal is greatly affected by the fMRI acquisition, which causes artifacts that at first completely conceal the myogenic potentials (Fig. 6). Synchronizer devices enable EMG sampling to be synchronized with fMRI volume/slice acquisition, allowing the later implementation of correction strategies to remove artifacts [e.g. 43, 44]. Moreover, slight displacements of the leads inside the magnetic field can cause further unpredictable artifacts. It is therefore recommended to place sandbags above and soft pads below the amplifier and the electrode leads to attenuate the vibrations of the scanner and to limit major facial movements as much as possible (e.g. use liquid instead of solid food). Lastly, we advise to keep the electrode leads and the amplifier in the center of the scanner borehole, where the magnetic field will be less distorted due to gradient movements.

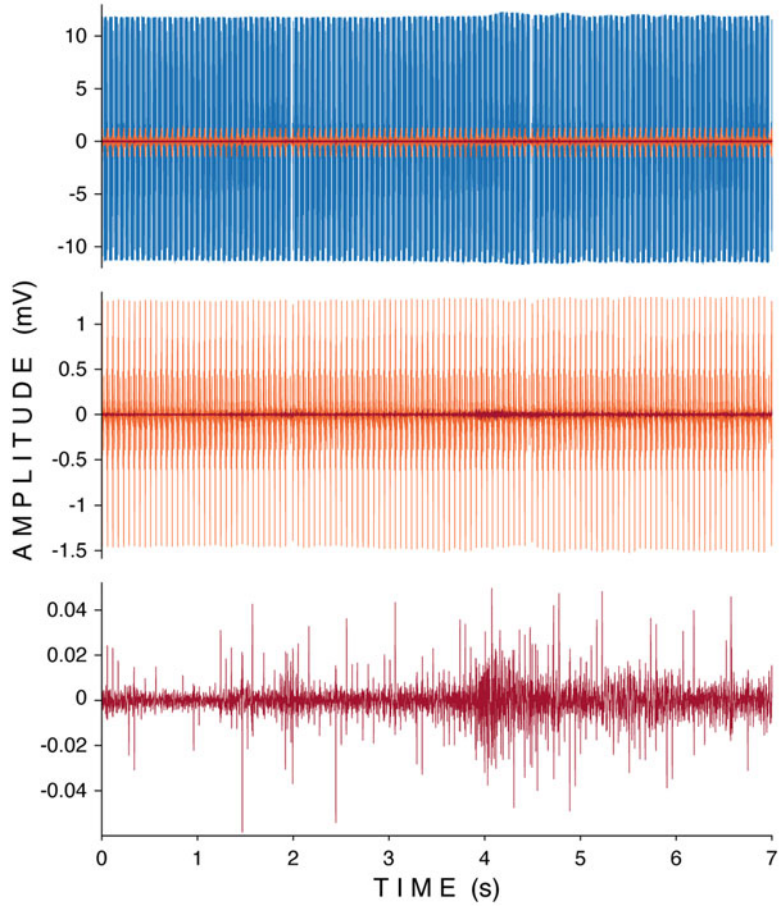

Fig. 6 Specimen of EMG signal processing acquired during fMRI from the ZM muscle. The fMRI artifact was removed using the AAS method [44]. The graphs represent 7 s of food stimulus delivery as in the task described in [29]. The top panel shows the superimposed plots of the raw (blue), the filtered (orange) and the clean (red) EMG trace. In the middle panel, superimposed plots of the filtered and clean EMG trace. In the lower panel, the plot of the clean EMG trace. Notice how filtering and cleaning allow to remove the large-amplitude fMRI artifact and recover the smaller-amplitude EMG, making an increase in ZM contraction accompanying milk swallowing (starting at 4 s) visible

4 Notes

1. Most of the instruments described here are also suitable for other techniques measuring electrophysiological activity (e.g., electroencephalography).
2. Because they substantially determine signal quality, we report some critical specifications of EMG amplifiers. According to recent guidelines [27] input impedance at 50/60 Hz should be ≥300 MΩ (80 MΩ for battery-powered amplifiers); common

mode rejection ratio should be ≥90 dB; gain should be programmable or generally ≤1000 to avoid saturation; and input referred noise of 1–2 μVRMS in the bandwidth of interest (i.e., approximately 10–500 Hz). Besides a TMSi amplifier, we have also used in our group a g.USBamp amplifier (g.tec Medical Engineering GmbH, Graz, Austria) and a BrainAmp ExG MR system (Brain Products GmbH, Germany), which is MRI compatible (Fig. 5).

3. The surface of ordinary EMG electrodes is of silver/silver chloride (Ag/AgCl) sintered nonpolarizable material. These may outperform gold-based electrodes which are less prone to oxidation but polarizable and may alter signal filtering. Of common usage are also disposable wet hydrogel, direct contact, or floating electrodes. For facial EMG the use of small electrodes (recording diameter of 3–5 mm, housing diameter 7–10 mm) is preferable to optimize interelectrode distance (and minimize cross-talk effects), but also to prevent excessive high-frequency removal [45]. Despite the small size of the electrodes the placement of several electrodes on a relatively small area is discouraged since facial movements may be hindered and subjects may experience odd haptic sensations interfering with the object under study.
4. Electrode-to-amplifier cabling should be as short as needed to prevent the production of movement artifacts due to cable swinging and antenna effects picking up power line noise.
5. The recording software is usually provided together with the hardware by manufacturers. Options are manifold, also open-source tools, for example Matlab- or Python-based programs, can serve the purpose and can operate on Linux systems too [46].
6. Disposable hydrogel electrodes are usually pre-gelled, while the application of conductive electrode gel or paste with nondisposable electrodes (e.g. Multitrode or ED6) is necessary.
7. Liquids were preferred to solid foods to avoid mastication, which involves major recruitment of the facial muscles – in our case the ZM would be extensively engaged. The swallowing of liquids generates facial movements of lower intensity, and subsequent muscle relaxation is quicker in most individuals (Fig. 2). The delivery and the dosage of liquid stimuli is also easier to control employing electronic pumps. Considering the repetition of trials, administer rather small quantities of food, which makes it easier to consume and taste the food, but also prevents its rewarding properties to decline and satiety effects to set in too rapidly. Thus, in each trial 2 mL of liquid was delivered. At the end of the trial 2 mL of water was provided to rinse the mouth and prepare the following trial. To compare

the hedonic experience between different foods, and rule out an important confounding factor, sugar was added to the milk to equalize its caloric content to that of the cocoa-milk.

8. A baseline period of a few seconds (we suggest at least 2 s varying randomly between trials) allows the subject to rest and to relax muscles before a new external event takes place. Further, for offline analysis the EMG acquired during this phase constitutes a baseline condition that is fundamental to remove nonspecific drifts of voltage, due to internal or external causes. This is obtained through baseline correction, generally subtracting the signal from the baseline period, or expressing the signal as percentage of the baseline (Figs. 2 and 3c).
9. Especially with naïve or skeptical participants, emphasize the fact that electrodes are for recording, and that no electrical shocks will be delivered. Note also that some participants or (sub)clinical populations (e.g. people on the autism spectrum) may not feel comfortable when touched, especially over the face by unknown experimenters during skin cleansing procedures and electrode montage. In these cases, inform adequately the participant before taking part in the experiment.
10. Facial hair may undermine the successful application of the electrodes; hairs can reduce the adhesive properties of the pads and form irregularities on the skin surface that cause skin-electrode air gaps leading to higher impedance. Therefore, in the case that target muscles (e.g., ZM) lie below dense hairy skin such as beard, we suggest to ask participants to come to the lab clean shaven (or shave such portion of face using foam and disposable razors).
11. Digital filters can be applied post-hoc for offline analyses to attenuate specific ranges of frequencies and thus clean the EMG signal from artifacts and nonmyogenic potentials (Fig. 3a, b). Typically, baseline drifts due to eye movements, perspiration, DC offset and low frequency artifacts occupy the low frequency spectrum at approximately 0–25 Hz and can be removed with high-pass filters. Considering that EMG signal power is minimal and negligible above 350–400 Hz, low-pass filters can remove high frequency noise. Therefore, in normal conditions, to remove artifacts and clean the signal, apply a high-pass filter at 20 Hz (for facial muscles) and a low-pass filter between 350 and 500 Hz [30].
12. The resolution-range trade off depends on the gain and the bits of data acquisition capability of the digitalization system. In fact, although higher resolutions are preferable, the resulting voltage range will be reduced and narrower range may lead to signal saturation, especially in case of strong muscular contractions or high environmental artifacts (e.g., fMRI acquisition artifacts).

13. Because of the interweaving and overlapping of muscles, the potential recorded from one electrode may have low muscular specificity. The exact placement of electrodes allows for the maximal accuracy and improves external validity through standardized systems. We placed ZM electrodes midway of the line connecting the cheilion and the auricular tragus, approximately 3 cm apart, slightly off the intersection with the line descending from the exocanthion. Eventual presence of beard will require shaving. CS muscle site is on the forehead, a little above the eyebrow; place the electrodes on an oblique line originating from the glabella ~1 cm above the nasion slightly off the midline, about 2 cm apart (Fig. 4).
14. Gentle but firm cleansing is recommended, since hard friction can cause long-lasting reddening and excoriation, which are particularly undesirable on the face.

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Chapter 16

Measuring the Postauricular Reflex as an Indicator of Appetitive Processing

Yoann Stussi, Sylvain Delplanque, and David Sander

Abstract

The postauricular reflex is a muscular microreflex behind the ear that is elicited by brief and abrupt sounds. Although it is vestigial in humans, it has been shown to have a potentiated response to the eliciting sound during the presentation of stimuli usually considered as pleasant relative to neutral or unpleasant stimuli across different sensory modalities; this response being even more prominent for appetitive stimuli, such as food and erotic images. This reflex can thus serve as a psychophysiological indicator of appetitive processing and could be a valuable tool for food science. Here, we describe a protocol that can be used to measure and analyze the postauricular reflex in humans. Our goal is to provide information on the materials and methods needed to record the postauricular reflex and to illustrate the preprocessing steps and quantification strategies that can be implemented to score this reflex.

Key words Postauricular reflex, Appetitive processing, Emotion, Positive affect, Psychophysiology, Electromyography

1 Introduction

The postauricular reflex is a muscular microreflex occurring behind the ear; it pulls the ear upward and backward [1] in response to brief and abrupt sounds, such as loud noises or noise clicks. It was first reported in humans as an electrical potential over the postauricular muscle evoked by click sounds [2]. The postauricular reflex is generally imperceptible in humans (i.e., it does not produce visible motion in the pinna) and measured using surface electromyography (EMG) by placing electrodes over the postauricular muscle (*see* Fig. 1) behind the ear [3]. This reflex has a typical response latency of 9–11 ms following the onset of an acoustic probe, showing a rapid response that is even faster than other reflexes similarly triggered by sudden and intense sounds such as the startle eyeblink reflex (latency of 45–50 ms) [4] (*see* **Note 1**). The neural circuitry of the postauricular reflex involves a multisynaptic pathway from

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Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_16,

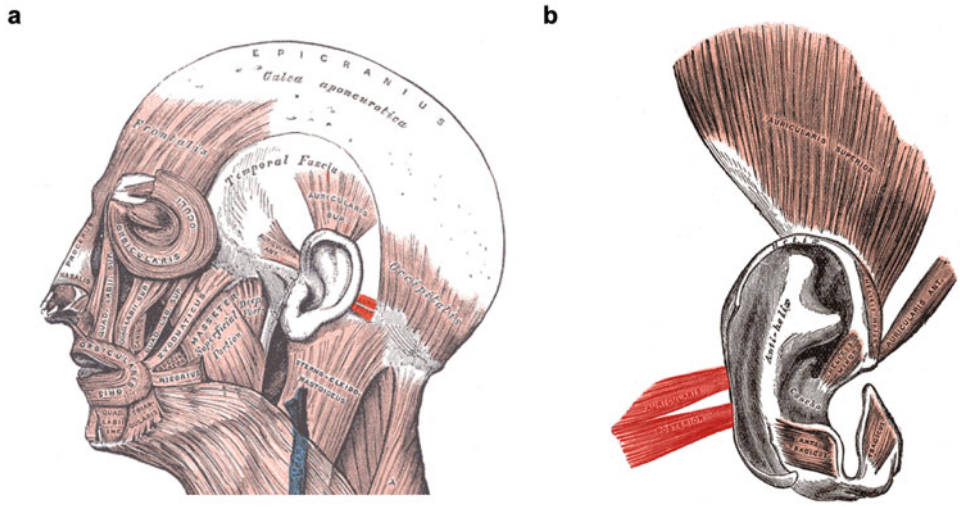

Fig. 1 Illustration of the postauricular muscle. Location of the postauricular muscle (highlighted in red) in relation to (**a**) the facial muscles and (**b**) the auricular muscles. (Modified from ref. (36). In the public domain)

the ventral cochlear nucleus to the trapezoid body in the caudal pons to the medial subdivision of the facial motor nucleus, which in turn activates the postauricular muscle [5, 6] (*see* Fig. 2). Importantly, even though the postauricular reflex is vestigial in humans, it is still modulated by multiple attentional and affective factors and has the potential to provide information about a variety of psychological processes [6].

For instance, studies in humans have shown that attention can modulate the amplitude of the postauricular reflex [4–7]. It has been reported that the postauricular reflex is larger behind the attended ear in comparison to the unattended ear in an auditory detection task [4]. The postauricular reflex also exhibits the attentional and sensory phenomenon of prepulse inhibition [4, 7]–in which the reflex is inhibited by the occurrence of a weaker stimulus (prepulse) preceding the reflex-eliciting stimulus (pulse)–at short lead intervals (i.e., time between the prepulse and the pulse; e.g., 100 ms). Longer lead intervals (e.g., 300 ms) conversely elicit prepulse facilitation of the postauricular reflex [7, 8]–which consists in the opposite reaction wherein the reflex is potentiated following the presentation of a less intense prepulse stimulus. Attention to the prepulse stimulus, however, does not enhance the prepulse inhibition of the postauricular reflex, thus differentiating it from the startle eyeblink reflex [4] and possibly reflecting its simpler neural circuitry [9]. Moreover, continued attention to visual or auditory foreground stimuli inhibits the postauricular reflex, the more complex the foreground stimulus the greater the postauricular reflex inhibition [6]. Indeed, evidence shows that the sound-evoked postauricular reflex amplitude is generally smaller during

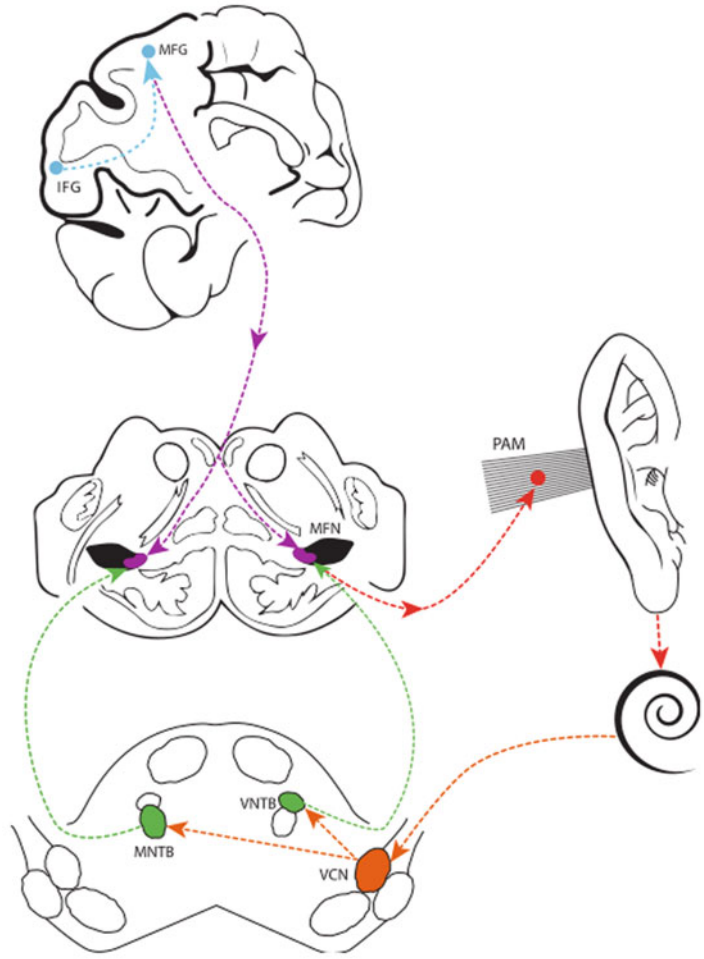

Fig. 2 Posterior view of a model of the postauricular reflex neural circuitry. The lower half (orange, green, and red arrows) shows the neural circuitry generating the postauricular reflex. The upper half (blue and purple arrows) depicts the putative neural activity that potentiates the postauricular reflex amplitude during pleasant versus neutral stimuli (see Ref. (6)). The spiral shape on the figure represents the cochlea. VCN = ventral cochlear nucleus, VNTB = ventral nucleus of the trapezoid body, MNTB = medial nucleus of the trapezoid body, MFN = medial facial nucleus, PAM = postauricular muscle; IFG = inferior frontal gyrus (left), MFG = middle frontal gyrus (left). (Figure by Natosha D. Benning and Stephen D. Benning (2017); available at https:/doi.org/10.6084/m9.figshare.4233098 under a CC-BY4.0 license and adapted from Ref. (37))

simultaneous picture or sound processing than during intertrial intervals containing only a fixation cross [10–12].

In addition to its attentional modulation properties, a key characteristic of the postauricular reflex is its sensitivity to affective modulation. Research has shown that the postauricular reflex in

response to the eliciting sound is specifically potentiated during the presentation of pleasant stimuli compared to neutral or unpleasant stimuli across different sensory modalities [8, 10–18]. More precisely, in the visual domain, the postauricular reflex magnitude is the largest when viewing appetitive images, such as images of food or erotic scenes, and larger than when viewing other types of positive images that do not directly relate to appetitive (approach) motivation, such as images of adventure or pleasant nature scenes [6, 18]. This indicates that the postauricular reflex can provide a psychophysiological index of appetitive processing in humans [6, 10, 11, 18]. Importantly, a growing body of evidence further suggests that the postauricular reflex is modulated not only while experiencing the appetitive or pleasant stimuli themselves [10, 12, 13, 18] but also in response to visual cues that predict the occurrence of a rewarding outcome (i.e., in anticipation of the reward) such as a pleasant odor [11] or juice [19]. Of note, the postauricular reflex potentiation to the cues extinguished when the reward was no longer delivered [11, 19], thus highlighting that the postauricular reflex is sensitive to appetitive contingencies. These findings suggest that the postauricular reflex possibly tracks the current reward value of the stimulus, which likely reflects the interplay of multiple reward components and not exclusively positive valence [20, 21]. In that sense, the postauricular reflex appears to be a relevant psychophysiological measure in the study of affective processes related to food stimuli and could be a valuable tool for food science. For instance, specific eating disorders such as binge eating and restrictive eating have been shown to increase and decrease, respectively, the postauricular reflex reactivity in response to food images [17, 22], highlighting that the postauricular reflex can be used to assess appetitive motivation to food. More generally, the postauricular reflex could offer an indirect psychophysiological index to characterize the appetitive properties of food stimuli without relying on (or in addition to) subjective reports.

In this vein, we report here a protocol that can be used to measure the postauricular reflex. This protocol is based on our work characterizing this reflex as an indicator of appetitive olfactory conditioning in humans [11], wherein the sound-elicited postauricular reflex is potentiated during the presentation of a stimulus predicting the delivery of a pleasant odor compared to a stimulus paired with odorless air. We first detail the materials and procedures used to record the postauricular reflex, then highlight the important aspects to consider to adequately measure it, and finally describe and illustrate the signal preprocessing steps and the quantification strategy that can be implemented to analyze the postauricular reflex.

2 Materials

2.1 Electromyography Apparatus

1. Amplifier system: A BioSemi ActiveTwo amplifier system (BioSemi Biomedical Instrumentation, Amsterdam, The Netherlands) with a sampling rate of 2048 Hz at direct current (*see* **Note 2**) is used to collect, amplify, digitize, and store the postauricular EMG signal (bandwidth 0.1 to 417 Hz).
2. Electrodes: Four 4 mm contact diameter Ag-AgCl active electrodes are used, two to measure the postauricular reflex and the two others serving as a recording reference and ground electrode (see http://www.biosemi.com/faq/cms&drl.htm for further information).
3. Alcohol-imbibed cotton pads: An alcohol-imbibed cotton pad is used to clean the site of the electrode placement and reduce skin impedance before positioning electrodes, while a second alcohol-imbibed cotton pad is used to remove the gel from the electrodes after their use.
4. Electrolyte gel: A commercially available conductive electrolyte gel (SignaGel Electrode Gel®, Parker Laboratories Inc., Fairfield, NJ) is applied on the electrodes to provide a conductive medium between the participant's skin and the electrodes, facilitating the passing of the current from the skin to the electrodes.
5. Wet cotton swabs and distilled water: Wet cotton swabs and distilled water are used to remove the gel from the participant and the electrodes after their use.

2.2 Acoustic Probe and Audio Apparatus

1. Acoustic startle probe: An acoustic startle probe consisting of a 50 ms white-noise burst (105 dB; *see* **Note 3**) with a nearly instantaneous rise time (<1 ms) is used to elicit the postauricular reflex.
2. Loudspeakers: Loudspeakers are used to present the acoustic startle probe binaurally (*see* **Note 4**).
3. Presentation software: MATLAB (version 7.8; The Mathworks Inc., Natick, MA) with the Psychophysics Toolbox extensions [23, 24] is used to control the delivery of the acoustic startle probe (*see* **Note 2**).

2.3 Data Preprocessing and Quantification

1. Analysis software: BrainVision Analyzer software (version 2.1; Brain Products GmbH, Gliching, Germany) is used to preprocess and quantify offline the postauricular reflex (*see* **Note 2**).

3 Methods

3.1 Participant and Skin Preparation

1. Invite the participant to seat in a comfortable chair (*see* **Note 5**).
2. Ask the participant to turn off their phone to avoid any possible magnetic interferences with the EMG apparatus and to remove any piece of jewelry (e.g., earrings) that might interfere with the electrode placement.
3. Give the participant an alcohol-imbibed cotton pad and ask them to gently rub it behind their left ear (*see* **Note 6**) and at the top of their forehead to clean their skin. The goal of this procedure is to reduce the impedance between the skin surface and the electrode gel by removing the oil on the skin surface as well as the dead skin cells [25, 26].

3.2 Electrode Preparation and Placement

1. Fill the electrodes with the electrolyte gel (*see* **Note 7**).
2. Ask the participant to pull their left pinna forward and place the electrodes on each side of the tendon of insertion for the postauricular reflex (*see* **Note 6**). This tendon is easily identifiable in most cases as a fibrous strip that connects the pinna and the scalp [10]. Place one electrode directly posterior to the tendon on the pinna surface and place the other electrode on the scalp over the postauricular muscle [3, 9, 10] (*see* Fig. 3; *see* **Note 8**). Crucially, the position of the electrodes on the postauricular muscle can significantly impact the recording of the postauricular reflex [3], which stresses the importance of a consistent and optimal electrode placement across participants.
3. Position the reference and ground electrodes next to each other at the top of the forehead near to the hairline, a site that is relatively inactive electrically [25].
4. Once the electrodes are placed, check their offset potentials. If the offset is outside the [−40, +40] mV interval (for the BioSemi active electrodes system; *see* **Note 9**), remove the electrodes, and repeat the skin preparation and electrode placement procedure.
5. Finally, check the EMG signal for noise artifacts (e.g., 50 or 60 Hz interference from power lines; see Ref. [25]) and to ensure its proper recording.

3.3 Experiment Design and Procedure

1. Once the electrode placement procedure is over, explain the experimental tasks to the participant and give them the instructions.
2. Remind the participant to refrain from moving during the experiment and to look toward the computer screen.

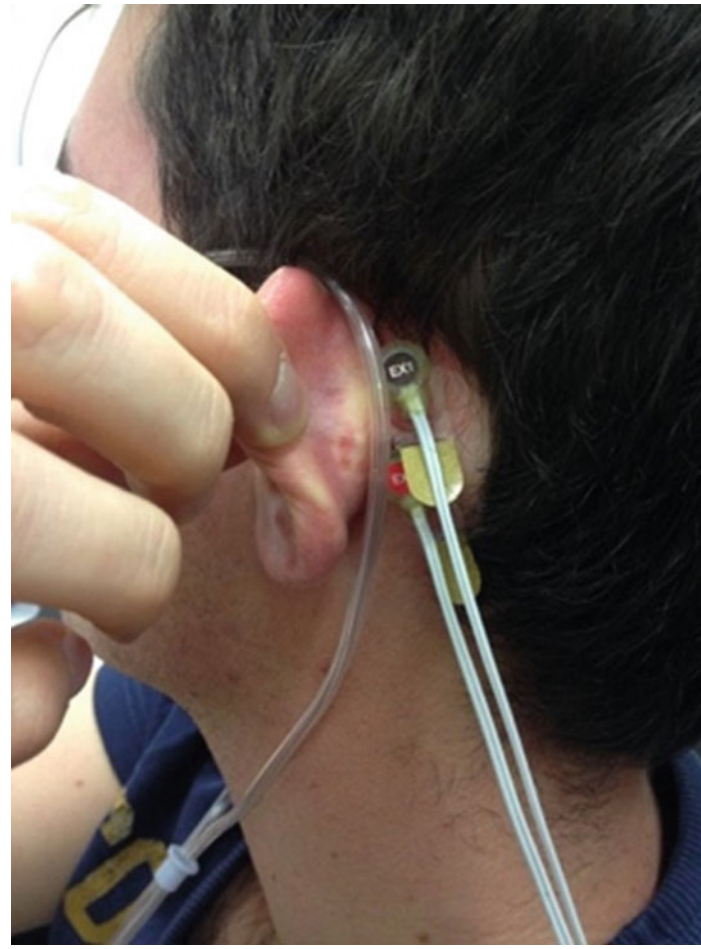

Fig. 3 Placement of surface electromyography electrodes to measure the postauricular reflex in humans. One electrode is typically placed directly posterior to the tendon of insertion on the pinna surface (red electrode) and the other electrode is placed on the scalp over the postauricular muscle (black electrode)

3. In order to habituate the participant to the acoustic probe, start the experiment with the presentation of 10 acoustic startle probes with an interstimulus interval randomly varying between 10 and 20 s (*see* **Note 10**).
4. After the startle habituation phase is completed, start the experimental task. Here, we describe the specific task used in our previous study [11] as an example (*see* **Note 11**). It consists of an appetitive olfactory conditioning procedure, during which a pleasant odor and odorless air are associated with two different geometric cues (*see* Fig. 4a), respectively, across three conditioning phases (habituation, acquisition, extinction; *see* Fig. 4b). The habituation phase comprises four unreinforced presentations of each geometric cues. During the acquisition phase, each cue is presented nine times, and one cue is

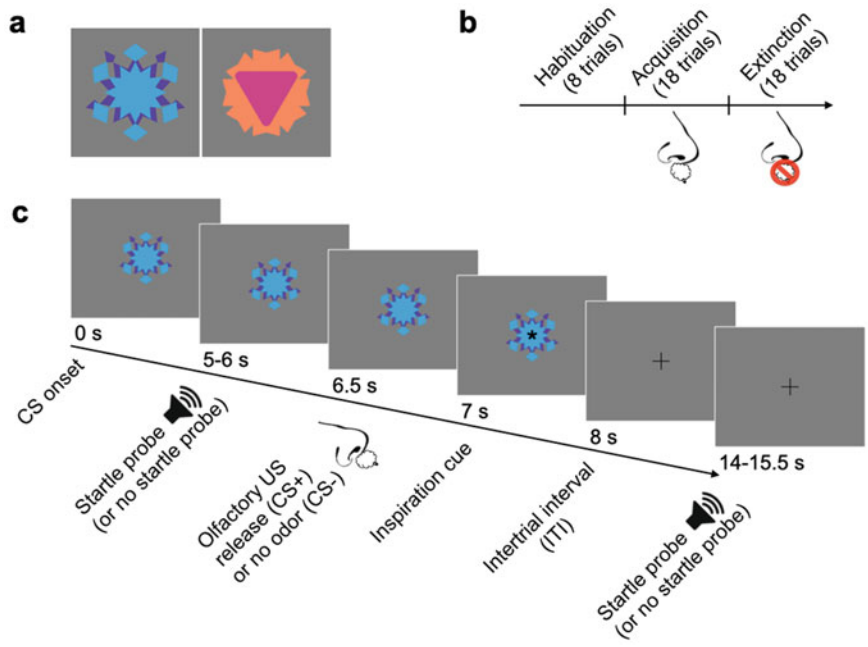

Fig. 4 Illustration of the appetitive olfactory conditioning task. (**a**) Geometric cues used as conditioned stimuli. (**b**) Conditioning phases. (**c**) Trial structure during the acquisition phase. (Reproduced from Stussi et al. (11) with permission from John Wiley and Sons)

systematically paired with the delivery of a pleasant odor (the CS+), whereas the other cue is always associated with odorless air (the CS−). The associations between the cues and the stimulus types are counterbalanced across participants, and their order of presentation is pseudorandomized into two different orders. Extinction includes nine presentations of each cue, and the pleasant odor is no longer delivered during this phase. During all the conditioning phases, a trial (*see* Fig. 4c) starts with the presentation of the cue for a duration of 8 s. The acoustic startle probes are delivered between 5 and 6 s after the cue onset in an equal number of trials for each cue and in approximately 2/3 of the trials (*see* **Note 12**). The pleasant odor is delivered 6.5 after the CS+ onset during acquisition and released via a custom-made, computer-controlled olfactometer with an airflow fixed at 1 L/min delivering the olfactory stimulation rapidly, without thermal and tactile confounds through a nasal cannula [27], for a duration of 1.5 s. An inspiration cue indicating the participant to breathe in evenly is next shown 7 s after the cue onset for 1 s. Each trial is followed by an intertrial interval ranging from 12 to 15 s, during which a fixation cross is presented onscreen (*see* **Note 13**). Additional acoustic startle probes are delivered between 6 and 7.5 s after the cue offset during 1/3 of the intertrial intervals to decrease their predictability. In total, the task includes 44 trials, ranging from 2 (for

the conditions of noninterest during habituation) to 6 (for the main conditions of interest during acquisition and extinction) trials per condition during which an acoustic startle probe is delivered (*see* **Notes 14** and **15**). The appetitive olfactory conditioning task has a total duration of approximately 16 min. Of relevance for the measurement of the postauricular reflex, there are three important factors to consider in particular: (1) the stimulus duration, (2) the time interval between the stimulus onset and the acoustic probe onset, and (3) the number of trials per condition. It is important to take into consideration sensory effects such as prepulse inhibition and facilitation when determining stimulus duration. The interval between the stimulus onset and the acoustic probe onset should be specifically adapted to whether these phenomena want to be prevented (i.e., by implementing a stimulus duration sufficiently important so that the interval can be long enough to prevent the stimulus from acting as a prepulse) or elicited (i.e., by using an interval enabling the stimulus to act as a prepulse). In addition, enough trials should be included to obtain a reliable estimate of the postauricular reflex magnitude within each experimental condition (*see* **Note 14**).

5. At the end of the experiment, remove the electrodes from the participant and give them a cotton pad to clean the gel off of their skin.
6. To remove the remaining gel on the electrodes, clean them using a cotton swab and gently pass them under distilled water and then carefully dry them (to avoid water electrolysis). Lastly, clean the electrodes with an alcohol-soaked cotton pad.

3.4 Acoustic Probe Delivery

1. In order to synchronize the delivery of the acoustic startle probes with the EMG recording, use the presentation software (e.g., MATLAB) to send triggers (i.e., bytes) via the parallel port to the EMG apparatus each time an acoustic probe is delivered.
2. Be careful to time the triggers to be sent right before the command to deliver the acoustic probe is executed (*see* **Note 16**). This procedure allows for registering the time-stamps of the acoustic probe onsets, which is a critical step given that the postauricular reflex is an extremely rapid reflex [4] and its analysis is time-locked to the acoustic probe delivery (i.e., event-related analysis).

3.5 Postauricular Electromyography Signal Preprocessing

1. The postauricular reflex signal preprocessing procedure we describe here follows general EMG preprocessing guidelines [25, 26]. First, calculate a conventional bipolar montage by subtracting the recorded activity of the electrode placed on the pinna surface from the activity of the neighboring electrode placed over the postauricular muscle (*see* Fig. 5a, b).

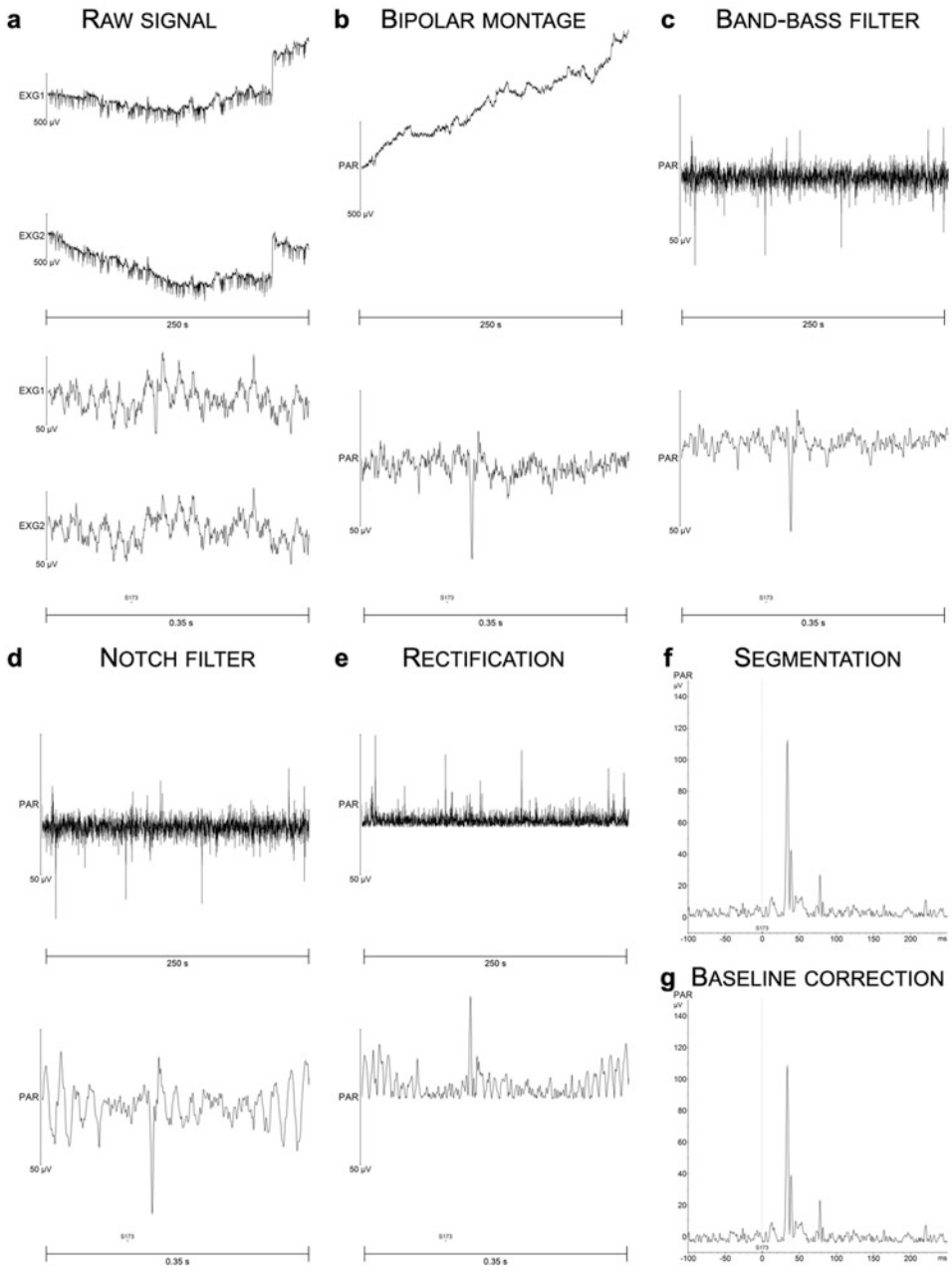

Fig. 5 Postauricular reflex (PAR) signal preprocessing steps. The electromyography (EMG) signal (**a**) is recorded by two electrodes placed over the postauricular muscle (EXG1) and on the pinna surface (EXG2), respectively. A conventional bipolar montage (**b**) is calculated by subtracting the recorded activity of the second electrode (EXG2) from the activity of the first electrode (EXG1). The raw postauricular EMG signal is then band-pass (10–400 Hz) filtered (**c**) and notch (50 Hz) filtered (**d**). The filtered signal is then rectified (**e**). Finally, the signal is segmented (**f**) into epochs starting from 100 ms before to 250 ms after the acoustic probe onset (dotted line), and baseline-corrected (**g**) with the baseline calculated as the average signal during the 50 ms prior to the acoustic probe onset. For panels **a**–**e**, the upper plot depicts the postauricular EMG signal over a period of 250 s, and the lower plot illustrates the signal in response to an acoustic probe within a 350 ms time window

2. Then, filter the raw postauricular EMG signal to minimize noise that is not caused by muscular activity (i.e., low- and high-frequency artifacts that are not comprised within the EMG signal frequency band) and hence increase the signal-to-noise ratio. Specifically, use a digital infinite impulse response band-pass filter (e.g., Butterworth zero-phase filter; 10–400 Hz) (*see* Fig. 5c; *see* **Note 17**). If necessary, apply a notch filter (e.g., Butterworth zero-phase filter; 50 Hz) (*see* Fig. 5d) to reduce noise from alternating current power lines (*see* **Note 18**).
3. After filtering, rectify the postauricular EMG signal (i.e., the EMG data points are converted to absolute values; *see* Fig. 5e). The rectification is performed to avoid the negative and positive components of the postauricular EMG signal waveform cancelling each other out during signal averaging [25] (*see* **Note 19**).
4. The next preprocessing step consists in segmenting the postauricular EMG signal into epochs starting from 100 ms prior to the acoustic startle probe onset to 250 ms after the probe onset (*see* Fig. 5f). We suggest that each segment be then baseline-corrected (*see* Fig. 5g). The baseline can be calculated as the average postauricular EMG activity in the 50 ms preceding the acoustic startle probe onset. Visually inspect all segments and remove by hand segments that are identified as containing excessive baseline shifts (in our study, 96 out of 2310 trials were discarded on this basis, which corresponds to 4.16% of the trials) from the analysis (*see* **Note 20**).

3.6 Response Quantification

1. Given its low signal-to-noise ratio as a microreflex, we suggest quantifying the postauricular reflex by averaging the signal of the rectified waveforms [4, 8–10, 12, 14–17]. For each individual participant (*see* **Note 21**), we recommend averaging the preprocessed postauricular EMG segments across trials within each separate experimental condition (*see* **Note 22**).
2. Score the postauricular reflex magnitude on the resulting aggregate waveform as the baseline-to-peak amplitude for each condition. The peak amplitude is computed as the maximum postauricular EMG activity that occurred within a 5–35 ms time window following the acoustic startle probe onset (*see* **Note 23**).
3. Peaks can be detected automatically using BrainVision Analyzer, but it is important to carefully check them manually afterward. The raw unstandardized extracted peaks (in μV) can for instance be used in the data analysis (*see* **Note 24**).

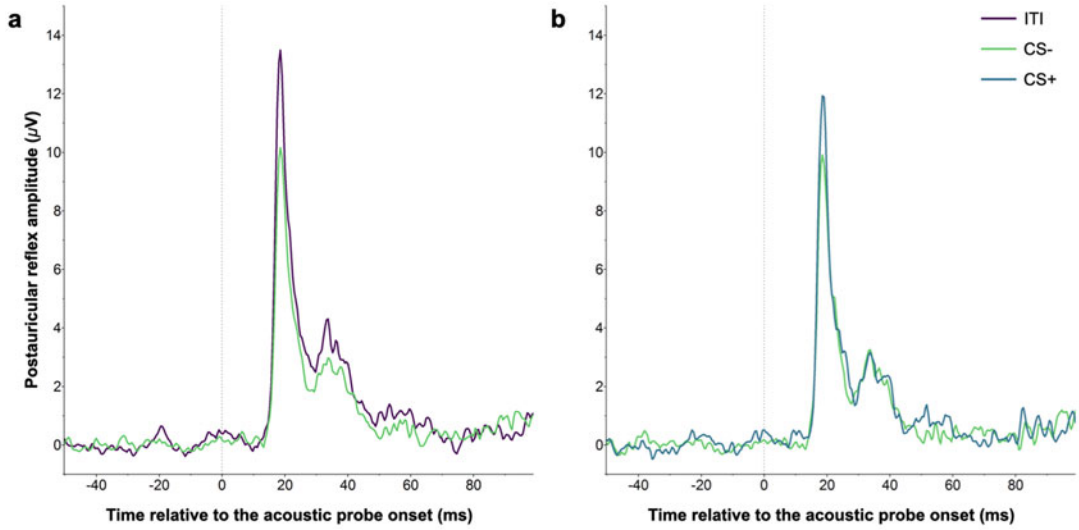

Fig. 6 Modulation effects of the postauricular reflex. (**a**) Grand-averaged postauricular reflex waveforms (14 trials by 55 participants) in response to a 50 ms white-noise acoustic probe at 105 dB during intertrial intervals (ITI) versus the presentation of a geometric cue associated with odorless air (CS−). These results illustrate the inhibition of the postauricular reflex during image processing. (**b**) Grand-averaged postauricular reflex waveforms (6 trials by 55 participants) in response to a 50 ms white-noise acoustic probe at 105 dB during the presentation of a geometric cue predicting the delivery of a pleasant odor (CS+) versus a geometric cue associated with odorless air (CS−). These results show the affective modulation of the postauricular reflex, as reflected by a higher amplitude when viewing a stimulus associated with an appetitive outcome compared to a neutral stimulus. (Adapted from Ref. (11))

To illustrate the modulation effects of the postauricular reflex that are typically found, Fig. 6 depicts the resulting grand-averaged (i.e., averaged across participants) rectified postauricular reflex waveforms for attentional and affective modulation. Specifically, Fig. 6a illustrates the inhibition of the postauricular reflex during a visual foreground stimulus compared to during intertrial intervals where only a fixation cross was presented, thereby reflecting attentional modulation of this reflex by continued attention during image processing. Figure 6b shows the effects of affective modulation of the postauricular reflex, as indicated by a higher postauricular reflex amplitude during the presentation of the geometric cue associated with a pleasant odor than during the presentation of the geometric cue associated with odorless air during appetitive olfactory conditioning.

Finally, Fig. 7 further illustrates the large variability in the postauricular reflex that can be observed across individuals by showing the average postauricular reflex amplitude during the intertrial intervals separately for each participant.

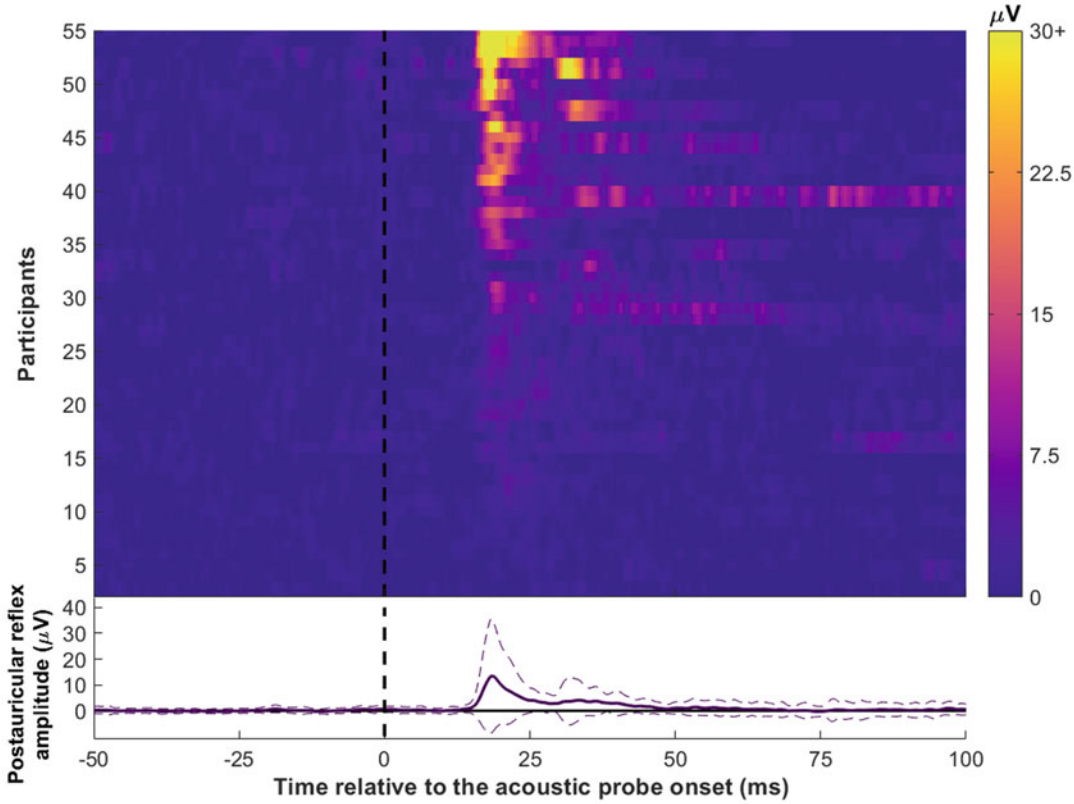

Fig. 7 Interindividual variability in the postauricular reflex. Upper panel: Ranked individual averaged postauricular reflex amplitudes in response to a 50 ms white-noise acoustic probe at 105 dB during intertrial intervals across 55 participants (14 trials). The amplitude was calculated as the maximum voltage within the 5–35 ms time window after the acoustic probe onset (vertical dashed line). Lower panel: Grand-averaged postauricular reflex waveform during intertrial intervals. The solid line represents the average postauricular reflex amplitude across participants and the dashed lines represent ±1 standard deviation

4 Notes

1. The postauricular reflex is often measured at the same time as the startle eyeblink reflex because these two reflexes can be elicited in response to the same acoustic startle probes and require the same materials to be recorded. Some authors describe the postauricular reflex as a component of startle–as is the eyeblink reflex–and more specifically as a vestigial startle response [5]. Of particular interest, the postauricular reflex furthermore presents an opposite pattern of modulation to the startle eyeblink reflex, the latter being potentiated in response to aversive stimuli compared to neutral stimuli and, in some cases, inhibited in response to appetitive stimuli [28]. For this reason, the postauricular and the startle eyeblink reflexes are often considered as being complementary in that they can be used to index appetitive and aversive processing, respectively.

2. The specific equipment described here corresponds to the one we used in our study [11]. However, other EMG recording systems, sampling rates (though a sampling rate of at least 1000 Hz is generally recommended; see Ref. [25, 26]), stimulus presentation software, and analysis software can be used depending on their availability and the researcher's preference.
3. The postauricular reflex can be evoked by both soft clicks and loud noises [8]; however, the postauricular reflex amplitude increases with the acoustic probe's intensity or volume [6, 29]. Accordingly, this aspect should be considered when selecting the intensity of the acoustic probe and weighed against a heightened aversiveness to louder noises, while making sure the participant's hearing safety is not compromised. Another element to consider is the level of background noise in the testing environment, which may act as a prepulse (or alternatively mask a prepulse) [25]. Therefore, it is important that the participant be isolated from environmental noises to control for unwanted influences thereof.
4. Note that most of the studies investigating the postauricular reflex typically used headphones to deliver the acoustic probes [4, 7, 8, 10, 12–14, 17–19]. The calibration of the sound intensity is usually easier to accomplish with high precision using headphones rather than loudspeakers [25]. Headphones might also be preferred in laboratory environments where the delivery of loud noises can be problematic. By contrast, loudspeakers are preferable when headphones might interfere with the electrodes or other sensors [25].
5. Although it has been reported that an upright posture increases background activity in the postauricular muscles [4, 14], participants should ideally feel comfortable enough to be able to perform the entire study with minimal neck and eye movements. In fact, flexing the neck forward [4] or tucking the chin toward the neck [30] have been reported to increase the postauricular reflex amplitude. Similarly, rotation of the eyes toward the sound-eliciting source has been shown to amplify the postauricular reflex and has been suggested to represent a critical factor that can account for a large part of the inter- and intraindividual variability found in the measurement of this reflex [30]. If visual stimuli are used during the experiment, adjusting the chair height is recommended so that the participant's gaze coincides with the center of the computer screen when possible. To control for eye movement in studies that do not use visual stimuli, it may be worth considering using a blank screen with a fixation cross that participants can fixate on when stimuli are presented. A supplementary option to mitigate the possible impact of eye rotation is to record eye

movements via (vertical and horizontal) electro-oculography and exclude from the analysis trials that are contaminated by excessive eye movements.

6. In most cases, psychophysiological studies measuring the postauricular reflex recorded the postauricular muscle EMG activity from both ears [3, 4, 7–10, 12, 14, 15, 17], and typically, the signal is averaged across them. Interestingly, no systematic difference has generally been observed when comparing the postauricular reflex of the left versus the right ear [12].
7. It is particularly important to pay attention that no air bubbles are introduced when filling the electrodes with the electrolyte gel. Such air bubbles can impede the conductive power of the gel, which may eventually interfere with the EMG measurement. To that end, the use of a plastic syringe is suggested. First, fill the syringe with the gel and then slowly push the plunger until the air is out of the syringe, while using a tissue to wipe off the excess gel coming out of it. This should help remove the air bubbles inside the gel before it is eventually injected into the electrodes. It is also important to avoid overfilling the electrodes with gel and to wipe off the excess gel remaining on the skin surface after the electrode placement to prevent the residual gel from creating a conductive bridge between the two electrodes, which may weaken or eliminate the recorded EMG signal [25].
8. Because the postauricular reflex is measured as a difference in electric potential, it is recommended to consistently use the same site placement for each respective electrode (i.e., the first electrode is always placed on the scalp over the postauricular muscle, and the second electrode is always placed posterior to the tendon on the pinna surface, or vice versa).
9. For passive electrode systems, the electrode impedance should be checked and should preferably be below 5 kΩ [25] or below 10 kΩ as the maximum upper limit.
10. Because the postauricular reflex is resistant to habituation (i.e., its amplitude does not decrease across time [29] or the number of acoustic probes presentations [7]), the inclusion of a startle habituation phase is not mandatory but may nonetheless help participants get used to the probe. The inclusion of such a habituation phase is highly recommended when the postauricular reflex is measured at the same time as the startle eyeblink reflex to reduce initial reactivity of the latter.
11. The information provided here is to be merely taken as an illustration of an experimental task where the postauricular reflex is measured as an indicator of appetitive processing. For the readers interested in measuring the postauricular reflex

in their own study, the task design and specifics will of course depend on the nature of the experiment and the research question.

12. In case the trial-by-trial trajectory of the postauricular reflex is central to the research question, it is suggested that each trial be probed. However, such a type of analysis might not be generally recommended because of the low signal-to-noise ratio of the postauricular reflex [4, 9].
13. The relatively long stimulus presentation duration in our procedure was implemented because of the concurrent recording of electrodermal activity [11], which can be affected by the acoustic startle probe when the time interval between the stimulus onset and the probe is too short. Moreover, a long intertrial interval was used to allow the electrodermal activity–which is relatively slow–to return to a baseline level before the start of the next trial. Shorter stimulus presentation (e.g., 6 s), stimulus onset-acoustic probe onset interval (e.g., 3–5 s), and intertrial interval (e.g., 3 s) durations [8, 10, 12, 17] can be implemented when only the postauricular reflex (or a combination of the postauricular and startle eyeblink reflexes) is measured.
14. Although the minimal number of trials required to obtain a stable and reliable measure of the postauricular reflex remains to be established, it has been suggested that including at least 12 trials per condition appears to produce a robust estimate of the postauricular reflex [31].
15. The decision to include six trials per experimental condition of interest was based on typical Pavlovian conditioning procedures used in the field [32] and constraints related to the overall duration of the experiment. But it is recommended to include as many trials per condition of interest as possible whenever it is warranted and feasible.
16. It is crucial to assess the potential occurrence of any time delays between the moment the trigger is sent, the moment the command to deliver the acoustic probe is executed, and the actual acoustic probe onset. It is strongly recommended to check this aspect before starting data collection. If a fixed time delay is detected, this delay should be considered and corrected (e.g., by systematically recoding the triggers) after data collection to appropriately score the postauricular reflex. In the event the delay between the command and the onset is variable, an option is to record the sound delivered through the loudspeakers (or headphones) using a microphone and to subsequently use this recording to detect and appropriately code the acoustic probe onsets for analysis, which is especially important when using signal averaging.

17. Across the literature, different band-pass filter ranges have been used to filter the postauricular EMG signal, typically varying from 3–300 Hz [14, 15] to 0.05–1000 Hz [13]. Importantly, the postauricular muscle has been shown to produce electrical activity that has frequency components primarily in the 25–300 Hz range [3].
18. Note that the power line frequency is typically 50 Hz in large parts of the world (Europe, most of Africa, most of Asia, and Oceania); by contrast, it is typically 60 Hz in North and Central America, parts of South America, and parts of East Asia. The use of a notch filter is generally not recommended when the EMG signal can be recorded under conditions that minimize 50/60 Hz power line interference [25]. This is because notch filters have the disadvantage of removing noise in the 50 Hz or 60 Hz range and also true EMG signal [25].
19. In contrast with the startle eyeblink reflex [25], the postauricular reflex signal is generally not smoothed (e.g., by using a low-pass filter) after rectification.
20. While trial exclusion based on visual inspection involves an inherent subjective component, an inter-judge reliability approach can be implemented to mitigate this aspect. This approach consists in having multiple researchers perform the visual inspection to determine which trial exclusions are agreed upon. Additionally, it is strongly recommended that the criteria adopted for rejecting a trial always be reported, along with the percentage of excluded trials using these criteria [25]. A rejected trial should furthermore not be considered equivalent to a trial in which there is no response or wherein no distinguishable response can be detected [25].
21. It should be noted that there exists a substantial variability in the postauricular reflex both across (*see* Fig. 7) and within individuals [33]. Moreover, a study reported an absence of a measurable postauricular reflex in at least one ear in 32% of their sample and bilaterally in 7% of their sample [34]. On an anatomical level, the postauricular muscle has been reported to be present in 95% of the studied population [35]. As previously mentioned (*see* **Note 5**), it has, however, been argued that a large portion of this individual variability may stem from the influence of eye rotation on the measurement of the postauricular reflex [30].
22. Although signal averaging is generally needed and recommended to reveal the postauricular reflex [6], some studies have scored the postauricular reflex using a trial-by-trial approach [18, 31].
23. Various time windows have been used to score the postauricular reflex magnitude. In general, these time windows are situated within an interval ranging from 5 to 50 ms following the acoustic probe onset [8, 10–13, 16–19].

24. Due to the large interindividual variability in the postauricular reflex, the postauricular reflex magnitudes are sometimes standardized within participants across conditions or trials (e.g., using Z-scores or T-scores) before data analysis [8, 12, 13, 31].

5 Conclusions

In this protocol, we reviewed and described how the postauricular reflex can be measured and quantified in humans. Our goal was to provide information on the materials and methods that can be used to record the postauricular reflex and illustrate the preprocessing and scoring of the recorded signal. We also aimed to demonstrate the value of the postauricular reflex as a psychophysiological tool for the study of appetitive motivation processes, thereby highlighting its relevance for food science.

Nonetheless, it is important to note that the affective modulation of the postauricular reflex by appetitive stimuli has mainly been investigated during the presentation of visual stimuli usually considered as pleasant that either consist in representations of a food reward (e.g., food images) or are associated with a food reward. Accordingly, it remains to be established whether such effects likewise occur during the consumption of the food reward per se. An interesting avenue for future research would therefore be to examine whether the postauricular reflex in response to an acoustic probe is potentiated during the presentation of pleasant olfactory or gustatory (e.g., tastes) stimuli, and whether these affective modulation effects are similar or even larger than those observed during the presentation of appetitive visual stimuli. In that respect, we hope that the present protocol will contribute to sparking further interest in the use of the postauricular reflex as a psychophysiological index of appetitive motivation, which could ultimately foster insights into the psychological and physiological mechanisms underlying food reward processing in humans.

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Chapter 17

Peripheral Nervous System Responses to Food Stimuli: *Electrode Placement and Measures*

Carmen C. Licon and Miguel A. Pedroza

Abstract

Recordings from the peripheral nervous system signals such as facial expressions, heart rate, electrodermal activity, and respiration are increasingly being used to measure emotions evoked by food and beverages. These measurements rely on the installation of electrodes that can record physiological activity from specific body parts while participants consume foods. This protocol aims to provide standard guidelines for electrode placement and signal transducer setup for data acquisition.

Key words Electrodermal activity, Heart rate, Electrocardiography, Electromyography, Pleasantness, Aroma, Taste, Food emotions

1 Introduction

Food preference decisions by consumers depend on the perception of key sensory attributes such as texture, taste, and smell [1, 2]. Preference is often associated with affective states that are characterized by having arousal (intensity) and valence (pleasantness) as primary dimensions; these affective states influence the perception of sensory attributes, behavior, and physiological reactions [3]. The latter are characterized by changes in the peripheral nervous system (PNS) signals such as electrodermal activity (EDA), electrocardiography (ECG), respiration, and facial electromyography (EMG). For EMG, it is important to note that several sites on the face can be studied with a particular interest in two sets of muscles: the *corrugator supercilii* muscle (associated with frowning and negative emotions) and the *zygomaticus major* muscle (in the area of the cheek, associated with positive emotions) [4, 5].

PNS measurements can be used as complementary variables to explicit measures such as numerical ratings of pleasantness, liking, acceptability, arousal, familiarity, and/or intensity of sensory stimuli [6, 7]. For example, PNS measurements have been used for

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exploring affective and perceptual dimensions of sensory stimuli (e.g. hedonic valence, emotional intensity) [8–11], to understand food aroma perception [8], to compare breakfast drinks [12], to study aroma in beer [13], and to characterize emotional perception of aroma compounds in wine [14] and cheese [15].

An important aspect to be considered in research linking food science and emotion studies concerns the replication of results from one study to another. Peripheral nervous activity is particularly concerned by this issue because of the inherent physiological variability between panelists and the methods used by different laboratories for positioning PNS sensors. Therefore, the purpose of this protocol is to provide standard procedure to position EDA, ECG, EMG, and respiration sensors in preparation for food sensory studies (*see* **Note 1**).

2 Materials

The present list is based on the BIOPAC Systems Inc. technology, but the user may also refer to other psychophysiological systems such as ADinstruments [16] or Thought Technology [17]. The probes or sensors work under the same principle; however, the transducers or amplifiers need to be compatible with the electrodes and the recording software.

2.1 General List of Materials

This protocol is based on a MP160 System from Biopac

- Bionomadix® receivers (wireless or wired). The number of receivers will vary depending on the number of transmitters. For this protocol, three receivers are used: EMG2-R, RSPEC-R, and PPGED-R.
- BioNomadix® Transmitters. For example, BN-ECG2-T for electrocardiogram, BN-EMG2-T for electromyogram, NN-RSP2-T for respiration, NF-PPGED-T for electrodermal activity can be used as wireless transmitters. Please note that some transmitters are also receivers.
- Acknowledge® software.
- PC or laptop with USB port, Windows or OS.
- Computer monitor.
- Electrode impedance checker (EL-CHECK Biopac ®).
- Deionized water.
- Cotton swabs.
- General-purpose scouring pads.
- 70% isopropyl alcohols pads
- Disposable gloves of various sizes: S, M, and L.
- 10 mL syringe with needle
- Medical tape.

The following list of materials is based on one participant, and if multiple participants are assessed at the same time, multiply the number of electrodes and electrode leads by the number of participants. Consult with your local specialist for more information about a customized setup if needed [18].

2.2 Electrodermal Activity (EDA)

- One 2-electrode clip lead (available at different sizes: 15 cm, 30 cm or 45 cm).
- Two electrodes:
  - Disposable pre-gelled Ag/AgCl-coated electrodes (27 mm wide × 36 mm long × 1.5 mm thick) or.
  - Reusable Ag/Ag Cl electrodes (6 mm diameter contact area).
- 0.5% NaCl gel (called isotonic) if using reusable electrodes.

2.3 Face Electromyography (EMG): Corrugator Supercillii *and* Zygomaticus Major

- One 2-electrode clip lead and one 3-electrode clip lead (available at different sizes: 15 cm, 30 cm, or 45 cm).
- Five reusable Ag/Ag Cl electrodes (4 mm diameter and 2 mm deep gel cavity).
- Adhesive collars.
- 5% NaCl electrode gel.

2.4 Electrocardiography (ECG)

- One 3-electrode clip lead (45 cm).
- Three disposable circular pre-gelled Ag/Ag Cl electrodes (11 mm diameter).

2.5 Respiration

- Respiration transducer.
- Amplifier or wireless transducer.

3 Methods

3.1 General Consideration

As with any study involving human subjects, it is essential that the research protocol is approved by a competent institutional review board or ethics committee prior to starting data collection, and that participants provide informed consent about their participation in research.

To optimize the measurement of PNS activity, the participant must be neither surprised by the installation of the electrodes nor in an altered emotional state. Thus, before positioning the electrodes and sensors, it is important that participants have the entire procedure explained to minimize anxiety, stress, or surprise prior to data collection (*see* **Note 2**). Moreover, ambient temperature should be temperate (not too cold or too warm) to avoid excessive sweat on the participants' skin (face, hands) or shaking due to excessive cold (*see* **Note 3**).

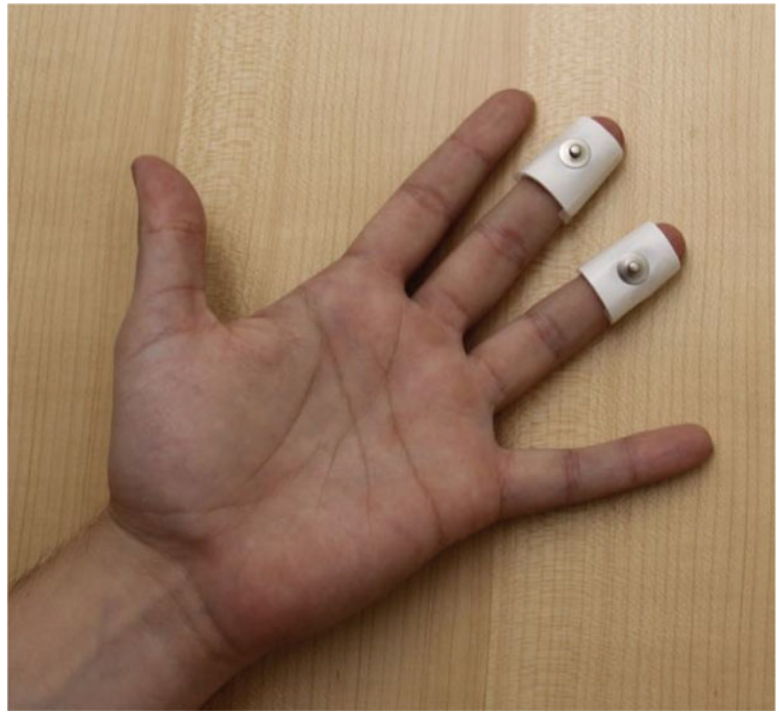

Fig. 1 Electrodermal activity electrode placement on the third phalanx of the middle and ring fingers of the participant's nondominant hand

3.2 Electrodermal Activity

Electrodermal activity (EDA) is defined as the activity of eccrine sweat glands that causes sweat to rise to the skin surface and change the electrical properties of the skin [19]. A common practice is to record EDA from the distal (third) phalanx of the middle and ring fingers (*see* Fig. 1). To optimize signal quality, participants should have their hands cleaned and sanitized using 70% ethanol or isoamyl alcohol pads prior to attaching the EDA electrodes. Reusable electrodes should be prepared by adding a small amount of 0.5% NaCl gel (the size of a pea, approximately) (*see* **Notes 4** and **5**). Reusable or disposable electrodes are placed on the participant's nondominant hand to avoid movement artifacts during data recording. Besides this, medical tape (3 cm approximate) may be used to secure the electrodes. The electrodes are then connected to the transducer, and the order of the wires is arbitrary since the signal measures the voltage difference between both electrodes (*see* **Note 6**). A quick test to evaluate the electrode setup is to startle the participants by clapping near them and see if the skin conductance signal increases after 3 or 4 s.

3.3 Facial Electromyography: Corrugator Supercilii *and* Zygomaticus Major

Soak abrasive sponge (1 cm × 2 cm) with 30 mL of potable water. Apply the adhesive collars to the electrodes without peeling the adhesive paper. While the sponge is soaking, fill the five electrodes with approximately 0.5 mL of the electrode gel by using a 10 mL syringe with a needle. *Do not touch* the electrode with the tip of the needle. Take the sponge and drain excess water with your fingers. Next, proceed to prepare the area of the skin where the electrodes are going to be placed as follows: the attachment areas of the skin are first exfoliated using the sponge and then wiped clean and dried with an alcohol pad. Peel off the adhesive paper from the electrodes and proceed to place them as follows: two electrodes are placed along with the *zygomaticus major muscle*, with an additional one attached by the ear just under the temple as a zero reference (Fig. 2a). Two electrodes are used to detect activity in the *corrugator supercilii* muscle; the first electrode is placed on the center of the superciliary arch, immediately above the eyebrows (approximately 2 cm above the right pupil); the second electrode is placed roughly 1 cm lower and towards the center of the face at a 45° angle, avoiding the skins folding near the glabella (Fig. 2b). Once the electrodes are in place, they can be connected to the transducer. For the *zygomaticus major muscle*, the ground wire must be connected to the electrode placed near the ear, while the other two wires (positive and negative) can be connected arbitrarily to the electrodes closer to mouth. For the *corrugator supercilii* muscle, the positive wire is placed on the sensor near the supraorbital forehead, while the negative wire is attached to the electrode placed near the glabella (*see* **Note 7**). If using this configuration, there should be only one ground connection per transmitter, regardless of measuring the activity of one or two muscles. Impedance levels

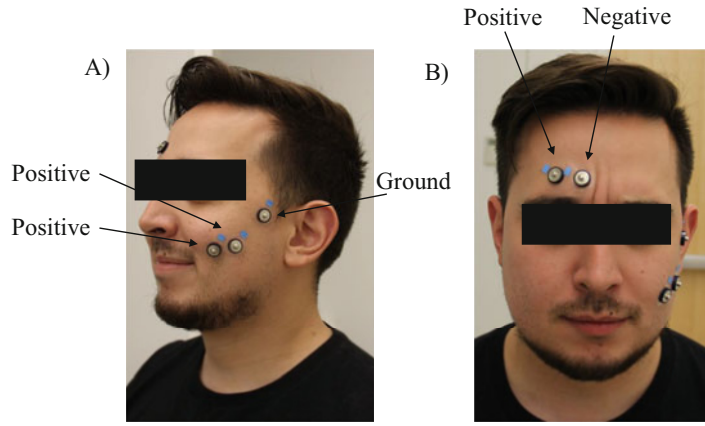

Fig. 2 Face Electromyography (EMG) electrode placement. (**a**) Zygomaticus major muscle and (**b**) Corrugator Supercilii

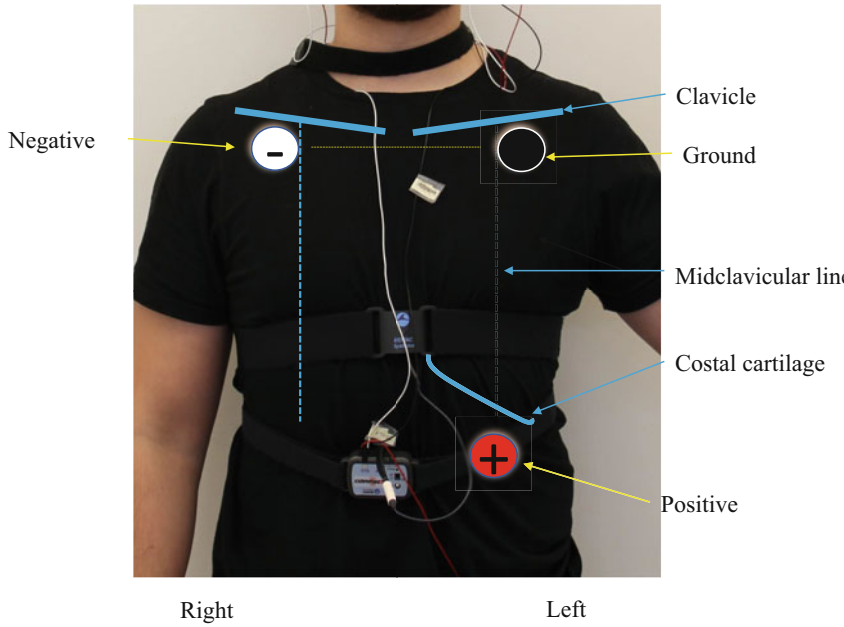

Fig. 3 Electrocardiography (ECG) electrodes placement

should be checked for the electrodes prior to starting data collection (*see* **Note 9**). After this, participants may be asked to frown and smile to verify that the sensors are responding to muscular activity.

3.4 Electrocardiography

Three electrodes, disposable or reusable, are placed on the participants' torso or the wrist and ankles. This protocol will only refer to the torso placement. Two electrodes should be placed symmetrically under the left and right clavicle bones next to the midclavicular line, and a third electrode is placed under the left costal cartilage near to the midclavicular line (Fig. 3). Electrodes should not be placed over bone. If using reusable electrodes, fill the electrode with gel using a syringe following the same procedure as for facial EMG (*see* Subheading 3.2). The attachment areas of the skin are dried with an alcohol pad before attachment. The positive wire (red in this configuration) is attached to the electrode placed on the bottom of the ribcage while the negative wire (white) is attached to the electrode placed under the participant's right clavicle. The ground wire (black) is attached to the electrode on the left clavicle. Once the electrodes are in place, impedance levels should be checked (*see* **Note 9**), and the leads can be connected to the transducer (*see* **Note 8**).

3.5 Respiratory Activity

The belt should be secured under the participant's diaphragm (Fig. 4). Ask the participant to breathe to align the placement of the belt. Once the participant exhales, the belt should tighten. The fitting should be checked when participants are seated to make sure

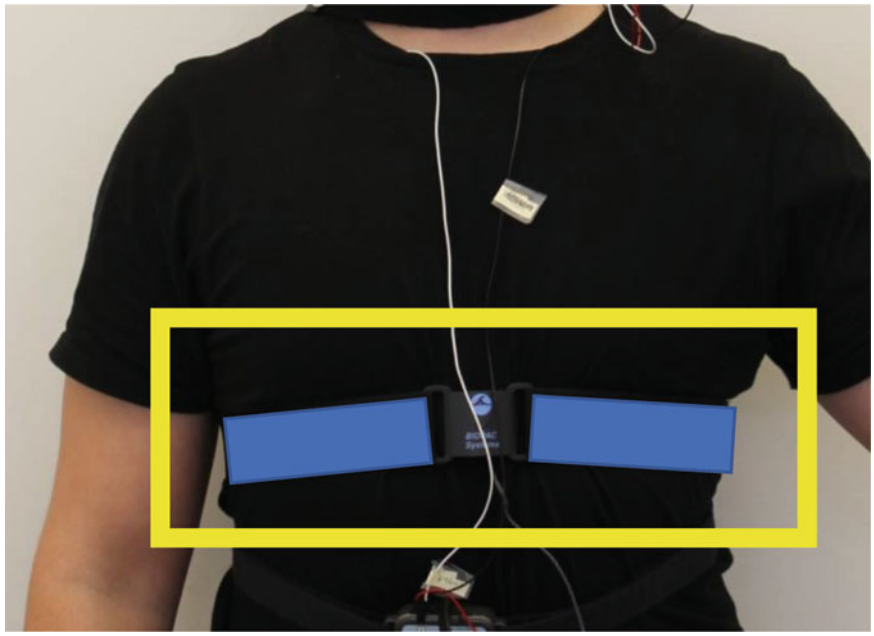

Fig. 4 Respiration belt placement

the belt is not loose or too tight and that they can breathe normally. As a suggestion, the experimenter should still be able to put a finger between the belt and the body to ensure it is not too tight and that the participant feels comfortable. Once the belt is in place, it can be connected to the transducer.

3.6 Example of Acquired Data

Figures 5 and 6 shows an example of the acquisition of different psychophysiological variables in response to the olfactory perception of cheese aromas presented for 5 s under the nose of a volunteer. The facial EMG activity from the *corrugator supercilii* and *zygomatic major* muscles, expressed in millivolts (mV), allows to extract the maximum or average amplitude; the ECG (mV) can be transformed into beats per minute (bpm) or heart rate; the EDA channel shows the electrodermal response curve (in microsiemens, μS), which allows to isolate the latency, rise time, amplitude, and recovery time; the respiration channel (in volts, V) can be transformed into respiration rate, expressed in cycles per minute (*see* **Note 10**).

Correct electrode placement is essential to maximize the signal to noise ratio of each signal and minimize the presence of artifacts such as discontinuous signals and outliers [20, 21]. After placing the electrodes and starting the experiment, analysts should constantly monitor all the signals shown by the software and verify that they are being recorded until the end of the experiment.

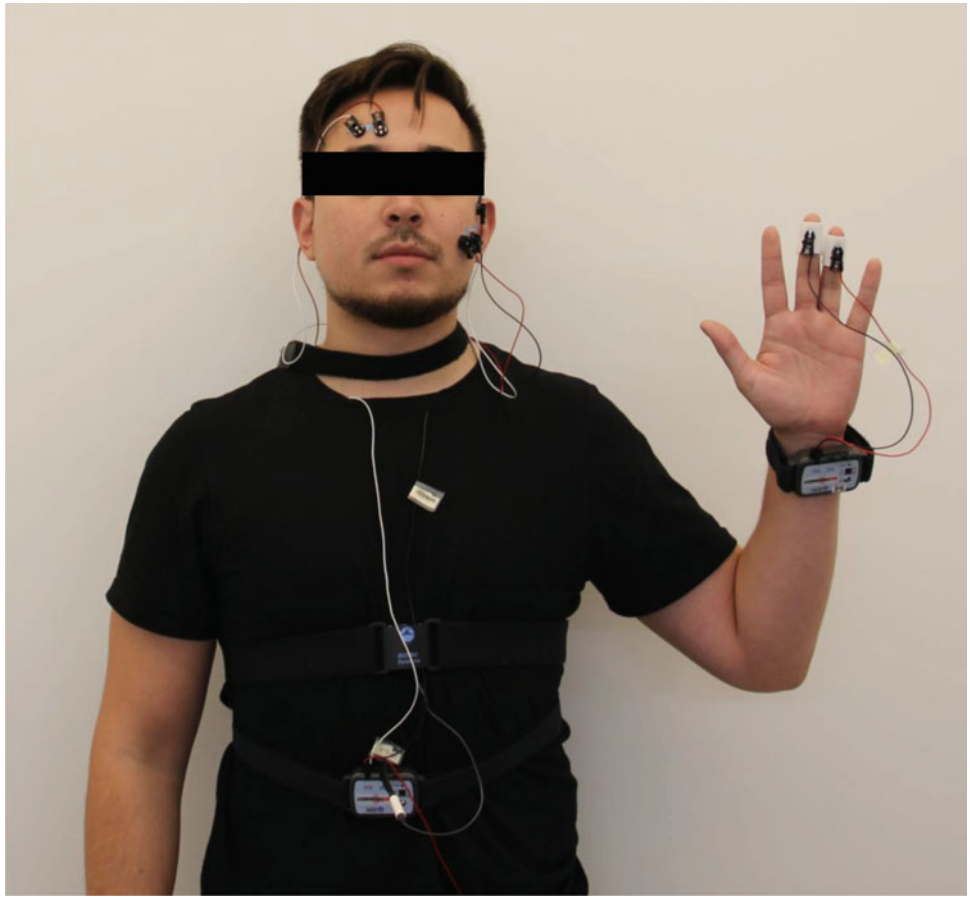

Fig. 5 Participant's electrode placement

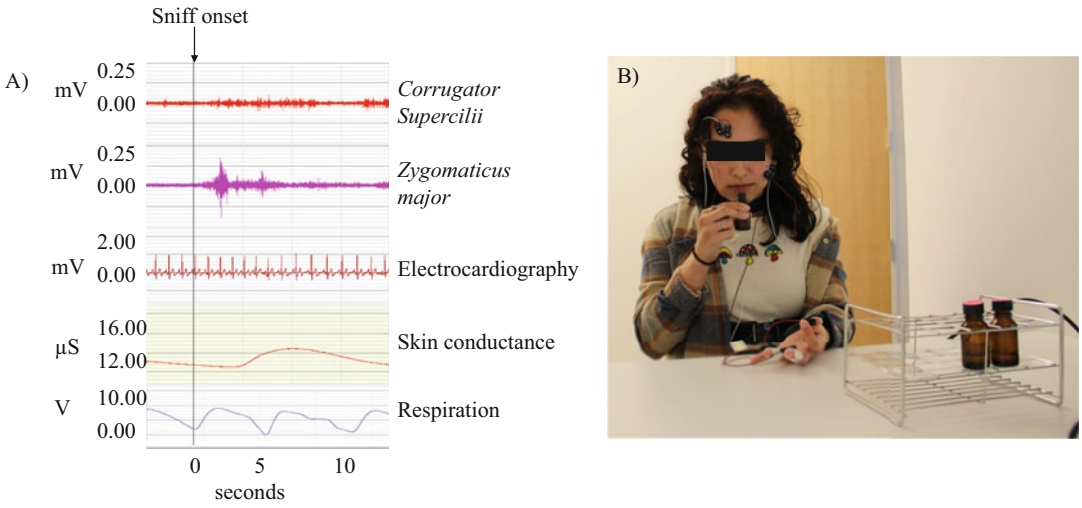

Fig. 6 (**a**) Acquisition of different psychophysiological variables in response to the (**b**) olfactory perception of cheese presented for 5 s under the nose of a volunteer

4 Notes

1. It is important to note that the activity of the PNS is not limited to the facial motor activity and the activity of the autonomic nervous system (EDA, ECG, respiration). Other parameters can be measured, such as skin temperature.
2. The temperature of the experimental room should be controlled to be around 25 °C. Participants should be seated on a comfortable chair, in a space painted in neutral colors without wall decorations, and an environment free of distractions, extraneous noise, and odors.
3. Before starting the physiological recordings, it is important to stabilize the signals for about 3–10 min until the researcher can observe a stable baseline.
4. To clean the reusable electrodes after each use, immerse them in clean water for approximately 1 h. Use a swab to remove any residual gel; this step is essential to extend the life of the reusable electrodes. Once they are clean, gently dry them with a paper towel before storing them in a dry place.
5. Fill the electrodes with the gel just before the experiment as the gel may dry out.
6. Wireless transducers should be charged or have enough battery before each recording session.
7. For EMG, gently rub the face of the participant before placing the electrodes by making an "X" mark where the electrodes will be placed.
8. Men should have no/few face or chest hair in the zones of electrode placement as it may impair signal acquisition.
9. Once electrodes for EMG and ECG are placed on the skin, an impedance check using an impedance meter should be made to determine the resistance between all the applied electrodes. Impedance levels should be 5 k ohms or less.
10. Synchronization between a software that coordinates stimulus presentation and the PNS signal recording is required to indicate precisely the onset of the stimulus for data analysis. Although it is less recommended, it is also possible to integrate a synchronization signal manually using a button-box or a keyboard.

Acknowledgements

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Chapter 18

Peripheral Nervous System Responses to Food Stimuli: *Analysis Using Data Science Approaches*

Maelle Moranges, Marc Plantevit, and Moustafa Bensafi

Abstract

In the field of food, as in other fields, the measurement of emotional responses to food and their sensory properties is a major challenge. In the present protocol, we propose a step-by-step procedure that allows a physiological description of odors, aromas, and their hedonic properties. The method rooted in subgroup discovery belongs to the field of data science and especially data mining. It is still little used in the field of food and is based on a descriptive modeling of emotions on the basis of human physiological responses.

Key words Emotions, Pleasantness, Odors, Aromas, Food stimuli, Data mining, Subgroup discovery

1 Introduction

The measurement of emotions is a real challenge for basic and applied research, especially in the field of food. Often, the analysis of emotions in relation to food stimuli is performed in a multimodal manner by combining subjective measures (valence or self-reported hedonic preferences) and more objective measures such as peripheral nervous system activity. Here, the challenge is to measure hedonic preferences related to odors, taste, visual aspects, texture of food products, or of the food as a whole, and to associate these verbal and declarative responses with more objective measures of emotion such as electrodermal or cardiac activity [1–5].

To date, most studies that have attempted to understand the physiological underpinnings of hedonic preferences to food sensory stimuli have used standard statistical approaches, such as comparing psychophysiological responses across different conditions (e.g., pleasant or unpleasant). Primarily used statistical methods included nonparametric tests (e.g., Wilcoxon, Kruskal Wallis) [6, 7] and parametric tests (analysis of variance, ANOVA) [8–11] depending on study design, data normality, and/or sample size.

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Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_18,

Today, complementary approaches from data science can shed different light on these data by allowing researchers in the field to test descriptive and predictive models of the relationship between subjective preferences and psychophysiological responses. These approaches may be particularly well-suited to the format of data generated in emotion and food science as they take into account large, heterogeneous, and complex data. Indeed, data science can be applied to different types of data and signals (heart rate, skin conductance, chemical data, MRI, etc.). It allows the treatment of a large number of stimuli (thousands/billion) and a large number of attributes in parallel (in our example four dimensions of the skin conductance will be analyzed together).

Data science is a general term used to describe the various aspects of data processing, with the aim of extracting meaningful information and relevant knowledge [12]. It includes data preprocessing (cleaning, normalization, discretization, etc.), data modeling, and data visualization. Within data modeling, we distinguish two main families of algorithms: those referring to machine learning, which are often predictive, and those belonging to data mining, which are often descriptive. Thus, whereas machine learning allows us to predict a variable (e.g. hedonic preference) on the basis of one or more variables (e.g. physiological responses) (*see* **Note 1**), data mining allows us to build descriptive models. The latter are often explanatory in the sense that they explain by explicit association rules how a pleasant food flavor is characterized physiologically compared to another less pleasant food for example.

The present protocol, inspired from a previous study [13], aims to provide researchers and students in the field of food science with a framework for using these data mining methods, especially subgroup discovery methods, in the context of research on food-related emotions. We will use electrodermal responses (e.g., skin conductance or SC) and ratings of pleasantness collected from human individuals in response to various olfactory stimuli as an example of data [14]. Using these example data, we provide the user with a step-by-step protocol that allows for a physiological description of odors and aromas characterized by their hedonic value.

2 Materials

1. A computer (*Specifications: Windows 7 or later, Mac OS X 10.11 or higher, or Linux RHEL 6/7).*
2. Python 3 SDK software: It is an interpreted, multi-paradigm, and multi-platform programming language (*see* **Note 2**). It supports structured, functional, and object-oriented imperative programming.

3. A Python development environment: Jupyter notebook; to be able to view the notebook created and read it as a tutorial.
4. The following libraries should be installed on Python:
   (a) scikit-Learn (version = 0.24.1) [15]*:* it is a library that offers various possibilities in terms data science methods,
   (b) matplotlib (version = 3.1.1): to handle graphical representation,
   (c) pandas (version = 1.2.3): to visualize and to manipulate tabular data,
   (d) pysubgroup (version = 0.7.2) [16]: offers several data mining algorithms,

   One can install Jupyter notebook and all of the above libraries by installing a Python distribution named Anaconda and by installing the pysubgroup package with the following command: *pip install pysubgroup*.
5. A database with subjective and psychophysiological data. As an example, we will use a dataset that combines SC responses to pleasant and unpleasant odors (see Licon et al. [14]). The dataset is downloadable from a public repository (Dataset name: "*PsychophysioDataset.xlsx*", available at https://github.com/mmaelle/Psychophysio-Analysis). Each dataset row is an observation for a specific odor with its subjective pleasantness (rated using a scale from 1, very unpleasant, to 9, very pleasant) and its associated SC response described by four parameters (amplitude, latency, rise time, and number of events in a period following stimulus onset). The database contains 2398 observations (109 observations for 22 individuals).

3 Methods

The protocol contains two steps developed below: (1) preprocessing and (2) data mining analysis. An overview of the whole analytical process is illustrated in Fig. 1.

3.1 Preprocessing

Preprocessing involves all the operations that precede the analysis step. Preprocessing is an important step that should not be neglected: it prepares the dataset so that it is as clean (without error), as simple as possible, and is adapted to the algorithms that will be used in the following analysis (modeling and data format compatible with the input parameters of the algorithm). To do this, noisy, outlier or irrelevant data must be corrected or rejected so as not to bias the study.

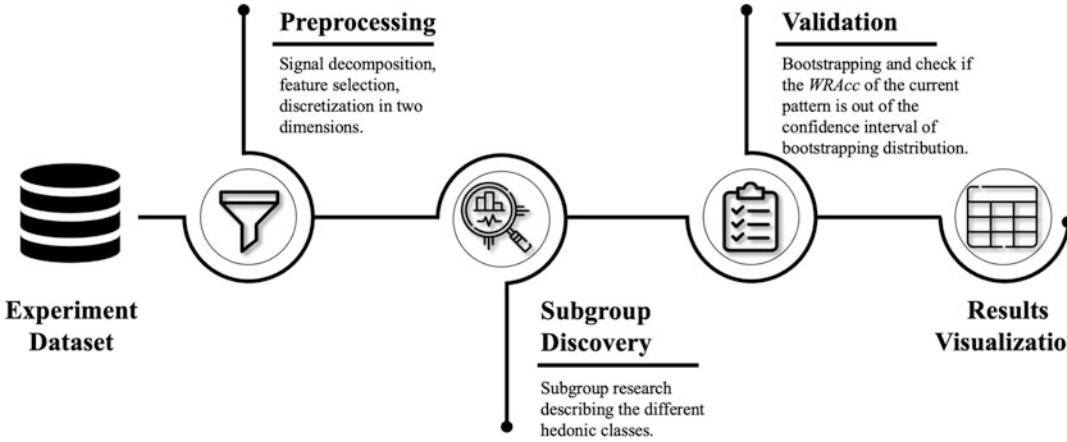

Fig. 1 Steps of the workflow

3.1.1 Skin Conductance Decomposition and Data Importation

In a first step, we start by importing the data — available in the excel file "PsychophysioDataset.xlsx" — into a python dataframe. The data set contains the following information: participant number, CID (Compound Identification number of the odorant), latency, rise time, amplitude, number of events in a time window following stimulus onset, and ratings of odor pleasantness. Note that if no peak has been identified, the attributes latency, amplitude, and rise time are equal to 0. Moreover, the number of events is calculated by subtracting the number of events before the presentation of the odor from the number of events after the presentation of the odor (the value can therefore be negative). For more details, refer to the second session in Licon et al. [14].

```
import pandas as pd
data = pd.read_excel('PsychophysioDataset.xlsx', sheet_name='Psychophysio')

print(data.head())
```

The preceding command displays the first lines of the Psychophysio dataset (Table 1).

3.1.2 Feature Selection

In a second step, irrelevant data (such as CID) that are not directly helpful to answer our question can be removed as follows.

```
df = data[['Subject', 'Latency', 'Rise-Time', 'Amplitude', 'Events', 'Pleasantness']].copy()
```

Table 1
Initial dataset

	Subject	Stimulus	Latency	Rise-Time	Amplitude	Events	Pleasantness
0	1	1	2.98046875	3.01171875	0.19736884	1	5
1	1	2	1.88671875	6.46875	0.91188065	2	4
2	1	3	2.0859375	4.74609375	0.27328656	1	0
3	1	4	1.69921875	4.515625	0.61869071	−2	5
4	1	5	0.80078125	3.4375	0.08303131	0	3

Incomplete data, such as trials for which participants did not provide perceptual scores or for which SC data are missing, can also be rejected.

```
df = df.drop(list(df[df['Pleasantness']==0].index))
nan_cols = [i for i in df.columns if df[i].isnull().any()]
for c in nan_cols :
 df = df.drop(list(df[df[c].isnull()].index))
```

Based on your data set, you will decide which variables are of interest and which should not be included in or should be rejected from your analysis.

3.1.3 Discretization

In a third step, we seek to label our data as "pleasant odors" and "unpleasant odors" for pleasantness. We will therefore discretize the self-reported scores into two classes. Having two discrete classes instead of scores allows one to deal with binary responses and to clearly separate pleasant from unpleasant odors on the one hand and weak from strong odors on the other hand. We choose here to discretize the scores using the clustering algorithm called *KMeans* [17] because this algorithm allows one to get rid of the subjectivity linked to the different scoring strategies (*see* **Note 3**). To obtain two groups (unpleasant and pleasant), we use $K = 2$.

We thus obtain the preprocessed dataset presented in Table 2. It is available in csv format in the file "*PsychophysioPreprocessed.csv*".

3.2 Data Mining Analysis (Subgroup Discovery Analysis)

3.2.1 General Information

We propose to use a data mining approach based on a subgroup discovery (SD) algorithm [18]. SD allows the discovery of patterns that are discriminating for a target class. Indeed, it finds population subgroups that are statistically "most interesting" from a population of individuals (or items). These subgroups are identified by conditions on the descriptive attributes. In this regard, we seek to obtain the largest possible subpopulations that have the most unusual statistical distributional characteristics.

Table 2
Preprocessed dataset

	Subject	Latency	Rise-time	Amplitude	Events	Pleasantness_class
0	1	2.98046875	3.01171875	0.19736884	1	Pleasant
1	1	1.88671875	6.46875	0.91188065	2	Unpleasant
2	1	2.0859375	4.74609375	0.27328656	1	Pleasant
3	1	1.69921875	4.515625	0.61869071	−2	Unpleasant
4	1	0.80078125	3.4375	0.08303131	0	Pleasant

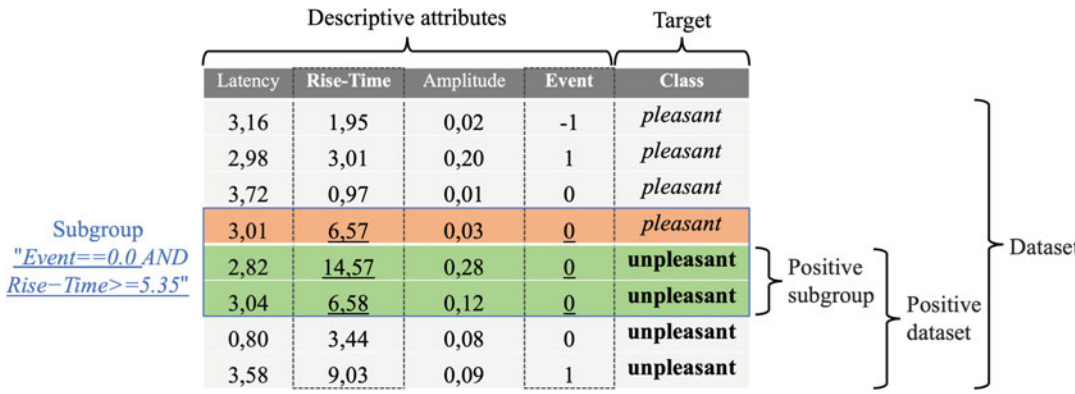

Fig. 2 Dataset example for the pattern described by the "*Event==0.0 AND Rise-Time>=5.35* → *unpleasant*" rule

For example, we obtain a pattern described by the rule "*Event==0.0 AND Rise-Time>=5.35* → *unpleasant*". The conditions on the physiological attributes form the property of interest "*Event==0.0 AND Rise-Time>=5.35*". This property describes the subgroup identified as having exceptional behavior: the distribution of the target (unpleasant) is high in this subgroup compared to the rest of the dataset. This means that having a rise time greater than or equal to 5.35 s and at the same time a constant number of skin conductance peaks is significantly more present for the "unpleasant" trials than for the other so-called pleasant trials. This pattern is illustrated by the dataset depicted in Fig. 2. The subgroup consists of the three colored rows in the dataset that verify the conditions present in the rule. In the dataset, rows belonging to the target class form the "positive dataset" (in bold in Fig. 2) and the other rows form the "negative dataset" (in italics in Fig. 2). Note that in the "positive dataset," the items that are part of the subgroup are called "positive subgroup" (or "positives_sg").

3.2.2 Processing

First, we import the data set "pandas" library for viewing and manipulating tabular data and a library for subgroup discovery analysis called "pysubgroup".

```
import pandas as pd
import pysubgroup as ps
df = pd.read_csv("PsychophysioPreprocessed.csv", sep=',')
```

Next, we define the search space in which the algorithm should search. We remove the target column 'Pleasantness_class', so that it is not considered as a feature and does not give us the rule "Pleasant implies pleasant". Setting the nbins parameter to 20 means that the algorithm must discretize the variable values into 20 classes. The

greater this value, the greater the number of rules generated and the more precise the rules.

```
searchspace = ps.create_selectors(df, nbins=20, ignore=
['Pleasantness_class'])
```

We now need to specify the target class: in our case, it is the 'Pleasantness_class' column, and we will start with the "unpleasant" odorants.

```
target = ps.BinaryTarget('Pleasantness_class', 'unpleasant')
```

Then, we create a Subgroup Discovery Task to identify the five best patterns (*result_set_size*) using the Weighted Relative Accuracy (*WRAcc*) value (*qf*). This task creates rules with a maximum of four conditions (*depth*) in the description of the subgroup. A condition can be written in different ways: either by a strict equality (e.g., "*Event*==0" for "the number of events is zero"), or by an interval (e.g., "*Latency:[1.84:2.11[*" for "the latency is between 1.84 and 2.11 ms"), or by a minimum or maximum value (e.g., "*RiseTime*>=*5.35*" for "the rise time is greater than or equal to 5.35 ms"). When several conditions are combined, they are separated by the "AND" operator, in this case the different conditions must all be true for a trial to be included in the subgroup.

```
task = ps.SubgroupDiscoveryTask (df, target, searchspace,
result_set_size=5, depth=4, qf=ps.WRAccQF())
```

Now we can extract the patterns. The algorithm, by default, is the 'BEAM' search which performs a beam search exploration. It returns a "SubgroupDiscoveryResult" type object that can be converted into a dataframe and viewed in Table 3. We can then inspect the found subgroups and their characteristics.

```
result = ps.BeamSearch().execute(task)
unpleasant = result.to_dataframe()
```

We can also export the results to a csv file.

```
unpleasant.to_csv('unpleasant_result.csv', index=False,
sep=',')
```

Then we can do the same search for "pleasant" odorants.

```
target = ps.BinaryTarget('Pleasantness_class', 'pleasant')
task = ps.SubgroupDiscoveryTask(df, target, searchspace, re-
sult_set_size=5, depth=4, qf=ps.WRAccQF())
```

```
result = ps.BeamSearch().execute(task)
pleasant = result.to_dataframe()
pleasant.to_csv('pleasant_result.csv', index=False, sep=',')
```

3.2.3 Example of Results

The top five results obtained for the attribute pleasantness and its targets can be viewed in Table 3 in the same format as that generated by the pysubgroup library. The best model for unpleasant has the following rule: "*Rise-Time*> = *5.35* → *unpleasant*". The subgroup size is 114 (size_sg) which corresponds to 5% of the dataset (relative_size_sg or size_sg / size_dataset). Of these 114 trials, 72 are classified as "unpleasant" (positives_sg) and 42 as "pleasant" (size_sg- positives_sg). So we see that we do not get a 100% accurate rule (without error), but information like "if we have a Rise-Time> = 5.35, then the smell is more likely to be unpleasant". To know how correct this rule is, we use quality measures.

The quality of the model is calculated as the frequency of the rule in the subgroup relative to the frequency of the rule in the entire data set. The score generally used is the Weighted Relative Accuracy (*WRAcc*) value. The *WRAcc* value is the probability of having the target in the subgroup minus the probability of having the target in the data set, multiplied by the probability of the target in the data set.

$$WRAcc = size\_sg/size\_dataset \times (positives\_sg/size\_sg - positives\_dataset/size\_dataset)$$

The *Wracc* value cannot exceed 0.25 or be less than −0.25. The higher the absolute value, the more exceptional the description feature in the subgroup compared to the rest of the data. A 0.25 value corresponds to the extreme case where the dataset is balanced and the subgroup – with a size of ½ size_dataset – uncovers all objects from one target without uncovering the other target (i.e., true positive rate is 1 and false positive rate is 0). Another measure of quality is shown by the parameter *lift*. The *lift* is the probability of having the target *(*e.g.*, pleasant or unpleasant)* in the subgroup divided by the probability of having the target in the whole dataset.

$$lift = (positive\_sg/size\_sg)/(positives\_dataset/size\_dataset)$$

If *lift* is less than 1, the rule is not interesting because the target is less frequent in the subgroup than outside. If we obtain a *lift* of 3, it means that the target is 3 times more frequent in the subgroup than in the whole data set.

We have data with a positive *WRAcc* and a *lift* greater than 1, which means that trends are found. The two quality measures are illustrated in Fig. 3. One way to be sure that a pattern is meaningful and not due to chance is to perform a bootstrap validation (*see* **Note 4**).

Table 3
Top five results obtained with *pysubgroup* library (unpleasant and pleasant patterns are depicted separately)

Unpleasant pattern							
Pattern rank	**Quality (WRAcc)**	**Subgroup**	**size_sg**	**size_dataset**	**positives_sg**	**positives_dataset**	**Lift**
1	0.006387	Rise-time > =5.35	114	2278	72	1130	1.25
2	0.003314	Amplitude> = 0.50	114	2278	65	1130	1.13
3	0.003282	Event==2.0	259	2278	138	1130	1.06
4	0.002875	Latency: [1.84:2.11[	114	2278	64	1130	1.11
5	0.002751	Event ==0.0 AND Rise-time > =5.35	59	2278	36	1130	1.21
Pleasant pattern							
1	0.005683	Rise-time: [1.92:2.26[	113	2278	69	1148	1.23
2	0.004285	Event == − 1.0	319	2278	168	1148	1.06
3	0.003271	Rise-time: [3.03:3.41[	114	2278	64	1148	1.13
4	0.003053	Latency> = 3.20	115	2278	64	1148	1.12
5	0.002541	Latency: [0.09:0.43[	73	2278	42	1148	1.16

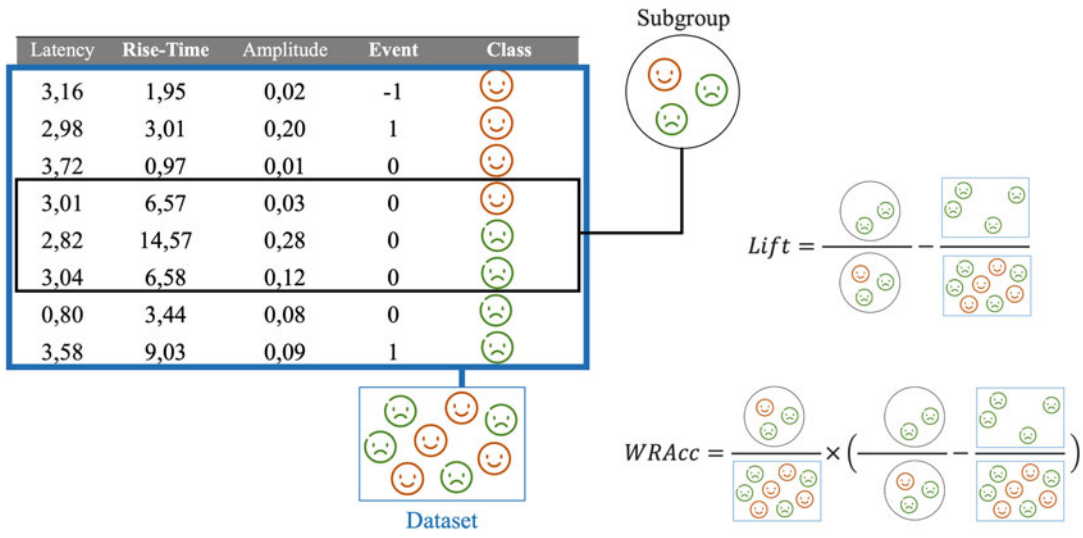

Fig. 3 Illustration of quality measures

3.2.4 Conclusions

Subgroup discovery methods have very little implementation in olfaction and food science, but when they have [13, 19–21], they provided descriptive models linking physicochemical or physiological parameters to hedonic odor and aroma perceptions. This approach is promising and will be useful when seeking to describe perceptual phenomena and emotional reactions related to food perception from a physiological perspective.

4 Notes

1. *Machine learning analysis.* To analyze the link between physiological and emotional responses, it is possible to use other data science methods such as predictive approaches called supervised machine learning. Supervised machine learning can be used to achieve two goals. The first is to automate a process, for example, to learn directly about a person's perceived emotions without having to ask them. The second is to know how the classifier separates the different groups in order to understand the underlying neural or physiological process better. To do this, the algorithm must be intelligible or, if it is a black box, a method must be found to allow for explanation [22]. The algorithm must also have a high prediction score, which can be difficult to achieve when trying to relate complex dimensions with high variability between individuals such as physiological responses and emotional responses. To make predictions, the scikit learn library available on Python is complete and offers multiple possibilities in terms of learning methods and algorithms. In R, there are libraries corresponding to

the classifier to be used (e.g., e1071, rpart, klaR, kernlab, CORElearn, Rweka, tree, caret ramdomForest, nnet, glmnet, gbm, rath, ipred, ROCR, mboost). In KNIME (menu Analytics/Mining), you can find a series of learning algorithms including neural network, decision tree, logistic regression. In Orange, you can find classification algorithms in the Model menu (Logistic Regression, KNN, Random Forest, SVM...).

2. *Other data mining tools.* Besides Python, different tools and platforms exist to perform data science analysis: graphical interfaces such as Weka [23] and ELKI [24] or software such as KNIME [25] or Orange [26], with which one can compose a specific workflow by assembling one after another module performing a specific operation. There is also an easy-to-use software with a graphical interface called Cortana [27]. Cortana is also available as a plugin for KNIME. For those with good R skills, for subgroup discovery, one can use rsubgroup [28] on R.
3. *Discretization.* In the literature, ratings are usually discretized by dividing the scale as used into 2 or 3 or by dividing into percentiles of equal size. However, the way in which emotions and preferences are provided is unique to each person: some people rate using a wide range of nuances, whereas other do not. It is important to separate the ratings into several categories while limiting this subjectivity. Therefore, we propose a partitioning method that is neither equi-depth nor equi-width: use *KMeans* clustering on each subject independently. This algorithm allows partitioning into k clusters such that the distance between intra-cluster points is minimized and the inter-cluster distance is maximized allowing the subjective data to be partitioned into categories as different as possible. We do not recommend discretizing the scores by dividing the scale into equivalent spaces (e.g. 1–5 vs. 6–10, for 2 groups and a scale ranging from 1 to 10), or partitioning into median because this method does not always reflect a person's assessment strategy. Indeed, with these methods, odors perceived in a similar way can be found in different categories and very different odors in the same category [13].
4. *Bootstrap validation.* To filter the patterns, we can add the *min_quality* parameter to the *SubgroupDiscoveryTask()* function in order to define the minimum accepted quality. However, this is not enough to guarantee that the pattern is significant. To validate the quality of a pattern, one can make a very large number of random draws of the same size as the pattern to be validated and look at the distribution of the quality measure of all the generated draws to verify that the pattern is outside the confidence interval of that distribution. For example, by calculating the *WRAcc* of 10,000 groups for

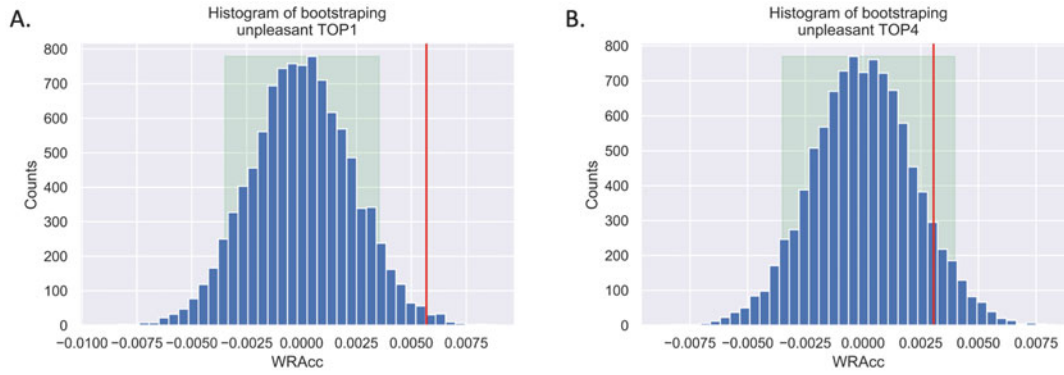

Fig. 4 Examples of validation with on the left a validated pattern (**a**) and on the right a rejected pattern (**b**). The blue barplot corresponds to the distribution of the WRAcc of the random sample, the green rectangle to the confidence interval, and the red vertical line to the WRAcc of the pattern to be validated

each pattern discovered, with the same support as the current pattern, drawn at random (with discount between each draw). If its *WRAcc* is outside the 90% random distribution, then the pattern is validated and can be considered as interesting. This interval validation avoids flagging subgroups indicating a *WRAcc* likely to be observed by a random subset of entities. Two examples are shown in Fig. 4.

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Chapter 19

Electroencephalography and Gustatory Event-Related Potentials Measures to Oral Stimuli

Emilia Iannilli

Abstract

Anatomically, the oral cavity is responsible for the reception of food stimuli. From there, a cross-modal interaction of somato- and chemosensory systems is required to induce the final flavor perception at the central nervous system (CNS) level. Important is also, however, the emotional component of flavor. In food science there has been increasing interest in studying how these different flavor components, including the hedonic one, are processed and combined to inform what is finally perceived by the CNS. In this chapter, we seek to provide a detailed, noninvasive methodology to measure brain-evoked activity to one of the stimuli composing flavor, namely gustatory stimuli. A dedicated delivery system, stimuli conditions, a feedback system, and the experimental design to record gustatory event-related potentials (gERPs) are presented. Moreover, information on how to integrate olfactory (retronasal) stimuli and trigeminal (irritation-like) stimuli in such procedure is included in the protocol notes. The methods also apply to gustatory event-related fields (gERFs) acquired in magnetoencephalography (MEG) system, as described in a separate note.

Key words Flavor, Smell, Trigeminal system, Olfaction, Gustation, EEG, MEG

1 Introduction

Eating behavior and consumer preferences are mainly driven by a food's chemosensory perception and the way an individual has experienced flavor learning throughout their lifetime. Flavor perception per se is the result of cross-modal interaction from tastes, odors, and temporally associated tactile/irritant sensations [1, 2]. In addition to this, overlapping cerebral processing structures belonging to the limbic system have been linked to the ability of flavor to evoke emotions [3], depending on personal experience. In recent food science focusing on the interaction between emotions and the senses, there has been rising interest in studying individual and integrated processing of the complex components of food (odor, taste, texture, etc.) to form a perception and specific associated affective states [4–7].

Moustafa Bensafi (ed.), *Basic Protocols on Emotions, Senses, and Foods*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2934-5_19,

Table 1
Schematic reduction to interaction-pairs of sensory systems involved in the detection of flavor. The interactions-pairs possible to study with the methods presented in this chapter are highlighted with a bold black border and are six in total

	g	**ro**	**T**	**tx**	**Irr**
g		g - ro	g - T	g -tx	g – irr
ro	**ro-g**		ro - T	ro - tx	ro – irr
T	**T - g**	**T - ro**		T vs. tx	T -irr
tx	tx -g	tx - ro	tx - T		tx -.irr
Irr	**irr - g**	**irr - ro**	**irr - T**	irr -. tx	

G gustation/taste, *ro* retronasal-olfaction, *T* temperature, *tx* texture, *irr* irritation

Food stimuli are received in the oral cavity to then be perceived through both somato- and chemosensory systems. The gustatory system, through the five principal tastants (bitter, sweet, sour, salty, and umami), olfaction by means of the volatile compounds through the retronasal route (the odor is presented in the ephipharynx of the soft palate, also called retronasal-olfaction:), and the trigeminal system's mediation of sensory (texture, temperature) and irritation sensation, work in concert. The difficulty, therefore, in studying the brain response of flavor perception simultaneously to its emotional responses lies in the complementarity of participating variables. To circumvent this and still study the cross-modal effects while keeping the other components of the chemosensory system constant, the decomposition of variables and the reduction into interaction-pairs is a convenient strategy. In Table 1, we show how this can be explored through a total of 10 interaction-pairs in the oral cavity. With the method presented in this chapter, it is possible to study six couples of these interaction-pairs, highlighted with a bold black border in Table 1: (1) retronasal-olfaction vs. taste, (2) retronasal-olfaction vs. temperature, (3) taste vs. temperature, and (4) irritation vs. taste, or (5) irritation vs. retronasal-olfaction, or (6) irritation vs. temperature. Stimulations involving texture are excluded due to the nature of experimental event-related design (*see* **Note 1**).

Contrary to psychophysics measurements, which are subjective, brain signal detection and interpretation are objective measurements and independent from subject bias. Electroencephalography (EEG) measures the immediate mass action of neural networks from a wide range of brain systems, elicited by the electric field generated by post-synaptic potentials from the apical dendrites of the pyramidal neurons [8, 9]. Thus, it provides a straightforward and noninvasive perspective into the human brain function.

In addition, EEG has excellent temporal resolution (milliseconds). Since some assumptions are needed to localize the sources of the electric fields, it was considered to have a relatively low spatial resolution compared to techniques such as magnetic resonance imaging (MRI – millimeters) in the past. However, this limitation has diminished in recent years. Now, powerful advances in computer technologies allow the simultaneous recording of over 256 high-density electrode channels. Together with a cutting-edge source analysis algorithm, this consents a spatial resolution comparable to that of MRIs.

As previously mentioned, flavor is a multimodal perception involving several forms of chemical sensations (among others). In this chapter, for simplification purposes, we aim to present a protocol to measure brain responses utilizing EEG to a family of chemical stimuli that make up flavor: taste stimuli. Nevertheless, detailed information on how other stimuli composing the flavor, in particular olfactory stimuli (retronasal) and trigeminal sensations (irritation Trigeminalirritation), can be integrated in the protocol to allow the study of other interaction pairs from Table 1, can be found in the notes.

In EEG acquisition, brain signals measured after an oral-event of interest that stimulate the gustatory system are called gustatory event-related potentials (gERPs). For this purpose, the stimulus onset must have well-defined, quasi delta-function characteristics in space and time [10]. It is crucial that the sensation we aim to elicit on the tongue, for example, the taste, should not be confounded by other mechanosensory, somatosensory, or thermal sensations. With these considerations, it appears clear that studying the electrical activity of the brain elicited by a taste stimulus requires a dedicated stimulus delivery system. In our lab, we use a computer-controlled oral stimulator to deliver pulses of a liquid solution of tastants at a controlled temperature. The device's characteristics will be described in Subheading 2.2 and referred to here on out as the gustometer [11].

All concepts here introduced for EEG are equally applicable to magnetoencephalography (MEG), which measures the magnetic activity produced by the firing neurons in the brain. Thus, with some specific modifications, the procedure can also be applied to gustatory event-related fields (gERFs) acquired in the magnetoencephalography (MEG) system. *See* **Note 2** reports all pertaining details.

2 Materials

In this section, the description of the materials and the specifications for EEG in a gustatory task used by Iannilli et al. [11–13] will follow. The description will focus on the specific EEG system used for most of our experiments; however, the setup can be generalized to any brand.

2.1 EEG Equipment

1. *Electrodes and cap.* ERP-EEG signals can be recorded with alow number of electrodes (<32 channels), which allows for classical time-component analysis, or with a high number of electrodes (>64 channels), that consent to study the biovoltage topographic pattern and the localization of brain sources.

   In our setup, we use Ag-AgCl active electrodes (BioSemi; 10/20 BioSemi–CAP; Amsterdam, Netherlands) that have the first amplifier stage integrated. This helps with problems associated with high electrode impedance and cable shielding. Electrodes can be placed using a head cap (BioSemi; Amsterdam, Netherlands) with standard 10/20 positions available in three sizes, fitted by each subject's head size prior to experimentation. External electrodes, eight in total, are added to the setup and to record six vertical and horizontal electrooculographic signals, essential to facilitate artifact rejection and correction during the signal preprocessing. The two reference electrodes are usually located on earlobes and mastoids, that in the case of active electrodes, as in our case, must be chosen post hoc during data import.
2. *Amplifier.* Each channel recording the scalp biopotential is connected to the amplifier (BioSemi ActiveTwo AD-box, BioSemi, Amsterdam, Netherlands), consisting of a low noise DC-coupled post-amplifier, with a first-order anti-aliasing filter, an oversampling rate of 64, and high-resolution 24-bit output. The analogic channel input is then converted into digitally multiplexed outputs and sent to the PC via a single optical fiber without any compression. Finally, a receiver is interposed before storing the data into the PC and converts the optical data to a USB2 output.
3. *Acquisition console and software.* Data are registered and stored on a PC disk. Therefore, it is essential to use a channel visualization layout that allows the user to check the quality of the data during the acquisition. In our setup, we use the acquisition software BioSemi ActiveView 605, which in combination with the USB2 receiver can be used to synchronously acquire additional signals coming from sensors such as respiration, temperature, stimulus onsets, and any other triggers involved in the experimental paradigm such as taste event trigger.
4. *Electrode gel.* In our setup we use active (or pre-amplified) electrodes, for this reason the cap can be applied directly on the scalp without the usual skin scrubbing to lower the impedance. We only use an electrode gel inserted in the cap holes (Signa gel; Parker Laboratories, Inc., Fairfield, NJ USA) before pushing the active electrodes in the electrode holders.

5. *White acoustic noise.* To dampen ambient sounds (e.g., possibly noise from equipment in use) that can trigger unwanted auditory brain signals, subjects receive acoustic white noise of approximately 50 dB speaking level (SPL) via headphones during the experimental session.

2.2 Gustometer

1. *General.* The gustometer is a device dedicated to stimulating the oral cavity (Fig. 1) through liquid tastant solutions.
2. *Stimuli and modules.* The device has seven modules to use with the five different basic solutions (e.g., the five basic tastes: sweet, sour, bitter, salty, umami) plus two containing the solvent solution or the control condition. The five modules can each be further diluted with ten percentage levels (dilution).

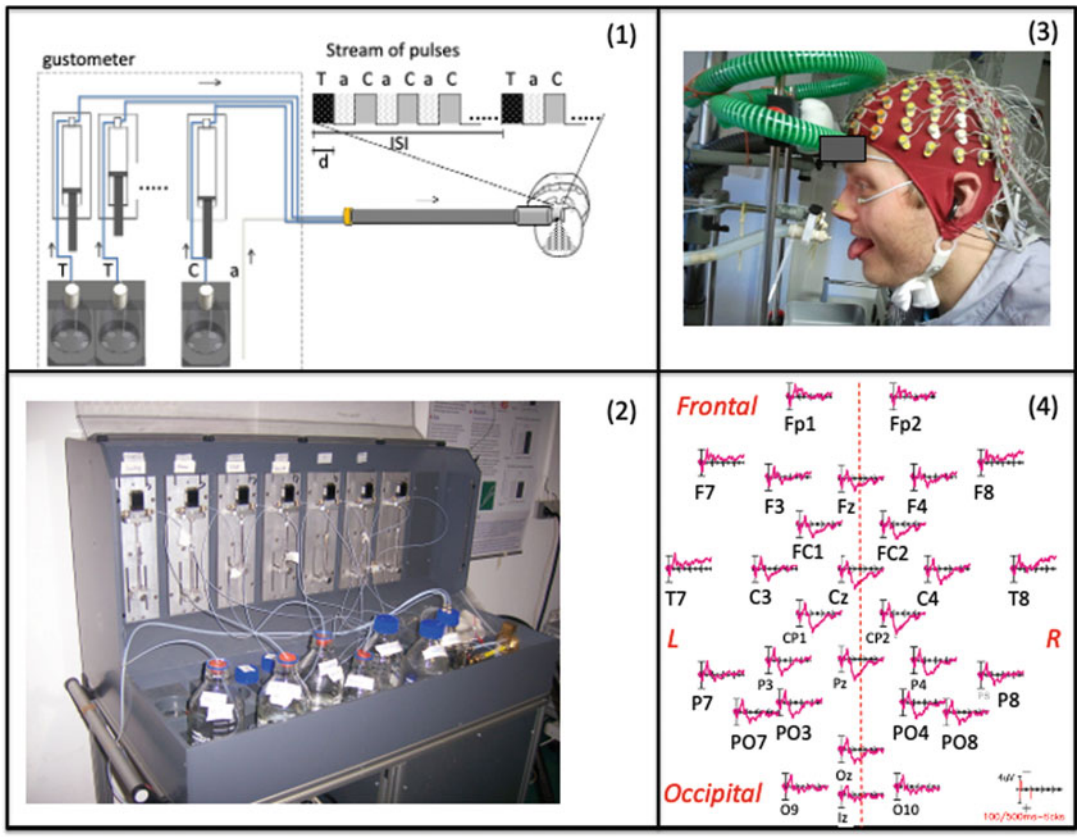

Fig. 1 (1) Schematic representation of the gustometer and the sequence of stream of pulses. The liquid solutions of tastants (T) are sucked through a system of computer-controlled syringes-pumps from the bottle containers into the syringes and then channeled in tubing collected in a thermoregulated water bath hose. The gustometer delivers the taste pulses (T) interleaved to tasteless pulses (C, control) and the airstream (a). d: pulse duration; ISI: interstimulus intervals. (2) A picture of the real gustometer. (3) The subject passively receives the stimulation while sitting comfortably. The gustatory event-related potentials (gERPs) are registered with the EEG system. (4) Example of gERPs on a 29 electrodes down-sampling of a high-density channel EEG system. R = right, L = left. (Reproduced from Iannilli 2017 with permission from Elsevier [13])

The solutions can be used independently or in combination for a final 250 decimal combination. The gustometer has a thermostatic temperature control to set the temperature to the required degree.

3. *Outlet*. The outlet of the gustometer produces a stream of pulses, constituted by a certain number of taste stimuli interleaved (decided by the experimenter) by a number of tasteless pulses and airstream pulses. The pulse stimulus is entirely computer-controlled (onset, duration, volume, interstimulus-interval), and its steepness (at 0.5 bar and 5 cm distance from the outlet the square wave reaches 90% of its amplitude in 22 s) is sharp enough (28,13 V/s) to evoke scalp potentials [11]. Typical parameters for the taste sequence are: pulse duration: 250 ms; interstimulus interval (ISI): from 28 to 30 s in order to minimize gustatory habituation [12, 13], and applied with randomized jitter to avoid stimulus expectation; solution volume delivered per pulse of 100 μl, and; repetition of conditions 60 times (*see* **Note 3**).
4. *Oral cavity*. Finally, the solution is sprayed onto the oral cavity. This method induces habituation to the tongue's pressure sensation, and when the temperature is set at 36 °C, the only elicited sensation is taste while the temperature effect is masked.

2.3 Feedback System

Together with the physiological measures, it is also important to implement a reliable computer-controlled feedback system that can return the subject's perceptions in synchrony with the EEG recordings. It is feasible to build a homemade script for this purpose. However, one available software that we prefer in our experiments is PsychoPy [14]. This is an open-source library running in Python designed for presenting stimuli and receiving subject feedback via mouse or keyboard interaction in neuroscience experiments. The toolbox has a high temporal precision (millisecond) if implemented into appropriate hardware, which is an essential feature when coupled with a signal acquisition system such as ERP-EEG. The stimulus onset, the psychophysical response, or other physiological activity must be synchronized and co-registered with the EEG signal.

2.4 Visual Tracking Task

In order to help the subject in maintain attention and vigilance during the experiment, it is necessary to set up a simple visual tracking task (e.g., a square field moving on a screen implemented in the PsychoPy paradigm).

2.5 System for Data Analysis

Many software packages are available to analyze EEG/MEG data, either with low or high electrodes resolution. In our lab, we use the free academic software CARTOOL by Denis Burnet (ref. web site:

http://brainmapping.unige.ch/CARTOOL) [15]. The program CARTOOL provides an analysis tool focused on reference-free EEG/MEG mapping techniques. The software is continuously up to date, and constantly increases in functionalities.

2.6 EEG Dedicated Room

EEG signal is susceptible to all electrical noise; for this reason, it is crucial to shield the experimental room where the acquisition is going to happen from electromagnetic sources. The best approach is performing the EEG in a separate space dedicated to only the participant, the outlet of the gustatory delivery system, and the EEG acquisition, with all acoustic and electrical noise shielded. At the same time, the rest of the equipment is allocated in a separate chamber. It is also convenient to have a camera with a speaker and microphone to communicate with the subject and supervise their overall behavior during the experiment.

3 Methods

In this section, a detailed description of the methodology used in our experimental setup to record gustatory g-ERP is presented [11–13, 16].

1. *The Overall Protocol*

   The protocol involves first preparing the experimental paradigm and the stimuli conditions. Then, the participant is trained and placed in a room dedicated to EEG. The subject's state of vigilance and sources of artifacts that could interfere with the EEG signal must also be monitored and controlled. Finally, once the protocol has been launched and the data collected, they must be stored and analyzed in an appropriate manner. All these aspects are described below.

2. *Prepare the Stimuli*

   (a) *Taste stimuli*. The five tastant stimuli, sweet, sour, bitter, salty, and umami, can be prepared according to [12]. In the study, a group of subjects (n.30) rated three different dilutions for each tastant solution on a scale from 0 to 100 a.u. (arbitrary units). The scale limits 0 and 100 a.u. were anchored respectively to "no taste perceived" and "very intense taste." Table 2 reports the relative concentration (c /C) per liter. We use water as solvents for injectable solutions (Aqua ad injectabilia, Braun, Melsungen, Germany). The choice to use saccharin, which is an artificial sweetener, instead of more commonly used sucrose to elicit the sweet quality of taste is justified in *see* **Note 4**.

Table 2
Concentration per liter (in c or C) used as basic taste stimuli

Tastant	Chemical name[a]	Chemical formula[b]	c (mM/l)[c]	C (g/l)[d]
Salt	Sodium chloride	NaCl	347.6824	20.3186
Bitter	Quinine hydrochloride	C20H24N2O2-HCl	0.7436	0.2683
Sour	Citric acid monohydrate	C6H8O7	40.0351	7.6919
Umami	L-glutamic acid monosodium salt monohydrate	C5H8NNaO4-H_2O	426.8045	72.1726
Sweet	Saccharine	C7H5NO3S	3.6067	0.6607

[a,b]Chemical name and formula as reported on the product by Sigma-Aldrich Chemistry, Steinheim am Albuch, Germany
[c,d]c/C = concentration respectively in mM/l and g/l. The table is based on work [12]

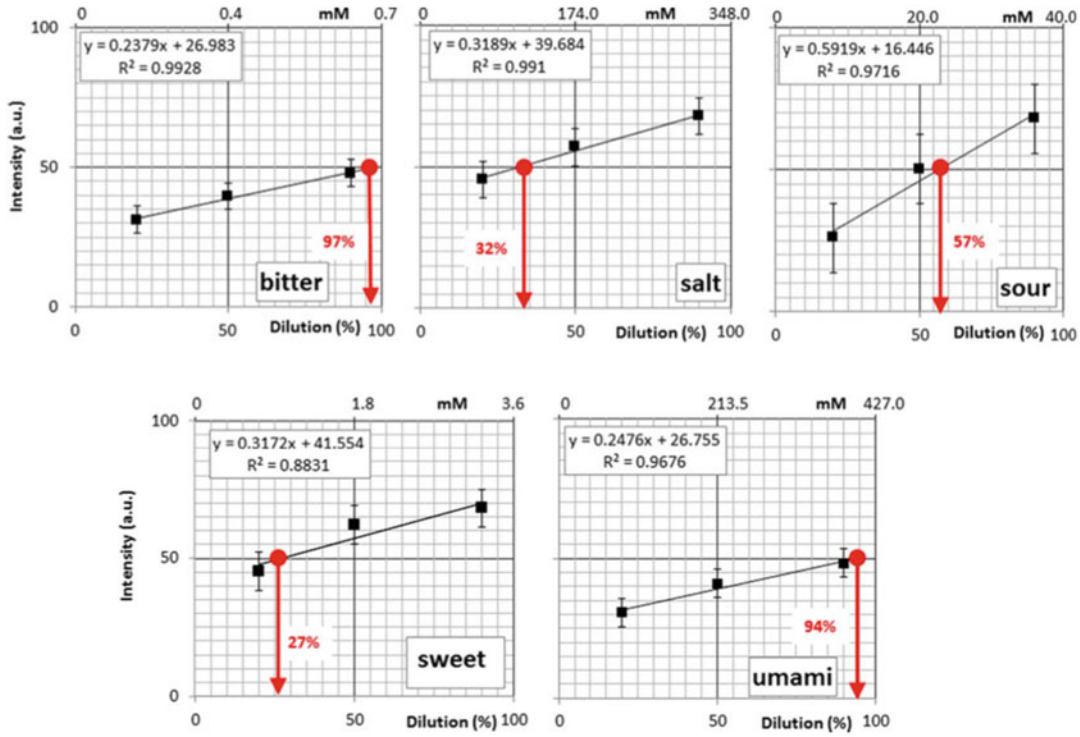

Fig. 2 Calibration curves showing the linear fitting of the mean intensity values (±SE) as rated by the group of subjects on a scale from 0 to 100 (a.u.) versus dilution of the solution expressed in percentage (lower axis) and millimolar per liter (upper axis). For example, to define an isointesity of 50 a.u. the relative dilution to use as parameter of the gustometer is identified by the red arrow and are 97% for bitter, 32% for salt, 57% for sour, 27% for sweet, and 94% for umami. The tastant delivery system can dilute the solution in percentage levels, where 100% corresponds to full basic concentration and 0 to only water. (Reproduced from Iannilli 2017 with permission from Wiley [12])

In the same work, final calibration curves (Fig. 2), generated through linear to the mean intensity values, were produced. These curves can help identify the intensity best used in an experiment. For example, to set an

iso-intensity of 50 a.u., for all the tastants, the calibration curve respectively identifies a dilution of 97% for bitter, 32% for salt, 57% for sour, 27% for sweet, and 94% for umami.

Although these calibration curves are useful for identifying a concentration for the initial experimental setup, it is good practice to reestimate the final concentration for every new study with the group of subjects included in the final experiment. For example, based on the calibration curve, three intermediate concentration levels can be chosen (such as 20%, 50%, and 90%). New fit curves can be generated, and the concentration levels then evaluated at the single subject or group level, depending on the necessities of the study.

(b) *Taste stimulus baseline (control condition)*. The control condition normally consists of an absent taste stimulus but with similar physicochemical characteristics. In experiments focused on food, it has been demonstrated that the use of water is problematic as it seems to induce taste-like activities in various insular areas [17]. When used as a control condition, it can mask brain areas involved in taste perception. A good practice is to use a solution containing the main components of saliva (artificial saliva), 25 mM KCl + 2.5 mM NaCO3 [18]. Therefore, in a food-related task, the baseline, also called control condition, is represented by artificial saliva (tasteless solution) with the same temperature and mechanosensory input of the taste condition and can be inserted in the sixth and seventh module of the gustometer. In this situation, the signal variation caused by the specific task is evaluated compared to the control.

(c) *Olfactory, trigeminal, or temperature stimuli*. To include olfactory or trigeminal stimuli into the protocol please refer to *see* **Note 5** (olfaction), **Note 6** (trigeminal), and **Note 7** (temperature).

3. *Setup a Training Session*

In order to make the subject familiar with the experimental procedures and the lab environment, it is important to invite the volunteer for a first training session in the lab. At this time, the subject is introduced to the lab equipment used for the experiment. They can take part in a mock session where they are trained to receive the oral stimulus, give eventual feedback, and properly perform, as trained, during the EEG acquisition. After that, the actual experimental session, with signal brain acquisition and the whole paradigm, can be planned for the coming days.

4. *Set Up the Participant in the Room*
   The subject must be set up in the experimental room dedicated to the EEG. Subsequently, it is necessary to maintain contact with the subject via the microphone system.
5. *Install the EEG Equipment*
   The electrodes must be positioned according to standard recommendations (*see* Subheading 2.1). The experiment during the ERP-EEG recording should be properly designed. An event-related paradigm, where individual trial events are measured, needs to be applied. In this paradigm, involving a gustatory task, a large number of trials (>30) are needed to have a significant signal-to-noise ratio (*see* **Note 3**). The feedback system provides the options to design an experimental paradigm to control and present multisensory stimuli in real-time, while simultaneously collecting the subject's behavioral responses and the brain signal or other physiological activity. Specifically, in the study of food-related emotions, a feedback system can present in synchrony with the stimulus delivery, a psychometric scale that refers to pleasantness, intensity, edibility, congruency between stimuli, or any other variable of interest.
6. *Install the Gustometer*. The liquid outlet is positioned at 2–3 cm from the location to stimulate, e.g., the tip of the tongue. A liquid spray, interleaved by constant airstream, comes out from the gustometer outlet, of, either tastant or tasteless solution. The subject sits on a comfortable chair and leans his or her forehead against a dedicated sustain with their mouth open to receive the taste stimulus. The subject must remain as still as possible while the liquid solution is collected in a bowl positioned under their chin.
7. *Ensure Subject Vigilance*. For an artifact-clean EEG acquisition, the subject must reduce eye movements and muscular contractions as much as possible. To help the subject accomplish this, it is recommended to implement the visual tracking task. Further, it aids to stabilize subjects' vigilance while sitting in a comfortable position during the whole session.
8. *Start the Study and Collect Data*. Once the subject is set up and all the instructions have been formulated, launch the experiment.
9. *Analyze the Data*. The purpose of this protocol is not to elaborate on how EEG data can be analyzed. However, we have provided some information on this topic in *see* **Note 8**.

4 Notes

1. *Texture.* To the best of our understanding, the study of olfaction and/or chemosensory interaction with food texture in the event-related design is not feasible. The reasons are related to the physical properties of food textures (high viscosity reduces the steep onset of the gustometer taste pulse and, hence, it does not elicit ERPs [11]) that are incompatible with the principle of the event-related design and the food delivery system (*see* Subheading 2.2). Functional magnetic resonance imaging (f-MRI) or resting-state (rs-) MRI in a block design is a preferable brain functional imaging technique to study food texture [10, 16].
2. *MEG.* The MEG system measures the variation of the magnetic field of the brain with sensors located on top to the scalp. Considering that the magnetic field generated by the earth is several orders of magnitude stronger than the field generated by the brain, the MEG system needs to be included in a highly magnetically shielded room. In addition, all the materials proper of the experiment, like gustometer outlet, liquid flows, and additional sensors, must not interfere with the measurement. Therefore, they have to be of nonmagnetic material, and it is recommended that before the participant enters the MEG room, an "empty room" recording of about 2-min duration with all additional devices in a fully operational mode utilized in the experiment is registered. Similarly, it is advisable to perform at least 2-min "resting state" recordings before and after the experiment with the subject fully prepared and positioned in the MEG system. All required equipment (gustometer outlet, microphone, camera, projector, etc.) should also be switched on.

   These operations give an overview of the noise level (and their changes) at the time of measurement and contribute to acquire valuable baseline data to identify problems and artifacts with the system in the new setup.

   In the specific, when using the gustometer in the MEG room, an outlet and a hose made in plastic, or equivalent nonmagnetic material, are required. The MEG room is typically equipped with a dedicated opening to funnel the hose with minimum measurement influence. It is also essential to properly select the volunteers and familiarize them with the magnetically shielded room and the procedures. A good practice is to have a mock experiment where the subject can learn how to behave correctly in the MEG environment during the gustatory task. The subject also needs to reduce eye blinking and remain as still as possible. Their head must be fixed under the dewar as close as possible to the MEG sensors (Fig. 3). During the gustatory task, a wood frame supporting the

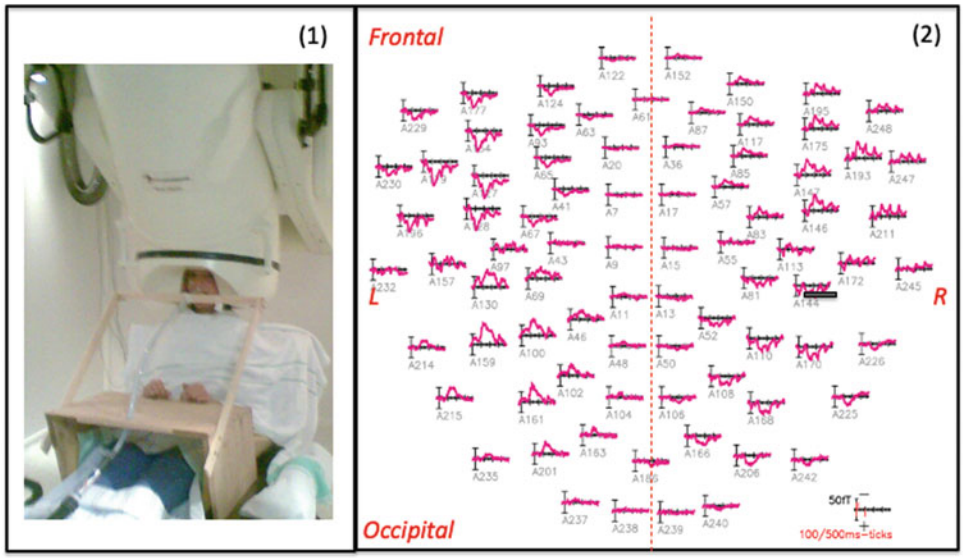

Fig. 3 Experimental setup of a gustatory task in a MEG system. (1) The subject is comfortably sitting in a magnetic-shield room with the head fixed under the dewar, where the MEG sensor are located. (2) Example of gustatory event-related fields (gERFs) on a 82 magnetometer down-sampling of a high-density channel MEG system (249 channels) . R = right, L = left. (Reproduced from Iannilli 2017 with permission from Elsevier [13])

gustometer outlet is positioned in front of the subject. He is positioned with his mouth open to receive the sprayed solution, not swallow, and let the excess liquid flow down his chest. The fluid is then collected by an absorbent cotton towel covering the subject's torso and lap [13]. In the MEG system, due to the recline position of the subject, to use an absorbent cotton towel is more convenient than bowl collecting the liquid, as in the case of EEG acquisition.

A delta function pulse-like output is needed to elicit the event-related field (ERF) measured with the MEG, similar to the ERP. Therefore, a system like the gustometer, with pulse-like output, is a suitable apparatus. Other parameters of the stimulus presentation sequence, such as pulse duration, interstimulus interval, solution volume per pulse, remain identical to those used for ERPs. Due to the low signal-to-noise ratio in the MEG system, a higher number of stimulus repetitions than the EEG is required. However, a compromise is indispensable due to the possible reduction of the subject's performance in an excessively long experiment.

For a gustatory event, considering that there are long interstimulus intervals (about 20–30 ms) applied to avoid gustatory habituation and recovery of the gustatory function, one cannot go over 80 repetitions per condition. 80 repetitions are equivalent to c.a. 48 min per condition; for this reason, if the experiment includes many conditions, it is also advisable to

have a protocol that involves multiple sessions that occur in separate days. This will avoid unreasonably long sessions for the subject and ineffectual results to the experiment.

Newer MEG-generation systems are often coupled with EEG equipment that allows for the registration of both signals simultaneously, which also helps in the definition of the electromagnetic sources. The interested reader can find extensive details about the MEG system and its use in more specific publications [9, 24].

3. *Session Duration*. In our experience, we have noticed that subjects can perform a task adequately and comfortably in a time interval no longer than 40–60 mins per session due to psychophysical limits. Other studies on efficient experimental design also support this [25].
4. *Sucrose*. Sucrose was not used due to the high viscosity of the solution. High viscosity reduces the steep onset of the gustometer taste pulse and, hence, does not elicit ERPs [11].
5. *Retronasal Olfactory Stimuli*. The volatile compound conditions need to be soluble, and can be added directedly into the gustometer jars that contain the taste conditions in the study. Another possibility is to add a retronasal cannula where a volatile compound is delivered with the help of an olfactometer [19]. The olfactometer is a device that delivers odorant-pulse stimuli, controlled in time, duration, and temperature similarly to the liquid tastants for the gustometer—more details in [26]. The positive aspect of using the olfactometer to deliver the retronasal odor or gas is the high level of time control on each stimulus independently, which also allows the analysis of synchronization or de-synchronization of two stimuli onset, significant in the study of flavor perception [1].
6. *Irritant Condition*. Some technical aspects can limit the choice of irritant conditions in our setup and need to be carefully chosen. For example, capsaicin is a typical irritant contained in foods. Therefore, adding capsaicin as an irritant condition in food research can be interesting. However, all the care needs to be taken when handling irritant substance in the lab. In the case of capsaicin, its use in one of the gustometer lines can critically contaminate the line itself. A solution could be to dedicate one line only to delivering conditions containing capsaicin. The human perception threshold for capsaicin is generally considered to be 0.7 μM [20]. Another possibility to deliver the irritant through the retronasal route is to use the olfactometer to provide gas irritants (*see* **Note 5**). A typical gas irritant used in beverages is CO_2, which is odorless and tasteless, and represents a very interesting condition for food perception. The relative human perception threshold for CO_2 delivered through retronasal route is about 4% of the gas volume [21].

7. *Temperature Condition*. The gustometer is built with thermoregulation based on a water bath, with a temperature range from 22 °C to 50 °C. The water bath makes the reach of the set temperature quite long (minutes, hours), inevitably longer than the interstimulus interval (typical in the range of seconds), making the change of temperature in the course of an experimental session impossible. This prevents the use of different temperature conditions in a pseudo-randomized paradigm during one session. However, it remains possible to organize the experiment in block design sessions at a different time of the day or days and later compare the different temperature conditions. To mask the temperature effect on a stimulus condition, we use a value of 36 °C, equivalent to the body temperature.
8. *Analyzing EEG Data*. EEG data can be analyzed in many ways. However, it is essential that the method of choice returns the desired outcomes and maximizes the paradigm's reliability. The pipeline suggested here focuses on scalp field maps and their characterization, which are important for spatiotemporal analysis and source localization.

   The ERP-EEG data are first preprocessed, a step that normally includes offline filtering (DC removal, 60 Hz notch filter), re-referencing and artifact rejection (artifacts associated with oculomotion, cardiac pulse, and muscle movement can be automatically identified and removed using independent component analysis (ICA) [22] and/or visual inspection). After that, the cleaned preprocessed data are averaged per subject and condition into a final grand average. Then, at the group level, we identify a set of voltage topographies that explain a maximum amount of the variance using cluster analysis. The resulting topographies can be fitted to the single-subject data. Finally the statistical tests, based on nonparametric randomization procedures, are used to quantify changes in scalp topography and their parameters. In search of olfaction- and gustation-related brain activities, source estimations are preferably based on undetermined (distributed) source models, with the Local Autoregressive Average (LAURA) inverse solution. This algorithm incorporates biophysical laws as constraints to the more general minimum norm algorithm [27]. The head volume conductor model is based on the Locally Spherical Model with Anatomical Constraints (LSMAC) [15, 28, 29]. More than 4000 solution points should be used as source space. The MNI (Montreal Neurological Institute) 152 brain will be used as a head model for the group analysis, with space solution constrained to the segmented MNI brain. The quality of the model and thus ultimately the quality of the source reconstruction will also greatly benefit from individual anatomical

information, e.g., using the individual anatomical MRI. It is beyond the scope of this chapter to discuss source reconstruction algorithms in detail, and for more information, we refer the reader to the relevant literature [23, 27].

Finally, statistical parametric mapping (SPM) on the tomographic EEG source images can be performed to determine group/conditions differences. The spatially extended statistical maps generated can be used to test hypotheses [18, 23].

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Chapter 20

Assessment of Food Odor-Evoked Emotions Using Functional Magnetic Resonance Imaging

Pengfei Han and Thomas Hummel

Abstract

Food sensory cues are prominent sources of emotions. The advancement of functional magnetic resonance imaging (FMRI) allows to noninvasively examine the human brain activation related to food-evoked affective experiences. This chapter describes a step-by-step protocol for assessing brain response in relation to emotions evoked by olfactory food cues (food odors) in humans.

Key words Food odor, Pleasantness, Functional magnetic resonance imaging, Protocol

1 Introduction

Foods are prominent sources of emotions [1]. The perception of food-related sensory cues (visual sense, odor, taste, or flavor) and related emotions influences food preference, choice, or consumption [2]. From an affective neuroscience point of view, emotions rely on an affective core, that is the pleasure system, which provides affective tones to emotions [3]. However, the theoretical grounding for restricting the description of food-evoked emotions to the unique scale of valence, liking, or pleasantness has been questioned [4, 5]. Thus, the emotional (or affective) experiences elicited by food-related stimuli can be structured in a multidimensional space, including (but not limited to) the two-dimensional space of valence (pleasure/displeasure) and arousal (level of salience) [6, 7].

Measurement that targets the physiological, behavioral, or the cognitive levels of food-related emotional processes can be distinguished into either explicit or implicit categories [8, 9]. The explicit measurements rely on the self-report of food-evoked emotions upon consumption, smelling, or seeing foods, using standardized

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scales (hedonic scales) or questionnaires (emotional lexicons, emotional pictures, or emojis) [8, 10]. The implicit methods refer to the direct reading of the automatic behavioral or physiological responses related to emotions, such as cardiovascular responses, electrodermal activity, or brain responses [11]. Compared to explicit assessments, the implicit methods can provide insight into whether an emotional experience has occurred and, to some degree, its nature. Moreover, implicit measures allow to assess equivalents of emotions while participants are tasting, smelling, or looking at pictures of food without the immediate need for a cognitive translation by the consumer after the experience [12].

The functional magnetic resonance imaging (FMRI) is a noninvasive neurophysiological technique for estimation of brain activity by measuring the regional hemodynamic changes (the blood-oxygen-level-dependency [BOLD]) in response to the presentation of certain stimuli [13]. The FMRI has high spatial resolution, which enables the location of cortical and deep brain structures involved in affective processing of food stimuli [14]. Previous research had shown hedonic or affective brain responses to various food cues including food pictures [15], tastes [16, 17], flavor [18, 19], or aroma [20]. These brain activations are commonly found in the orbitofrontal cortex, the insula, the anterior cingulate cortex, as well as primary sensory cortex [21–23], areas that overlap with the emotional network in the mesocorticolimbic brain structures [24–27]. Therefore, FMRI provides a unique noninvasive method for assessing and quantifying the neural activation related to food stimuli, which are regarded as early precursors of food-evoked emotional experiences.

It has been known for a long time that odor perception is intimately and strongly linked to emotions [28, 29]. Moreover, compared to food cues from other sensory modalities (e.g., visual modality), food odors have been shown to be potent stimuli with high ecological relevance and more affect-laden characteristics (e.g., hedonism or motivation) in the context of food appraisal [30]. In this chapter, we describe a method protocol using fMRI for determining brain activation patterns for a group of subjects in response to food-related odors varied in perceived pleasantness. The protocol provides a physiological measure of food odor-elicited affective feelings (i.e., conscious emotions). However, the protocol will not include instruction on the analyses of fMRI data generated by the study design. It has to be noted that inferring specific emotions based on a pattern of brain activation in response to food-related odors alone is generally a very risky (if not fallacious) endeavor, and that further converging evidence using multiple measures should be considered (see, [31] for a similar discussion).

2 Materials

The materials shown in this protocol are intended to be general guidelines and may vary according to specific experimental needs. All materials must be MRI compatible.

2.1 MRI Scanner and Head Coil

An MRI scanner with 3 Tesla (T) magnetic field strength (Siemens Sonata, Erlangen, Germany) and a 64-channel head coil (*see* **Note 1**).

2.2 Odorants

We chose odors based on their published averaged pleasantness ratings. The odor stimuli include an unpleasant fish odor (Fish flavor oil, Givaudan Inc., Geneva, Switzerland) [32], and a pleasant roasted peanut odor (Symrise, GmbH, KG) [33]. Additional stimuli include a pleasant (2-Phenyl-Ethanol, Sigma Aldrich, CAS 60-12-8) and an unpleasant non-food odor (Octanoic Acid, Sigma Aldrich, CAS 124-07-2) (*see* **Note 2**).

2.3 Solvent

Solvent (e.g., water, mineral oil, 1,2-propanediol, ethanol) is needed to dilute the odorant to proper concentrations. Here, 1,2-propanediol is used as the solvent for odorant solution preparation (*see* **Note 3)**.

2.4 Olfactometer

A computer-controlled multichannel olfactometer allows precise delivery of odorous stimuli (mobile MRI-compatible olfactometer with 5 channels [34]) (*see* **Note 4**).

2.5 Olfactometer Parts

About 5-meter long Teflon tubes (inner diameter: 2 mm) are led into the scanner room and connected to a nosepiece (inner diameter 4 mm; a single PTFE-flow line with a length of 5 cm). The 5-meter long tubes and the nosepiece are connected using several Y-shape joint plastic tubing connectors over a length of approximately 15 cm. At the end of the tubing a nosepiece for monorhinal stimulation, a mask or a Y-piece can be attached, to deliver odorants to one or both nostrils (*see* **Note 5**). The tubing reaches for approximately 1 cm inside the nasal cavity beyond the nasal valve with the participant lying inside the magnet bore. One-way stop-flow valves are required for protecting the back-flow of liquids into the olfactometer, and should be placed between the wash-bottle and the olfactometer (Fig. 1c). Gas-washing chambers are used as odor solution container.

2.6 Air Source

A constant clean air source is required to bring odor stimuli to the near-nose area of the participants. We use the built-in fresh air supply in the MRI scanner room with adjustable flowrate (*see* **Note 6**). A flowmeter is connected at the air outlet for setting and adjusting the flowrate.

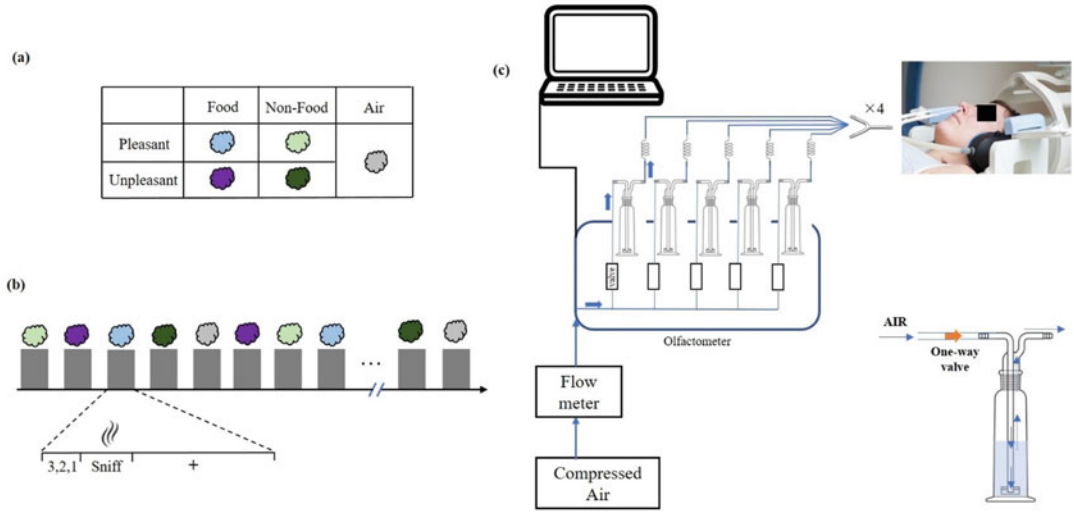

Fig. 1 fMRI odor stimulation paradigm. (**a**) Food and non-food odor stimuli used for the experiment; (**b**) the event-related odor stimulation design; (**c**) diagram of the odor delivery system with the main components: air source, olfactometer, computer, tubing and nosepieces, and washing bottles

2.7 Computer and Software for Visual Cue Presentation

The E-Prime MRI compatible presentation system with a computer installed with the E-Prime software (*see* **Note 7**). Visual cues include the 3-second countdown, the "Sniff" indicator, and the fixation cross between two odor stimuli (Fig. 1b).

2.8 Respiratory Monitoring System

Because the act of sniffing induces activations within primary olfactory cortex, which may interfere with the activation caused by affective value [35], the breathing patterns of each subject should be monitored online during the experiment. A breathing monitoring system with a respiratory belt is used to measure the "sniffs" of participants during the fMRI scan (e.g., the BioPac MP150 system, CA) [36]. Respiration is recorded with a breathing belt affixed to the subject's chest to record abdominal or thoracic contraction and expansion (*see* **Note 8**).

3 Methods

3.1 Participants Screening

A group of participants should be screened for the safety to be scanned in an MRI scanner (*see* **Note 9**).

3.2 MRI Hardwire Preparation

Prepare the hardwires outside and in the MRI room before arrival of the participant, including attaching the head coil to the scanner. In most of the MR setups, the MR scanner delivers the trigger (corresponding to the repetition time [TR] of the imaging sequence) to the simulator. This trigger helps to synchronize stimulus presentation and data acquisition.

3.3 Olfactometer Set-Up

Place the tubing into the MRI room, and keep the olfactometer and its control computer in the control room. Fill the gas-washing bottles with roughly 50 ml of odor solvent and place them in the olfactometer. Check the connections to ensure that all odorant containers are properly attached to the channel outlet of the olfactometer. Connect the long polytetrafluoroethylene (PTFE) to the gas-washing bottle and the nose pieces. Connect the radiofrequency trigger from the MRI system to the "trigger in" port on the olfactometer and the computer with E-Prime program to synchronize the odor/visual stimulation paradigm and fMRI image acquisition.

3.4 Briefing and Practice

Participants should remove all belongings and get changed before entering the MRI scanner room. Participants should be informed the general procedures of the experiment, and be trained to familiarize and follow the instructions of the odor perception task. Participants report their hunger/fullness and thirst levels on a 9-point scale (*see* **Note 10**).

3.5 Place Participant

Guide the participant into the MR room and position the participants. Have the participant wear the earplugs (for protecting them from the noise) and the respiratory monitoring belt before lying supine on the MRI examination bed. After the participant lies down, manually adjust the tightness and placement of the belt holding the respiratory sensor according to the respiration pattern seen on the display. Then place and fix the nose piece properly (using medical adhesive tape) on the subject's near-nose area (e.g. back of the nose) to ensure that the air blows into the nostrils (*see* **Note 11**). Move the participant into the MRI scanner and confirm that the participant can view the entire display using the mirror.

3.6 Start the Experiment

Start the fMRI image acquisition on the MRI console (*see* **Note 12**); the odor stimulation paradigm with visual cues for odor sniff should start as soon as image acquisition starts.

3.7 Odor Stimulation Task

In our past studies, we used an event-related design for odor stimulation during fMRI scanning. Assign duration for the stimulus (the opening of a specific channel) as well as duration for the channel to be closed. A countdown from 3 s is shown on the screen followed by "SNIFF". The odor is presented for 4 s (approximately one respiratory cycle with inspiration and expiration). Then a "crosshair" is displayed for 11 s before the next countdown starts (*see* **Note 13**). The fMRI scan is divided into 3 separate runs. Each run should last 10 min and 30 s. During each run, each odor is presented for 7 times, and in total each odor condition is presented for 21 times (*see* **Note 14**). The order of the odor stimuli presentation is randomized (*see* **Note 15**).

3.8 Rating of Odor Stimuli

Participants taking part in the fMRI study rate the odors for pleasantness, intensity, familiarity, sweetness, and edibility on a 9-point Likert scale. The rating phase is preferred inside the scanner while participants are in their lying position. (*see* **Note 16**).

3.9 Complete Experiment

As the session ends, switch off the olfactometer. Move the participant out of the magnet bore and remove the tap and the nozzle.

3.10 Data Storage and Analysis (See Note 17)

Copy the raw image from the MRI computer and perform data analysis. The frequently used programs for fMRI data analysis include the Statistical Parametric Mapping (or SPM), Software for analysis and Visualization of Functional Magnetic Resonance Neuroimages (or AFNI), and Advance in functional and structural MR image analysis and implementation (FSL) [37].

4 Notes

1. MRI scanners with 1.5, 3, or 7 Tesla magnetic field strength are cleared for human neuroimaging research [38]. Currently, 3-Tesla is the most convenient and most widely available field strength for acquiring reliable data in human participants.
2. These odors are just examples, and this could be changed according to the aims of the study. Choose odors with different pleasantness for the experiment. The odor intensity, pleasantness, familiarity, sweetness, food association (edibility), and irritation should be pre-evaluated by a group of participants using 9-point scales. Selected odors should only differ in terms of valence/pleasantness with all the other characteristics matched. Ensure that all containers have the same amount of space, same amount of solution, and same surface area for the solution. For example, use six 250 mL size glass bottles as odorant containers, with each bottle holding 50 mL of odor solution.
3. For odors that are water and oil soluble, we recommend mineral oil or propylene glycol as odor solvent to eliminate water bubbles caused by the air flow which may enter the tubes through the glass container. In addition, the solvents provide good solubility for the lipophilic odorants.
4. Any type of equipment, either custom-made or commercial products with similar capabilities, may be used in an analogous fashion. The technical details of the olfactometers described in the current protocol can be found in the original paper by Sommer et al. [34]. This olfactometer enables the supply of odor stimuli to the nose, with little thermal or tactile stimulation, in a precise and controlled manner.

5. The use of separate channels and tubes mostly prevent cross-contamination of scents in the odor presentation. However, potential odor (cross-) contamination needs to be considered. For example, the Y-shaped connector is a risk point where more than one type of odors are mixed up. Replacing the Y-shaped connectors and nosepieces for each participant is preferable. Besides, a constant high-flow vacuum applied to the rear of the head coil [39] or synchronizing the delivery of odors with the user's breathing pattern [40] has been shown as an effective way to prevent ambient contamination.
6. Airflow is at the very essence of stimulus delivery in olfactometry. A high temporal resolution is not crucial in fMRI studies due to the relatively slow nature of the hemodynamic response and thus a simpler stimulus delivery unit is enough to get fMRI results. The flow of air for most fMRI research is between 2 L and 15 L per min [34]. Therefore, high-powered air pump is not suitable for such purposes. The nonhumidified air can cause irritative sensation and crusting of nose skin at high flow. Based on results from recent studies, an aquarium air pump is suitable for achieving the airflow range [41].
7. Most of the MRI scanners are equipped with displays for visual stimuli/written information or instructions. The display will be visible to the participants (in a supine position) via the mirror fixed to the head coil.
8. Subject-specific sniff waveforms were baseline-adjusted by subtracting the mean activity in the 1000 ms preceding sniff onset, and then averaged across each condition. Sniff inspiratory volume, peak amplitude, and sniff duration (time to peak) were computed for each trial, sorted by odor stimuli conditions, and used for later analysis [42].
9. Ask the subject about medical history, including potential implants, claustrophobia, or other preexisting conditions that may interfere with the subject's ability to safely participate in the fMRI study. Additionally, perform a simple odor identification test [43] to ensure that the subjects have normal olfactory function and can smell the odorants during the experiment. Besides, eating disorders, restraint eating traits, or other known characteristics that may affect emotional or affective processing of food stimuli should be screened and excluded. The group of subjects are preferably at least 20 subjects.
10. The homeostatic state is deeply rooted in central-nervous information processing [44]. Hunger/thirst status can interfere food-evoked emotional experiences and related brain responses [45–47]. Therefore, it is important to ask the participants their hunger/thirst levels.

11. In olfactory neuroimaging studies, odors are directed into the nose, using a nozzle or nasal cannula, or are dispersed surrounding the nose using a nasal mask [48, 49]. For example, while odor delivered via a cannula allows for monorhinal stimuli with optimal temporal resolution, stimulation with high airflows with dry air at room temperature over a long period of time will cause nasal congestion and mucus secretion. Besides, the entire MRI head-coil can be transformed into a well-controlled odor delivery interface with a Teflon-coated Plexiglas canopy surrounding the head-coil, and a constant high-flow vacuum applied to the rear of the head coil. This odor delivery interface provides a more natural odor perception environment and shows good dissociable odor presence and absence with minimal added discomfort during the olfactory fMRI experiment [39].
12. Regarding the MRI sequence, a pragmatic recommendation given by Koopmans and Yacoub [50] can serve as a guideline: First, pilot an fMRI study using a gradient-echo BOLD sequence with the lowest resolution at which the effect of interest would still be expected to be seen; Then, verify if the effect of interest (for example the difference between two conditions) can be identified with these parameters. Only if this is not possible or if the results are uninterpretable, additional measures to increase resolution or removal of vascular effects should be investigated. As an example, functional images can be acquired by using a standard echo planar imaging sequence (repetition time TR/ echo time TE: 2000/20 ms, flip angle = 80°, voxel size: 3.0 × 3.0 × 2.5 mm^3, 40 slices, 64 × 64 base resolution, field of view = 192 mm), oriented parallel to the inferior edge of the temporal and frontal lobes. Structural images can be acquired with a T1-weighted 3D sequence (TR/TE: 1900/2.31 ms, flip angle = 9 °, field of view = 256 mm, voxel dimensions: 1 mm, isotropic 256 × 256 × 192 voxel). Meanwhile, some new or promising sequences may be suitable for studying food odor-evoked emotions. For example, recent study results found that short TR (or the combination of short odor stimulation and short TR) is associated with more pronounced signal increase in olfactory/emotion-related brain regions (e.g., piriform cortex, amygdala, insula, and orbitofrontal cortex) and shorter time to peak signals [51, 52]. This fast acquisition sequence with simultaneous multi-slice image collection is preferred for FMRI study of emotional brain responses to food odors.
13. Designs of odor stimulation may be modified according to the study objectives and data analytically approached. The event-related fMRI design consists of short stimulations alternating with rather long baseline (e.g. clean air) periods, while the

blocked design typically consists of blocks (with each 15 s to 30 s long) alternated with baseline blocks (same time length). The detection of activated areas is optimized by blocked design (higher statistical power) [53], while the event-related design enables to depict the temporal profiles of brain responses to certain odor stimuli (finite impulse responses) which is more suitable for exploration of dynamic changes of emotion-related brain activity in response to food odors.

14. In other sensory experiments, one would enhance the frequency of the stimuli to increase the brain activation signals and to improve the statistical power of the data. However, this cannot easily be done in olfactory experiments because of rapid desensitization of the olfactory system after repeated odor stimulation [54]. Besides, several studies reported the improvement of detectability and reproducibility of the olfactory fMRI signals in the olfactory cortex through the inclusion of short odor stimulation durations, or more ecological task design (e.g. synchronization of the breathing cycle with odor stimulation) [55]. Bitter and colleagues have shown that the optimum olfaction stimuli repetitions to reduce olfaction habituation and provide high quality brain activation results should not be set to more than 4 to 8 times [56]. However, it is recommended to include a greater number of trial repetitions to achieve a better signal-to-noise ratio.
15. Sequence effects, such as habituation [57], and contrast effects (e.g., presenting a pleasant odor right after an unpleasant odor; see [58], for an example with beverages) may have a strong influence on chemosensory perception. The duration of the stimulus typically comprised between 1 s and 3 s, with interstimulus intervals with enough length to return to a neutral emotion baseline. This interval typically ranges between 10 s and 30 s to prevent odor adaptation. To control for the contrast effect, odor stimuli sequence needs to be considered (e.g., alternation between odors). In addition, a trial-by-trial rating of the odor pleasantness can be obtained and used in later data analyses.
16. The inclusion of an odor rating phase in the fMRI experiment is also generally recommended, which ensures that the odors used are rated as pleasant or unpleasant by the subjects taking part in the fMRI study. Such a rating phase is important due to the high interindividual variability in odors' pleasantness evaluation.
17. As for the expected brain activations that can be typically obtained with the protocol described here, interested researchers can refer to a literature of meta-analyses of brain activation in response to olfactory-related food and nonfood stimuli, and pleasant vs. aversive olfactory stimuli [59].

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