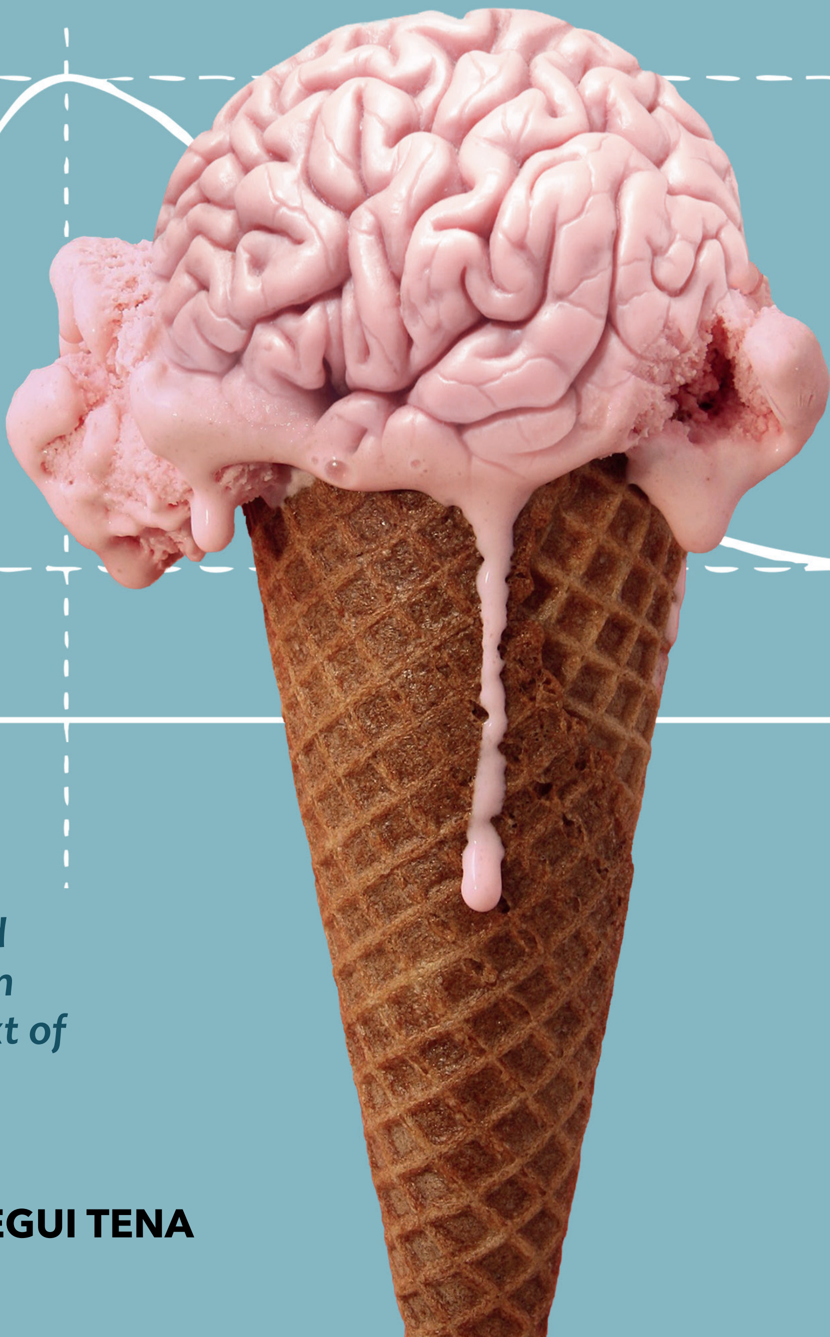
THE BODY AS A TATTLETALE



Physiological responses to food stimuli perception within the context of expectations

LUZ M. VERASTEGUI TENA

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This thesis was conducted under the auspices of the Wageningen School of Social Sciences.

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 12 November 2019
at 11:00 am in the Aula.

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The body as a tattletale: Physiological responses to food stimuli perception within the context of expectations.
170 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2019)
With references, with summary in English

ISBN: 978-94-6395-058-9
DOI: https://doi.org/10.18174/497178

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CHAPTER 1

General introduction

1.1. The perception of food

The evolution of the human diet is full with changes that have shaped human physiology, behavior and perception (Crittenden & Schnorr, 2017). Primates responded to food availability in an opportunistic way, ingesting only foods that were seasonably available. Later on, a shift towards a greater quantity of meat in the diet led to the elaboration of tools and cooking. Cooking itself helped change the digestibility and toxicity of certain foods, such as vegetables. Moreover, the extent and the time used in processing food diminished. As a result, there was a greater variety of food in the human diet. This was linked to changes in human dentition and brain size. Finally, the introduction of agriculture and animal domestication led to a total manipulation of the food sources. This led to changes not only in the human diet but also in the human environment (Chrzan & Brett, 2017). Nowadays, food availability is higher than that of our ancestors, as is the diversity of food (Kearney, 2010). The human interaction with food, its perception and subsequent consumption, is now among the most multisensory events in humans (Velasco, Karunanayaka, & Nijholt, 2018).

The interaction with food starts with the process of perception, which entails three basic stages: exposure, attention, and interpretation (see **Figure 1.1**). Exposure can be through stimuli such as visual cues, smells, sounds, and tastes present in an individual's environment (Solomon, Bamossy, & Askegaard, 2002). Only a few of these stimuli are noticed or even attended to. An individual's awareness to these stimuli will depend on his past experiences and current needs as well as on the intensity, duration, and relevance of the stimuli. Attention relates to how much individuals focus on the stimuli within their range of exposure. Saliency or incongruences in a stimulus can increase attention. Finally, interpretation relates to the meaning that is given to a stimulus. The preconceived opinions, needs, and experiences of the individual might influence said interpretation (Solomon et al., 2002). The final perception of a food product is, therefore, obtained through a multidimensional process in which sensory inputs (deriving from olfaction, observation, touch, gustation, and the trigeminal system) and inputs from the aforementioned factors (incongruences, saliency of the product, preconceived opinions, and needs of the individual) interact and lead to a response that can lead to the acceptance or rejection of the food product (Costell, Tárrega, & Bayarri, 2010).

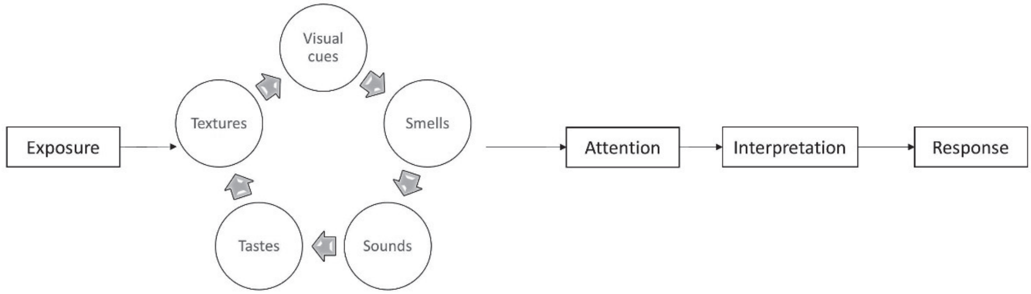


Figure 1.1. The perception of food (adapted from Solomon et al. (2002).

The integration of the sensory properties picked up by our senses can occur peripherally and centrally. Visual and auditory stimuli do not make physical contact with the areas in the body (retina, cochlea) that convert the sensory properties in information. Hence, these stimuli are integrated centrally in the brain. On the other hand, the properties of oral stimuli interact with oral and olfactory sources in the body. Many of these interactions between sensations happen first at the peripheral level. Thereafter, the information from the oral senses interact together centrally with those from vision and audition (Verhagen & Engelen, 2006). The information obtained helps organize behaviors (i.e. approach, avoidance, ingestion) through cognitive processes, affective reactions, and their linked physiological and/or motor responses (Pickard et al., 2010). Most research focuses on the affective reactions after the exposure to or consumption of a food product. However, as shown in this chapter, there is a richness of reactions in the human-food interaction that are yet to be explored and that can help reveal how individuals perceive the food in their environment.

1.2. The autonomic nervous system

The human nervous system (HNS) and the body are intrinsically and dynamically coupled. The body can respond to perception, cognition, and affective states and these can, likewise, respond to changes in the body (Critchley, Eccles, & Garfinkel, 2013). This happens through different branches of the HNS. The HNS is primarily composed by two branches. The first is the central nervous system (CNS), which is formed by the brain and spinal cord and is responsible for cognition, movement, the senses, and emotions (Jacobson, Marcus, & Pugsley, 2018; Tuladhar et al., 2015). The second is the peripheral nervous system, which is divided into the somatic nervous system and the ANS and is in charge of bringing information from and to the CNS. The somatic nervous system innervates skin and skeletal muscles. The ANS is formed by the sympathetic (SNS) and the parasympathetic systems (PNS) and innervates cardiac muscles, smooth muscles, and receptors of blood vessels and organs from the respiratory, digestive, endocrine, and urogenital systems. The SNS and PNS have opposing effects in these tissues and are dominant in specific conditions. The SNS is active during "fight-or-flight" reactions and during exercise while the PNS predominates during resting conditions. (Jacobson et al., 2018; McCorry, 2007). This makes the ANS important for the maintenance of physiological homeostasis, the response to acute stressors, and the regulation, integration, and orchestration of different physiological processes (Kenney & Ganta, 2014).

ANS responses are believed to not only indicate the changes related to the maintenance of the body's homeostasis but also changes related to affective states, motivation, attention, and preferences (Mendes, Harmon-Jones, & Beer, 2009). As ANS responses are not under voluntary control, such changes are believed to be unbiased. They are additionally believed to reveal information about people's attitudes and responses that they may not be able to articulate (Mendes et al., 2009).

ANS activity can be assessed through various measures. These include measurements of the cardiovascular, respiratory, and electrodermal systems. When it comes to measuring reactions to food, cardiovascular and electrodermal measures are often used. Skin conductance response is among the most commonly used measures of electrodermal activity while heart rate is among the most common cardiovascular measures (de Wijk & Boesveldt, 2016).

Skin conductance response refers to an increase in the electrical conductivity of the skin. It is mainly mediated by the sympathetic nervous system and is related to the activity of the sweat glands in the body. The body has two types of sweat glands. The apocrine glands are present in the hair follicles in the armpits and general areas. The eccrine glands are found in most of the body and the highest density can be found in the palms and soles of the feet. The function of the apocrine glands is not yet well understood. The eccrine glands on the other hand, are known for thermoregulation. Those located in the soles and palms, however, react more to affect-related stimuli such as pain, fear, or anxiety than to changes in temperature (Dawson, Schell, & Filion, 2000; Greco, Valenza, & Scilingo, 2016). Skin conductance changes are mediated by the amygdala and several areas of the prefrontal cortex (including the ventromedial, orbitofrontal, and dorsolateral area), the parietal lobe, and the anterior cingulate. They send inputs to the hypothalamus, which in turn controls the autonomic responses of the flight or fight response, including those of the eccrine sweat glands (Dawson, Schell, & Courtney, 2011). This would be the case, for example, when being presented to an unknown food. The unknown food would signal danger or uncertainty and lead to a fight or flight response that would increase sweating and, as a result, skin conductance.

Heart rate refers to the number of heart contractions (heartbeats) during a certain window of time (Barbieri, Matten, Alabi, & Brown, 2005). It is mediated by both sympathetic and parasympathetic nerves. Their control centers in the brain are located in the hypothalamus, brainstem, and spinal cord. Higher levels of the cortex, such as the limbic cortex, can also influence heart rate via signals to lower centers, such as the brainstem, spinal cord, and cerebellum. (Valderrama, Navarro, & Le Van Quyen, 2010). The action of the SNS and the PNS on heart rate depends on the circumstances. Situations of stress, fear, or anxiety like, for example, eating a food product considered disgusting, would make the sympathetic nervous system more active and lead to a higher heart rate. Restful conditions would lead to a higher activation of the parasympathetic nervous system and decrease heart rate. (Gordan, Gwathmey, & Xie, 2015).

1.3. Stimuli characteristics and the autonomic nervous system

Whether ANS responses are the basis for affective responses or if they occur independently of it is still a matter of debate. Three theories in particular have addressed this matter. The James-Lange theory, proposed separately by William James (1884) and Carl Lange (1885) on the same era, suggests that stimuli evoke ANS responses which in turn create the affect or emotion. This sequence was opposite to what was originally believed: that the ANS response is a product of affect itself. Challenging the James-Lange theory is the Cannon-Bard theory, which states that the ANS responses and affect are independent of each other. Stimuli characteristics are processed in the brain, which separately produces affect and the ANS responses. (Friedman, 2010). Finally, the Schachter-Singer theory or two-factor theory stated that first a stimulus creates arousal (and its subsequent ANS response), but that this response does not follow a particular pattern. The arousal stimulates a cognitive process to evaluate the arousing situation and determines the quality of the affective response (Reisenzein, 1983). There is still no scientific consensus on the relation between the assessment of stimuli, affect, and ANS responses, nor about the order in which they occur (Kreibig, 2010). Nevertheless, the link between them has become the basis for a body of research related to theories of adaptive behavior, emotion, and appraisal theories (Cacioppo, Berntson, Larsen, Poehlmann, & Ito, 2000; Frijda & Mesquita, 1998).

Stimuli can elicit ANS responses depending on factors such as relevance, novelty, valence, attention, and arousal (de Wijk & Boesveldt, 2016; Moors, Ellsworth, Scherer, & Frijda, 2013). Relevance refers to how significant the stimuli (or the context in which the stimuli are encountered) are for the well-being of the individual (Scherer, 2013). It is an initial evaluation stage and is intrinsically related to the novelty and valence of the stimuli (Olteanu, Golani, Eitam, & Kron, 2018). Both novelty and valence are often coded automatically. The detection of novelty is linked to attentional resources (Cohen, 2014). Novel stimuli are usually sudden and create a certain degree of arousal. These characteristics help them draw the attention of the individual (arousal itself is critical for attention as it can affect the capacity of attentional channels) (Cohen, 2014; Ellsworth & Scherer, 2002). Attention helps allocate the necessary processing resources in order to determine which actions should be taken (Coull, 1998; Ellsworth & Scherer, 2002). Valence will in part determine whether the type of reaction an individual will have will be that of attraction or aversion. The processing of valence depends in both organ specificities and memory. Given that this processing is fast, it is sometimes difficult to separate valence from attention (Ellsworth & Scherer, 2002).

Attention has been linked to cardiac deceleration since Lacey and Lacey observed these patterns when individuals asked to attend to simple or visual auditory stimuli while the opposite change (cardiac acceleration) was seen when individuals were in stressful stimulation or tasks involving cognitive elaboration (Carroll & Anastasiades, 1978; Lacey & Lacey, 2007). Arousal, on the other hand, has been linked to increases in skin conductance. Skin conductance is considered a robust marker of autonomic arousal (Rosebrock, Hoxha, Norris, Cacioppo, & Gollan, 2016).

Skin conductance increases have also been found in regards to novelty and relevance. During a repetition-change paradigm, pictures that were presented for the first time elicited significantly larger increases in skin conductance than pictures that were repeated. This change was seen for all pictures. However, pictures deemed as pleasant and unpleasant were related to larger changes than the neutral ones. This result was attributed to higher orienting as a function of the relevance of the stimuli with a valence (Bradley, 2009). The same study found that novelty and valence were related to cardiac changes. Novel pictures led to a pronounced cardiac deceleration, which became stronger when the pictures were unpleasant. This was most likely because the negative valence of the unpleasant picture led to an enhanced sensory intake and attention (Bradley, 2009).

Research on ANS responses to food have mainly looked at effects related to valence by looking at how ANS responses change when presented to stimuli that are liked or disliked (see **Table 1.1**). They, therefore, do not focus on the effects of the other aforementioned factors (relevance, attention, arousal, novelty). An exception would be the study by Delplanque et al. (2009) that looked at the processing of novelty and pleasantness when smelling different odors and found, similar to the results of Bradley (2009) that novelty leads to a cardiac deceleration and an increase in skin conductance. Nevertheless, the changes in heart rate and skin conductance responses obtained related to valence (measured as liking or pleasantness) in the food domain cannot yet be interpreted with confidence. For example, while He, Boesveldt, de Graaf, and de Wijk (2014) found that sniffing a disliked fish odor led to a higher heart rate and skin conductance than sniffing a liked orange odor, Beyts et al. (2017) found no differences when sniffing beer samples with aromas of different liking. Likewise, a study by de Wijk, He, Mensink, Verhoeven, and de Graaf (2014) in which breakfast drinks of different liking were tasted found that liking scores were positively associated with heart rate increases but a study by Horio (2000) that gave basic tastes found that heart rate increases and liking were negatively correlated for all tastes except the sweet one. Contradicting this data is the study by Danner, Haindl, Joechl, and Duerrschmid (2014) where juices of different liking were tasted. This study could not find any significant correlation between heart rate increases and liking.

Studies in food responses are not the only ones that have obtained contradictory results. At first, the contradictory data regarding ANS responses had been attributed to methodological problems such as failure to quantify the intensity of the response and the mistiming of the physiological recordings (Cacioppo et al., 2000). Nowadays, the use of different methodologies for quantification and comparison (including the use of different baselines and the small differences among stimuli) is considered as a potential source of the problem (Kreibig, 2010). Likewise, it is also being considered that the problem is not the methodology, but rather the fact that most studies have failed to rule out the possibility that the patterns observed reflect something separate to what is being studied. This because the differences that have emerged in autonomic responses seem to be more related to other components covering a set of simple factors like, for example, arousal (Barrett, 2006). Should this be true, it is possible that studies on responses to food find contradictory results because they look at the relation between ANS responses and valence alone while neglecting its relation with the other factors (i.e. novelty, arousal). Moreover, valence along with relevance, attention, and arousal can interact and conform a broader dimension. Such a dimension could be expectations.

1.4. Expectations and ANS responses

Expectations are key to the interaction with food. They provide an individual with an idea (based on previous experiences, pre-set ideas, beliefs, or even the context of consumption) of what the food product should look, smell, feel, and taste like (Tarancón, Sanz, Fiszman, & Tárrega, 2014; van der Laan, de Ridder, Viergever, & Smeets, 2011). Any deviation from this idea can change how a person responds to the product.

Deviations from expectations are assessed through predictive coding. All the contents that an individual perceives are constantly being checked, altered, and selected by prediction error signals in the brain (Piqueras-Fiszman & Spence, 2015). The brain will try to decipher whether the incoming signals and the prediction match the same object. Errors in this prediction will cause the higher level systems of the brain to adapt in order to try and reduce the disconfirmation (Piqueras-Fiszman & Spence, 2015).

The majority of the explanatory models related to disconfirmations of expectations deal with their effect in the self-reported ratings given by individuals. These include the assimilation, contrast, generalized negativity, and the assimilation-contrast model. During assimilation, individuals adjust their perception of the product to their expectations. Hence, their product ratings shift in the direction of their expectations. During contrast, individuals magnify the difference between expectations and product performance. As a result, the product ratings shift to the opposite direction of the initial expectations. Generalized negativity happens when the ratings given by individuals are always lower to the initial expectations, regardless of the performance of the product. Finally, the assimilation and contrast model states that assimilation occurs when disconfirmations between expectations and product performance are small and contrast occurs when the disconfirmations are large (see **Figure 1.2**) (Piqueras-Fiszman & Spence, 2015; Schifferstein, Kole, & Mojet, 1999).

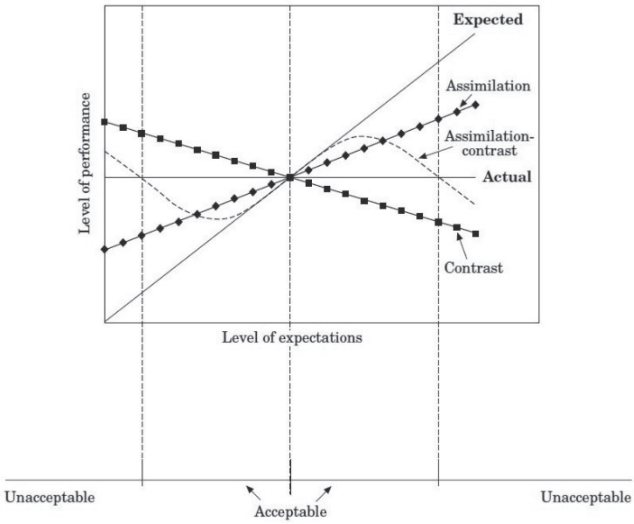


Figure 1.2. Schematic of assimilation, contrast and assimilation-contrast model. Figure obtained from Schifferstein et al. (1999).

The relation between the processes happening when dealing with disconfirmations in expectations and their potential effect in ANS responses was first addressed by Mandler in 1982 in his schema incongruity theory (see **Figure 1.3**). Mandler named these disconfirmations "schema incongruity", where "schema" relates to a preconceived idea or expectation. According to Mandler, a disconfirmation was sufficient for an autonomic response from the subject. The ANS response obtained from the disconfirmation would in turn lead to affect. The intensity of this affect would depend on the degree of ANS arousal. Assimilation would most likely happen in the case of small disconfirmations and would lead to weak ANS responses and an affect of low intensity. Individuals would have been able to accept the small deviations from their expectations and would, in general, value it as positive. In the case of large disconfirmations, accommodation (changes to the schema to include the new information) would most likely take place. Accommodation would lead to strong ANS responses and an affect of high intensity. Unsuccessful accommodations would usually be seen as negative. Successful accommodations could be positive or negative depending on how the new schema and the environmental evidence relate (Mandler, 1982).

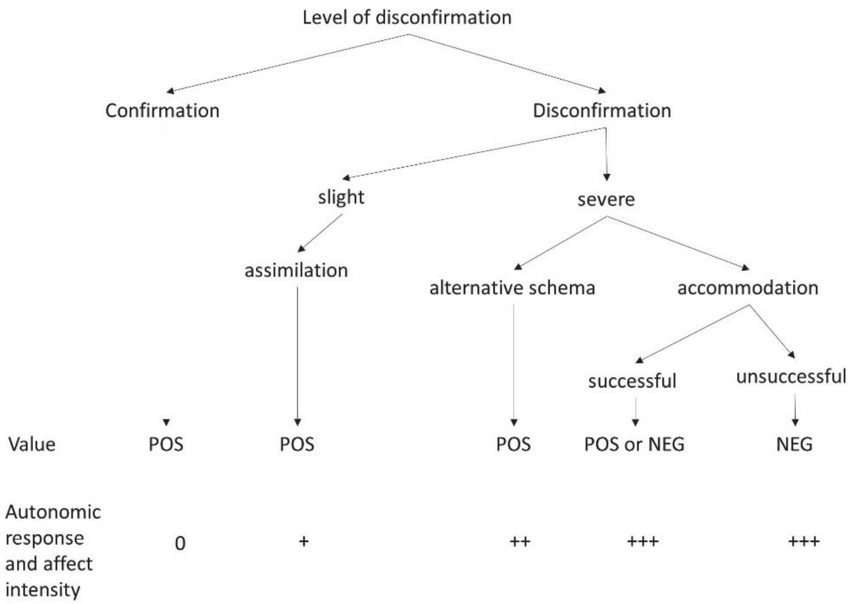


Figure 1.3. Mandler's schema incongruity theory. Adapted from Mandler (1982).

Mandler's theory does not look onto the effects of components such as relevance, novelty, valence of the stimulus, and attention. Moreover, Mandler's schema incongruity theory is still hypothetical. It has not yet been tested whether the ANS responses obtained when there are disconfirmations are indeed a product of the processing of the disconfirmation of expectations and if they follow the patterns suggested by Mandler. The purpose of this thesis is, therefore, to assess if ANS responses capture, as Mandler suggested, the processes related to the (dis)confirmation of expectations when individuals are presented to food products and the effect that other components (such as novelty, valence of the stimulus, relevance, arousal, and attention) might have in these responses.

Table 1.1. Studies with food-related stimuli of different valence and their results on heart rate and skin conductance.

Study	Sample population	Stimuli	Component looked at	Measure of heart rate and skin conductance	Results
Rousmans, Robin, Dittmar, and Vernet-Maury (2000)	Thirty-four, participants (17 males and 17 females).	Taste stimuli: Evian water (as control/neutral taste), sucrose solution of 0.3 M (sweet), quinine sulphate solution of 0.00015 M (bitter), citric acid solution of 0.02 M (sour), NaCl solution of 0.15 M (salty).	Valence (pleasantness).	Using simultaneous recording of the ANS responses skin resistance and instantaneous heart rate (IHR) were measured. Skin resistance measures: -temporal ohmic perturbation index [OPD]: Equivalent to the time in which a subject is responding to a stimulus. -amplitude of the skin resistance response (difference between the pre-stimulus mean skin resistance and the lowest skin resistance within 10 s post stimulus). IHR measure: -Delta between the pre-stimulus mean heart rate value and the value of the maximum increase.	The pleasant sucrose solution had smaller heart rate increases, OPD index, and skin resistance amplitude than the other solutions (rated as unpleasant). The bitter solution had the largest heart rate increase, OPD index, and skin resistance amplitude from all the solutions tasted.
Horio (2000)	Twenty-nine participants (gender not stated).	Taste stimuli: Taste solutions of different concentrations of sucrose,	Valence (liking).	Using data from simultaneous recording of heart rate, increases (%) in heart rate from	Compared to the pre-stimuli values, heart rate increased after tasting all solutions. The citric

		NaCl, citric acid, quinine-HCL, and monosodium glutamate.		baseline (30 seconds before presentation) were calculated. The relationship between heart rate and liking of the taste solution was also calculated. Increases in heart rate measure: -Comparison of the peak heart rate for each solution to the mean heart rate of the 30 seconds before each solution was presented (seen as 100%). Relationship between heart rate and liking of taste solution measure: Pearson correlation coefficient for the relation between liking score and heart rate.	solution had the largest heart rate increase. Heart rate increase and liking ratings were negatively correlated for all solutions except sucrose.
Inoue, Kuroda, Sugimoto, Kakuda, and Fushiki (2003)	Eight participants (5 men and 3 women).	Low and high intensity jasmine and Chinese green tea odors.	Valence (liking). Arousal (odor intensity).	Time course characteristics for the change in heart rate, high frequency component (HFC) and low frequency component (LFC) bands. The mean value before inhalation was set as baseline and each data value over time was given as the mean±SEM.	Heart rate decreased from baseline values and HFC increased when subjects that liked jasmine tea inhaled the jasmine odor. LFC increased from baseline values when subjects that did not like jasmine tea inhaled the jasmine odor. No differences were found between subjects with and

					without predilection when the odor used had a low intensity. Heart rate remained similar between subjects that liked green tea and subjects that disliked green tea when inhaling the green tea odor of high and low intensity. Inhalation of the low intensity jasmine teas as well as the high and low intensity green tea led to a decrease in heart rate from baseline values and an increase in HFC.
Robin, Rousmans, Dittmar, and Vernet-Maury (2003)	Thirty participants (14 male and 16 female).	Taste stimuli: Evian water (as control/neutral taste), sucrose solution of 0.3 M, 0.15 M NaCl solution, 0.02 M citric acid, and 0.00015 M quinine sulphate.	Valence (liking).	Using simultaneous recording of the ANS responses skin resistance and instantaneous heart rate (IHR) were measured. Skin resistance measures: -temporal ohmic perturbation index [OPD]: Equivalent to the time in which a subject is responding to a stimulus. -amplitude of the skin resistance response (difference between the pre-stimulus mean skin resistance and the lowest skin resistance	The unpleasant tastes (salty, sour, and mainly bitter) induced larger increases in heart rate and skin resistance than the pleasant sucrose solution. This applied for both female and male participants.

				within 10 s post stimulus). IHR measure: -Delta between the pre-stimulus mean heart rate value and the value of the maximum increase.	
Leterme, Brun, Dittmar, and Robin (2008)	Twenty participants (8 male and 12 female).	Taste stimuli: Evian water (as neutral taste), sucrose solution of 0.3 M, three different sweet flavors (orange, lemonade, and coke), and a NaCl solution of 0.15 M.	Valence (liking).	Using simultaneous recording of the ANS responses skin resistance and instantaneous heart rate (IHR) were measured. Skin resistance measures: -temporal ohmic perturbation index [OPD]: Equivalent to the time in which a subject is responding to a stimulus. IHR measure: -Delta between the pre-stimulus mean heart rate value and the value of the maximum increase. Relationship between the liking of the solution and ANS measures: Pearson correlation coefficient for the relation between the liking score for each solution	OPD index was higher for the NaCl solution, which was also rated as the most unpleasant solution. There was a heart rate increase after tasting all samples. The increases in heart rate remained similar between solutions. No correlations were found between the liking scores and any of the means of the autonomic responses.

				and IHR and skin resistance.	
Delplanque et al. (2009)	Eighteen participants (9 male and 9 female).	Odors: 16 pairs of unpleasant and 16 pairs of pleasant odors. Odorants included fruity odors (lime, fig, etc.), floral (lavender, geraniol), and animal (body odors, leather) odor.	Valence (pleasantness). Novelty (first presentation of odor).	Heart rate measure: Fifty heart rate scores were obtained by averaging the heart rate values within successive 200 ms periods after odorant presentation. The 50 scores were expressed as a percentage from the baseline (baseline: mean heart rate during the first 10 s before the odorant was presented). Skin conductance measure: Skin conductance responses scored as changes in skin conductance starting at 1 to 4 seconds after inhalation.	Larger skin conductance increase for the unpleasant odors than the pleasant odors. Larger skin conductance increase for novel odors than the repeated odors. Larger heart rate increase for repeated odors than for novel ones. Smaller heart rate decrease for unpleasant odors than pleasant ones.
de Wijk, Kooijman, Verhoeven, Holthuysen, and de Graaf (2012)	Fifteen 10-year-old children (10 male and 6 female) and 15 young adults (3 male 12 female).	Food products: For each participant, six personally disliked foods and three personally liked foods.	Valence (liking). Relevance (related to instruction: taste, smell or visual instruction).	Delta of the post-pre event mean heart rate and skin conductance.	Skin conductance had larger increases when looking for the first time at disliked foods than when looking at liked foods. Changes in skin conductance responses varied with type of instruction, with the largest skin conductance increases for the

					"taste" and "smell" instruction. Heart rate changes varied with instructions and age group. For children, the instructions "visual inspection" and "taste" led to a heart rate increase. The "smell" instruction led to a heart rate decrease. In adults, the instruction "visual inspection" led to a heart rate decrease while the "smell" and "taste" instruction led to a heart rate increase.
He et al. (2014)	Twenty-six female participants.	Odors: Fish odor (negative valence) and orange odor (positive valence).	Valence (liking). Arousal (odor concentration).	Time-averaged means for heart rate and skin conductance.	Time-averaged mean heart rate and skin conductance were higher for the fish odor than for orange odor. Heart rate increased with concentration but skin conductance did not.
de Wijk et al. (2014)	Nineteen participants (10 female and 9 male).	Breakfast drinks: Five commercially available breakfast drinks (three yogurt drinks and two fruit drinks).	Valence (liking). Arousal (taste intensity).	Heart rate and skin conductance changes during the first 1 to 5 seconds of tasting. Relationship between liking and intensity and ANS measures: PLS regression coefficients between liking and ANS measures (heart rate, skin conductance) and	Heart rate increased during the first seconds of tasting and gradually decreased. Skin conductance decreased during the tasting of all samples. Positive correlation between liking and heart rate and skin conductance increases. Negative correlation between taste intensity and heart rate increase.

				between taste intensity and ANS measures (heart rate, skin conductance).	Responses during the first seconds showed stronger correlations with liking while later responses showed stronger correlations with taste intensity.
Danner et al. (2014)	Eighty-one participants (35 female and 46 male).	Juices: Fruit and vegetable juices (banana, grapefruit, orange, mixed vegetables and pickled cabbage).	Valence (liking).	Delta between mean heart rate and skin conductance during tasting and baseline (before sample tasting). Skin conductance responses were additionally corrected for interindividual variance. Relationship between liking and ANS measures: Spearman correlation coefficient between liking scores and ANS (heart rate and skin conductance).	Larger skin conductance increases for disliked samples than for the liked ones. Heart rate responses remained similar. No correlation between liking and heart rate increase. Weak negative correlation between liking and skin conductance increase.
He, de Wijk, de Graaf, and Boesveldt (2016)	Twenty-eight female participants.	Odors: Seven food odors (mushroom, fish, chocolate, caramel, cucumber, orange, and apple) and one control (odorless).	Valence (liking).	Changes in ANS responses during the 4 s after odor presentation: Calculated by subtracting t=0 (mean value at the start of the odor presentation) to each of the 1 s post odor means.	Largest increases in skin conductance were for the fish and the mushroom odors (which were rated as unpleasant) as well as for caramel (which was rated as pleasant). The smallest skin conductance increases were for the cucumber, apple and chocolate pleasant odors.

					Heart rate showed a biphasic response with positive and negative peaks that remained similar among odors. However, the difference between peaks was smaller for the chocolate and the control odor than for the mushroom, fish, caramel, cucumber, orange and apple odors. Negative correlation between liking and skin conductance increase.
Beyts et al. (2017)	Sixty participants (32 male and 28 female).	Beer. Commercial lager (base beer) spiked with aroma compounds with Aroxa flavor capsules (isoamil acetate, lightstruck, diacetyl, hoppy, mercaptan, and hydrogen sulphide.	Valence (liking).	Heart rate measure: Mean heart rate for the 10 s after the sample presentation divided by the baseline (mean heart rate of the 10 s before sample presentation).	Heart rate did not differ significantly when sniffing the beers with aromas of different liking.

1.5. Thesis outline

The central research question of this thesis is:

How do the (dis)confirmation of expectations and its related components alter the ANS responses to food?

This thesis begins with the hypothesis that expectation (dis)confirmation will lead to ANS responses that follow the patterns suggested by Mandler (**Figure 1.3**). Confirmations will not present significant changes in ANS responses. Large disconfirmations of expectations will produce ANS responses of larger magnitude than that of small disconfirmations and confirmations.

As novelty, valence, relevance, arousal, and attention are linked to the responses to expectations, this thesis accounts for these components when testing Mandler's theory. In the cases in which our hypothesis is rejected, this thesis looks for other components that may explain the patterns obtained and further elaborates on them. Moreover, it evaluates if such components or processes could help understand better the human-food interaction and hence be of use for the research on reactions to food.

The thesis outline is as follows:

RQ1. How does the creation and (dis)confirmation of expectations alter the ANS responses elicited by stimuli? To what extent can context affect the relevance and subsequent ANS responses of these stimuli?

Chapter 2 answers this question by comparing the ANS responses evoked when stimuli create expectations to the ANS responses evoked when the same stimuli disconfirm expectations. In addition to this, it is evaluated whether presenting the same stimuli in a different context can affect how relevant it is deemed by individuals and lead to different ANS responses. To achieve this, ANS responses were measured when ingredient images of different valence were shown before tasting identical samples (creation of expectations) and when they were shown after the samples were tasted ((dis)confirmation). The effect of relevance was evaluated by comparing these responses to those of a second study in which the same images were observed in a context in which no tasting was involved.

RQ2. Within the context of expectations, which ANS patterns are related to novelty (first experience with a stimulus), valence (pleasant or unpleasant stimulus) and disconfirmations?

Chapter 3 follows on the findings of Chapter 2 and looks separately at patterns of ANS responses related to novelty, valence and disconfirmations. Novelty was measured by assessing the changes related to the first encounter with taste stimuli. Valence was manipulated by giving pleasant, unpleasant, and neutral taste samples. Disconfirmations were created through a mismatch between information presented and taste given. The differences in patterns related to these components and what they entail for the dimension of expectations are discussed.

RQ3. How does the degree of the expectation disconfirmation alter the changes in ANS responses?

Chapter 4 studies how ANS responses change when taste expectations are confirmed and disconfirmed. Two different degrees of disconfirmations are assessed: small disconfirmations, which can be perceived as according to expectations and large disconfirmations, which can be perceived as a strong deviation from expectations. In addition to this, the differences in ANS patterns to that of confirmations are evaluated.

RQ4. To what extent does attention and arousal influence the changes in ANS responses to expectation (dis)confirmation?

Following the findings of the previous three chapters, Chapter 5 aims to confirm whether the ANS responses found in the previous studies are indeed related to attention and arousal. This chapter additionally evaluates whether the ANS results of Chapter 2 can be replicated. The first part of the study followed a similar procedure to that of Chapter 2. The relation of the ANS responses to arousal was assessed by looking at the similarities of these ANS response patterns to those of the ANS responses obtained when observing images from the IAPS database with varying levels of valence and arousal. The link with attention was studied by comparing the patterns of ANS responses obtained with the results of a dot probe task using the same images.

Chapter 6 concludes the thesis. It discusses the limitations of the use of ANS responses for the measurement of the reactions to (dis)confirmations of expectations and whether some ANS responses are less variable than others. The ANS response patterns found for the components studied are also discussed. Finally, it is concluded that with the methodology used, differences in ANS responses can only be found when the study designs include highly relevant, novel, arousing, or contrasting disconfirmations or stimuli. As a result, ANS measurements may not be the best tool for the study of the human-food interaction and should be used with caution.

CHAPTER 2

The responses of the autonomic nervous system to visual food cues

This chapter can be found as *Verastegui-Tena, L., Schulte-Holierhoek, A., van Trijp, H., & Piqueras-Fiszman, B. (2017). Beyond expectations: The responses of the autonomic nervous system to visual food cues. Physiology & Behavior, 179, 478-486.*

Abstract

Self-report measures rely on cognitive and rational processes and may not, therefore, be the most suitable tools to investigate implicit or unconscious factors within a sensory experience. The responses from the autonomic nervous system (ANS), which are not susceptible to bias due to their involuntary nature, may provide a better insight. Expectations are important for the human-food interaction and should be considered. However, research using ANS responses has not focused thoroughly on expectations. Our aim was to investigate the mechanisms underlying ANS responses by evaluating the reactions to different images when expectations about a product are created (before tasting the product) and when they are confirmed and disconfirmed (after tasting the product).

In a first study, seventy-five participants tasted four drinks (three identical soy-based drinks and one rice-based drink) and were told that they would be shown their main ingredient either before or after tasting. For the three identical drinks, the images shown were worms, chocolate, and soy. Heart rate and skin conductance were measured during the procedure. The results showed that ANS responses followed similar patterns when images were presented before or after tasting. Heart rate decreased for all images, with the largest decrease found for chocolate and worms. Skin conductance increased, with the largest increase found for worms. To test whether the effects were solely caused by image perception, a second study was done in which forty participants only saw the images. The responses obtained were smaller and did not completely match those of the first study.

In conclusion, the ANS responses of the first study seem to be a result of the sensory processing and defense mechanisms happening during the creation and (dis)confirmation of expectations. The second study confirmed that visual perception alone could not account for these effects and that it led to smaller changes. Hence, it seems that the relevance of the context of use influences the patterns and magnitude of ANS responses to food cues.

2.1. Introduction

Food research relies on self-reports to measure and individual's perceptions of food products as well as to characterize such products. These self-report measurements are based on cognitive and rational processes and, therefore, cannot answer questions related to other unconscious and implicit aspects of the human-food interaction (Danner et al., 2014; Köster, 2003). Other routes might be able to give a better insight into the mechanisms underlying these unconscious processes.

There is a richness of reactions yet to be explored that may contribute to revealing how individuals perceive the world around them and in this context, food products. Among these reactions, the responses of the autonomic nervous system (ANS) may be a useful tool. The ANS is a component of the peripheral nervous system that is not under voluntary control. It is divided into a sympathetic and a parasympathetic branch, each relevant for specific situations. The first is relevant for emergency "flight or fight" reactions and exercise while the latter predominates in resting situations (McCorry, 2007). The ANS responses commonly measured include cardiovascular activity (heart rate, heart rate variability), electrodermal activity (skin conductance and skin potential), skin temperature, and blood pressure (Kistler, Mariauzouls, & von Berlepsch, 1998). The branch of the autonomic nervous system represented in the measurement depends on the ANS response used. Skin conductance responses are related to sympathetic activity, heart rate variability reflects parasympathetic activity, while heart rate and blood pressure represent a combination of both branches (de Wijk & Boesveldt, 2016).

ANS responses are not susceptible to self-report biases due to their involuntary nature. They are believed to precede awareness and, as a result, proposed to reveal the preferences of individuals (Mendes et al., 2009). These characteristics hold some potential when it comes to food products, as it is sometimes difficult for individuals to express why they react the way they do (He et al., 2014). Nevertheless, the understanding of ANS responses and their correct application in the food domain entails certain challenges. When using these measurements it is necessary to take into account the fact that the ANS is in charge of different tasks in our body. Bodily functions like breathing, the digestion of food, or even the movement of blood in our body may affect ANS responses as well as other non-affective and non-emotional responses, such as attention and mental effort (Quigley, Lindquist, & Feldman-Barret, 2014).

Researchers in the food domain have attempted to find ANS patterns that can reflect implicit factors such as emotions and liking/pleasantness but, unfortunately, the effects found are inconsistent and difficult to compare (Mauss & Robinson, 2009). For instance, in a study with breakfast drinks, de Wijk et al. (2014) found that there was a positive correlation between liking scores and increases in heart rate and skin conductance. Horio (2000) measured the heart rate of participants when tasting stimuli such as quinine-HCl (bitter), MSG (umami), citric acid (sour), and sucrose (sweet) and found a negative correlation between liking and heart rate increase for the first three but no significant correlation between heart rate increase and liking for sucrose. Danner et al. (2014) studied the implicit and explicit reactions to different juice drinks and found no differences in heart rate. However, they found an increase in skin conductance level (SCL) compared to baseline for a pickled cabbage juice (sauerkraut) that had a low liking score and a low SCL increase for a common orange juice. There is a lack of clarity in most published studies regarding skin temperature. Danner et al. (2014), He et al. (2014), and Leterme et al. (2008) did not find a significant difference in skin temperature between pleasant and unpleasant stimuli, while de Wijk et al. (2012) found higher temperatures for liked foods in both children and young adults and Robin et al. (2003) found a higher skin temperature amplitude for unpleasant stimuli.

ANS responses to expectations may lead to clearer patterns than the factors currently assessed in the food domain. Expectations are a key factor in the human-food interaction. Food cues such as the sight of food elicit an array of physiological, physical, and cognitive processes (van der Laan et al., 2011). Our brain interprets and integrates the information from previous experienced situations with the new information of the food product in front of us (Piqueras-Fiszman & Spence, 2015). Individuals already have a preconceived idea of the taste, texture, and other sensory characteristics of the food (sensory expectations) as well as how much they will like it before consuming it (hedonic expectations) (Tarancón et al., 2014). All foods are evaluated according to these expectations and, after this process, a judgment is given (Lawless & Heymann, 1999).

The hedonic evaluation of a food will not be affected if the food the individual is presented with matches their expectations. However, there might be a disparity between the expected experience with the product and the actual one; which would lead to a disconfirmation of expectations. In such cases, the following processes might take place: (a) a minimization of the difference and adjustment of the perception to what was expected (assimilation), (b) a maximization of the difference (contrast), (c) a negative evaluation of the product regardless of how it is perceived (generalized negativity), or (d) assimilation when there are small discrepancies and contrast as these discrepancies increase (Piqueras-Fiszman & Spence, 2015).

In research, expectations are usually manipulated by using verbal or non-verbal information. Some studies, for example, use simple sensory information like taste or visual cues to assess their effect on hedonic expectations. The moment this information is presented plays a role on the impact it will have on the individual (Cardello, 2007). For instance, the study by Lee, Frederick, and Ariely (2006) showed that the timing in which the information about an unpleasant ingredient present in a beer was received affected the overall experience of the tasting. Participants had to taste and give their preference between two beers, one adulterated with an ingredient perceived as unpleasant and one unadulterated. The beer with the unpleasant ingredient was less liked when participants knew the ingredient before tasting it (and hence had particular expectations about its flavor) than those that tasted it blindly or before getting the information. This is because obtaining information about a product prior to its consumption creates expectations about the sensory properties of the product (Davidenko et al., 2015). Our behavior is motivated to the same extent by the anticipation of pleasant situations, which we would like to experience, and the anticipation of unpleasant situations which we would like to avoid (Fonberg, 2008). The expectations that are created through food cues (visual, auditory, or olfactory), or even by the thought of eating, lead to a variety of anticipatory responses in the body. These anticipatory responses can prepare the body to facilitate the digestion of food or they can diminish the negative consequences that are expected from food intake (Nederkoorn, Smulders, & Jansen, 2000). Changes in cardiac activity, skin conductance, and blood pressure have been measured in some studies in response to food cues. Nederkoorn et al. (2000) looked at the physiological changes in normal subjects when exposed to liked foods and found that, compared to baseline, they led to an increase in heart rate, systolic blood pressure, temperature, and skin conductance level. De Wijk et al. (2012) looked at the ANS responses while looking at three liked and disliked foods before they were consumed and found that skin conductance response increased while looking at disliked foods compared to liked foods but that heart rate did not differ. Vögele and Florin (1997) found that food exposure led to an increase in skin conductance, blood pressure, and heart rate for both binge eaters and normal eaters compared to baseline and that eating led to a further increase in heart rate and blood pressure compared to food exposure. There are still some contradictions among the findings in studies. For example, Overduin and Jansen (1996) found that exposing fasting and non-fasting subjects to food did not increase heart rate, skin conductance and salivation. Moreover, the physiological responses when observing food were similar to the ones when observing soap. Another study by Nederkoorn and Jansen (2002), however, used a similar procedure with restrained and unrestrained eaters and found that unrestrained eaters showed an increase in heart rate when exposed to food compared to when exposed to soap.

While the aforementioned studies have looked at the anticipatory responses related to the creation of expectations, the efficacy of the ANS responses to capture them is still unclear. Moreover, the measurement of the changes related to the confirmation and disconfirmation of expectations has been neglected by such studies. A link between ANS responses and the disconfirmation of expectations has been previously hypothesized though not yet put into study. George Mandler stated that a disconfirmation of expectations would lead to an activation of the autonomic nervous system. In cases of assimilation, in which the disconfirmation is small, the activation will be low. However, the activation will increase in situations in which assimilation is not possible and it is necessary to modify an expectation (Mandler, 1982). Our study looks into Mandler's theory and evaluates the extent to which ANS responses can capture the reaction to specific visual stimuli as expectations are created and when these same expectations are confirmed and disconfirmed. For this purpose, two studies were conducted in which the ANS responses to the same visual cues were measured. For the first study, images of different valence were presented as main ingredients of food products that participants were asked to drink. The effect of the creation of expectations was measured through the anticipatory responses when the images were presented before consuming the drink. The confirmation and disconfirmation of expectations were assessed through the responses when presenting the images after consuming the drink. The incorporation of images of different valence would serve as an exploratory analysis to additionally test if the effect of the confirmation and disconfirmation of expectations would overrule that of valence. For the second study, participants saw the same images of the three ingredients but were not asked to drink the products. This was done to determine if the effects found were inherent to the image perception or a consequence of the creation or the (dis)confirmation of expectations.

Our hypotheses were: a) given the increase in physiological responses found when exposed to food cues and the effect that information before a product has on its evaluation (de Wijk et al., 2012; Lee et al., 2006; Nederkoorn & Jansen, 2002; Nederkoorn et al., 2000), the ANS responses during the creation of expectations would be stronger than the responses to the confirmation and disconfirmation of expectations; b) in line with Mandler's theory regarding expectations, ANS responses were expected to be stronger for the images that disconfirmed expectations than for those images that confirmed them. The responses would capture the effect of the initial disconfirmation rather than that of valence. Hence, the responses for positive and negative images that disconfirm expectations were expected to be similar; c) the differences in ANS responses would not be fully derived from the perception of the images. That is, the effects would only be seen when tasting was included in the design. Hence, we expected that in our second study we would not find the effects of Study 1.

In order to answer the aforementioned hypotheses correctly, certain subjective factors that can influence the intensity of the ANS responses were considered in the design. Given that our current focus is in the food domain, it was important to account for the fact that individuals with high food neophobia have a higher physiological arousal when presented to food stimuli (Raudenbush & Capiola, 2012). Other factors accounted for included that subjects with high sensitivity to body signals have been found to experience emotions more intensely and that individuals with high emotional intensity (more intense experience of emotion) present stronger emotional responses to stimuli than individuals with a lower emotional intensity (McFatter, 1998; Pollatos, Traut-Mattausch, Schroeder, & Schandry, 2007).

2.2. Study 1

2.2.1 Materials and methods

Procedure

Screening and selection of participants

Eighty-nine Dutch citizens ranging from 20 to 45 years of age were recruited from the Ede area and surroundings. Participants were excluded if they had a BMI higher than 24.9 kg/m^2 (calculated from self-reported height and weight), were color-blind or had any food-related allergies. Participants were given a summary of the procedure that would follow during the study and were asked to schedule an appointment if they agreed to participate. Ethical approval was obtained by the Social Sciences Ethics Committee of Wageningen University.

Experiment session

The study took place at Wageningen University, the Netherlands, in a well-lit white room. The room was equipped with a table, three chairs, and a computer with E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA).

Participants were instructed to refrain from eating or drinking (except water) for one hour before their appointment and to wear comfortable clothes for the study. The researcher explained the procedure to the participants, giving ample time for questions, and asked them to read and sign the informed consent. Once the informed consent was signed, the researcher started placing the sensor pads.

Heart rate and skin conductance responses were measured continuously throughout the whole study. For the measurement of heart rate, seven sensor pads (Kendall™ H98S8 60-mm micropore ECG electrodes) were placed on each participant; five were on the chest and two on the back. For the measurement of skin conductance, two sensors (Biopac® TSD 203 Electrodermal Response Transducer) were placed, one on the index finger and the other on the middle finger of the non-dominant hand. The researcher checked all signals to avoid any problems due to electrode misplacement. After all signals were checked, a baseline measurement was taken. For this measurement, participants were asked to remain still, to close their eyes, and to breathe normally for one minute.

The study consisted of tasting drink samples and observing the images of the alleged main ingredients of the samples. The cover story given was that the aim of the research was to study the effect that the bodily reactions had on specific associations when the samples were tasted. Participants were told that they would try four different non-dairy drinks with a similar flavor but with a different main ingredient. Participants were randomly assigned to one of two possible conditions, ensuring that the male: female ratio between them was as similar as possible. The moment the ingredient was shown on the screen varied depending on the assigned condition (**Figure 2.1**). In one condition, participants were shown the image of the main ingredient before tasting the sample (and so forth for the other three samples). For the other condition, the design was reversed: participants were shown the image of the main ingredient after the actual tasting of the sample. In reality, three of the samples given were the same and one was different to ensure the effectiveness of our design (see section "Tasting samples"). For the three identical samples, different images were randomly shown (one neutral, one positive, and one negative, see section "Visual Stimuli").

Participants were seated in front of a computer and instructed to taste the samples (25 ml) with their eyes closed, leave the sample in their mouth for 20 seconds and swallow it afterwards. We chose 20 seconds to give them the impression that the corresponding ingredient would be present in their mouth for a long time. Participants ate a water cracker (Carr´s Original table water, Carr's of Carlisle, UK) and took a sip of water before tasting each sample in order to cleanse their palate. To ensure that they were familiar with the procedure and that all samples were correctly swallowed, they practiced with a water sample first. All images were shown for five seconds and were preceded by a fixation cross that lasted three seconds. To avoid any influence from the movements done during the tasting, a gap of 10 seconds was added between the tasting of each sample and the presentation of the next image. Participants were asked to avoid any movement and to breathe normally while watching the images.

At the end of the study, participants filled out four questionnaires about the remembered sensory characteristics of each sample, the Food Neophobia Scale (FNS) (Pliner & Hobden, 1992), the Private Body Consciousness (PBC) Scale (Miller, Murphy, & Buss, 1981), and the reduced Emotional Intensity Scale (EIS-R) (Geuens & de Pelsmacker, 2002). The FNS scale consists of a 10-item questionnaire to measure the avoidance or reluctance to eat novel foods (Pliner & Hobden, 1992). The PBC scale measures on a 5-item questionnaire the sensitivity to internal bodily sensations (Miller et al., 1981). Finally, the EIS-R consists of 17 items representing different emotional experiences, 9 measure positive emotional intensity factors and 6 measure negative emotional intensity factors.

Once participants had finished, they were asked to guess the aim of the study and to give any additional comments. This was done to ensure that any lack of significant results was not due to the participants guessing the aim of the study. After giving their answer, participants were debriefed. They were told which information was false, the real nature of the samples they had tested, and the main objective of the study.

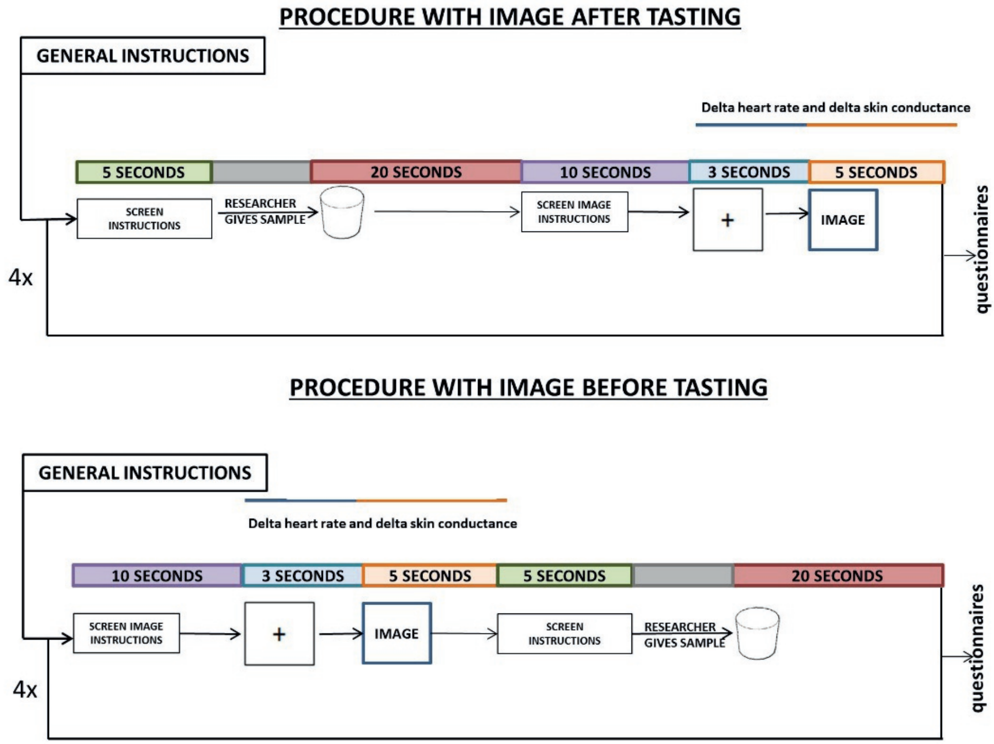


Figure 2.1. Procedure of Study 1. Heart rate and skin conductance were measured while participants looked at images which were shown before or after tasting a sample. The (+) symbol relates to the fixation crossed that preceded each image.

Tasting samples

In order to correctly prevent any influence related to the liking of the samples' flavor, we selected a drink whose flavor would be regarded as neutral. The three repeated samples given in this study consisted of a commercially available unsweetened soy drink (AH zachte soja drink ongezoet, Albert Heijn B.V., Zaandam NL). To improve the taste of the drink, one pill of sweetener (Natrena zoetjes DE Master blenders, Amsterdam the Netherlands) was diluted in 5 ml of hot water and then added to 250 ml of the soy drink.

Pretests with only this soy drink as the tasting stimulus revealed that participants could tell that the three samples were the same. Therefore, a rice drink sample was added and allocated randomly at either the second or the third position in order to prevent having the soy drink presented together three times. The rice drink was prepared in a manner that would ensure that, although participants could tell the sample was different, it was still similar to the soy one which further helped our cover story. It consisted of a combination of 200 ml of a rice drink (Rice Dream, Original organic, Hain Europe NV, Aalter Belgium) and 100 ml of the unsweetened soy drink (AH zachte soja drink ongezoet Albert Heijn B.V., Zaandam NL).

The aforementioned drinks were chosen after pretests with three drinks (sweet soy drink, unsweetened soy drink, and coffee milk) showed that the unsweetened soy drink was the most difficult one to recognize by participants. We also found that the unsweetened soy drink was disliked by people. Adding the sweetener, however, allowed us to increase the liking at a level in which it was not highly liked nor disliked (liking score= 4.9±1.8 on a 7-point scale) while still ensuring that the drink was not easily recognized. All samples were stored in the fridge and taken out one hour before the participant's appointment. The samples were served in 30 ml plastic cups.

Visual stimuli

Four images were presented depicting the supposed "main ingredient" of each of the samples. As mentioned above, for the three soy drink samples, three different images (one neutral, one positive, and one negative) were shown. The neutral image consisted of a picture of soy (opened soybean pods), which depicted the real ingredient of the drink. The positive image was a picture of chocolate (dark chocolate) and the negative image was a picture of worms (grub worms). We chose these pictures because insects and chocolate had previously been categorized as negative and positive in other studies (Berkman & Lieberman, 2010; Piqueras-Fiszman, Kraus, & Spence, 2014). The fourth image shown was rice (handful of rice), the main ingredient of the sample that was different from the others in our design. The soy, worms and rice images showed the raw versions of the ingredients. All images were presented in random order except for the rice image which, for the reasons stated previously (see section "Tasting samples"), was shown either in the second or the third position. The images used were standardized to a resolution of 450x600, 96 dpi sRGB format and set on a white background.

Physiological measurements

Heart rate and skin conductance were measured with the VU-AMS version 3.9 (de Geus, Willemsen, Klaver, & van Doornen, 1995). The ECG had a sampling rate of 1000 Hz and heart rate was obtained from the time between two adjacent R waves. Skin conductance was sampled at a rate of 10 Hz with a signal range between 0-95 µs. The signal was filtered both in forward and reverse direction with a low-pass filter with a cut-off frequency of 2 Hz.

Data treatment and analysis

Heart rate and skin conductance data were extracted and visually inspected for artifacts with the VU DAMS program (version 3.9). For each image, two labels were created: one for the three seconds in which the fixation cross was shown and the other for the five seconds in which each image was shown. The statistical software R version 3.2.2 was used for the analyses of the ANS responses data obtained from the VU DAMS labels. Only the data of the images that corresponded to the three soy samples were analyzed.

The deviation from the baseline for heart rate (delta heart rate) and skin conductance (delta skin conductance) was calculated for each image. We used the mean heart rate and mean skin conductance from the fixation cross preceding each image as a baseline. To test if the ANS responses differed between images and conditions, the image effect on heart rate and skin conductance was analyzed by means of a mixed model anova stating subject as random factor and the variables image, condition (seeing the image before or after tasting), order of presentation of the images, as well as the interactions between the image and the order, and the image and the condition as fixed factors. The variables gender, age and BMI were assessed in separate models, as previous literature has found differences in ANS responses related to these factors (Antelmi et al., 2004; Carrillo et al., 2001; Kopacz & Smith, 1971). These variables were only added to the main model if their inclusion affected the general outcome of the model. Post hoc analyses for image effects were performed using Tukey's HSD test for multiple comparisons. Additional permutation tests were carried out for the heart rate and skin conductance models when they did not fulfill the normality assumption.

Relationship of ANS with reported food neophobia, body consciousness and emotional intensity

To test the effect of food neophobia, participants were divided into high food neophobics (score higher than 35) and low food neophobics (score lower than 35) on the FNS scale. For both delta heart rate and skin conductance response, a mixed model anova with image, food neophobia and the interaction between the image and food neophobia as fixed factors and subject as random factor was used. To test the effect of private body consciousness, participants were divided into high PBC (higher than the median) and low PBC (lower than the median) and the same model that was used to test the effect of food neophobia was applied. Likewise, for the EIS-R scores the positive and negative subscales were used to divide participants into high and low groups for positive and negative emotions respectively and the effect of both was tested with the same mixed model anova used for FNS and PBC.

2.2.2 Results

Eighty-nine participants completed the study. Seven participants guessed the main aim and were removed from further analysis. The data of four participants could not be used: two due to mistakes during the execution of the study, one due to an error in the electrode placement and one due to a mistake in the software. Two participants showed a higher than normal quantity of ectopic beats and were therefore also excluded from the data analysis. One participant decided to stop with the study. In total, the data of 75 participants, 44 females (mean age=28.3±6.7, mean BMI=21.6±1.6 kg/m^2) and 31 males (mean age=30.4±6.9, mean BMI=22.5±1.4 kg/m^2) were used. The demographics of the sample, divided by condition, can be found in **Table 2.1**.

Table 2.1. Demographics of the sample of Study 1, divided by condition (N=75).

	Image after tasting	Image before tasting	*p*-value[a]
N	41	34	-
Gender			
-Female	24	20	0.953[1]
-Male	17	14	
Age (years)	28.7 ±7.0	29.7 ±6-6	0.238[2]
BMI (kg/m²)	21.9 ±1.5	21.9 ±1.7	0.744[2]

[a] *p*-value calculated with Welch's t test[1] or chi-square test [2].

Effect of the images during the creation and confirmation/disconfirmation of expectations on heart rate and skin conductance

The following section describes the results of the analyses done to assess the changes in ANS responses caused by images of different valence when expectations are created (presentation before tasting) and when expectations are confirmed and disconfirmed (presentation after tasting). Results for the mixed model analysis for the effect of ingredient images can be found in **Table 2.2**. The addition of gender, age and BMI did not affect the main outcome. As a result, the main model (see section "Data treatment and analysis") was used.

Heart rate

Delta heart rate was significantly different between images (p=0.011). Post hoc tests revealed that the main differences for the images were between the worms and the soy images and the chocolate and the soy images, with a larger decrease for the chocolate and worms images than for the soy image. There was no significant effect for condition (p=0.350) nor for the order of presentation between the images (p=0.454). We found no significant effects for any of the interactions. These results indicate that observing the positive (chocolate) and negative (worms) images led to a stronger decrease in heart rate than observing the neutral image (soy). Contrary to what was expected, the moment the image was presented (either before or after tasting) did not have an effect in these responses.

Skin conductance

Delta skin conductance was significantly different for each image (p=0.012). Post hoc tests revealed that the main difference for the image effect was between the worms and soy images, with the worms showing a higher skin conductance response. The difference between the worms and the chocolate was not significant (p=0.07). We found a significant effect for the order of presentation of the images (p<0.001) but not for the interaction between the image and the order of presentation (p=0.270). There was no significant effect for condition (p=0.787) or for the interaction between the image and the condition (p=0.694). These results indicate that, contrary to what was expected, only observing the negative image (worms) led to a higher skin conductance than observing the positive (chocolate) and the neutral image (soy) and that this effect was found regardless of the moment the image was presented (either before or after tasting).

Effect of food neophobia, body consciousness and emotional intensity

From our sample of 75 participants, only five showed high food neophobia (score higher than 35) on the FNS scale. It was, therefore, not possible to perform a valid analysis of the effects of food neophobia. Regarding PBC, 38 participants scored high on the body consciousness scale and 37 scored low. The mixed model anova showed no effect of PBC on any of the ANS responses (p=0.908 for delta heart rate and p=0.566 for delta skin conductance). Analyses of the effect of emotional intensity showed no significant effect for both positive (p=0.188 for delta heart rate and p=0.254 for skin conductance) and negative (p=0.950 for delta heart rate and p=0.105 for skin conductance) emotions.

Table 2.2. Results of the mixed model anova considering image, condition, order of presentation, and two-way interactions with the image (N=75).

	Image			**Before or after tasting**			**Order of presentation**			**Image* before or after tasting**			**image*order**		
	df	F	*p*	df	F	*p*	df	F	*p*	df	F	*p*	df	F	*p*
Delta heart rate (bpm)	2	4.680	0.011**	1	0.882	0.350	3	0.877	0.454	2	0.803	0.450	6	0.797	0.574
Skin conductance response (µs)	2	4.5	0.012**	1	0.07	0.787	3	8.1	<0.001***	2	0.4	0.694	6	1.3	0.270

**Significance at $p<0.05$.

***Significance at $p<0.001$.

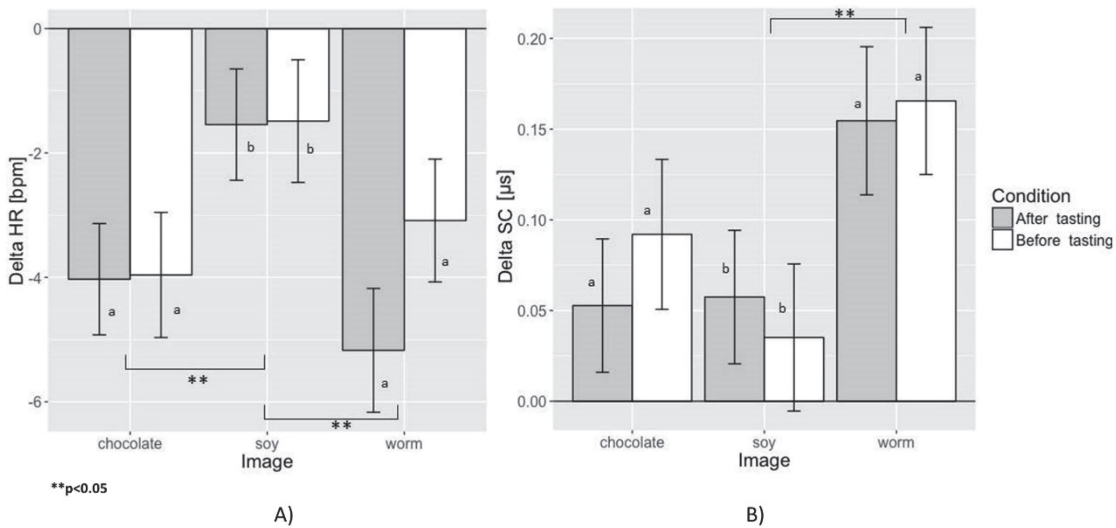


Figure 2.2. Adjusted mean (± SE) of (A) delta heart rate (beats per minute) and (B) delta skin conductance (µs) during the presentation of the images before (N=34) or after tasting (N=41). Different letters indicate a significant difference between the group images (Tukey's HSD test, $p<0.05$).

2.3. Study 2

2.3.1 Materials and methods

Procedure

Screening and selection of participants

A sample of forty Dutch participants ranging from 20 to 45 years of age was selected. Participants were students and staff members recruited from the Wageningen area and surroundings. They were excluded if they had participated in Study 1, had a BMI higher than 24.9 kg/m^2, were color-blind or had any food related allergies. The Social Sciences Ethics Committee of Wageningen University approved the study.

Experiment session

The study took place in the same room as in Study 1. Participants were instructed to wear comfortable clothes to ensure there would not be any problems with the measurements. On the day of their appointment, participants signed an informed consent before the researcher started placing the sensor pads. All sensor pads were placed as specified in the procedure of Study 1. Participants were informed that they would be shown some images on the computer screen. Before the presentation started, participants were asked to remain still, to close their eyes, and to breathe normally for one minute.

The study consisted of a presentation of the images, in random order, shown in Study 1 (chocolate, soy, rice, worms). OpenSesame version 3.1.2 (Mathôt, Schreij, & Theeuwes, 2012), a software similar to that of Study 1, was used for the presentation of the images. Participants were told that they would be shown images of ingredients used in newly-developed soy-like drinks. Before the presentation, the researcher made sure participants understood there was no tasting involved. All images were shown for five seconds and were preceded by a fixation cross that lasted three seconds. Heart rate and skin conductance responses were measured throughout the whole study. Participants were asked to avoid any movement and to breathe normally while watching the images. At the end of the presentation, participants filled out a questionnaire that contained questions about the images shown. Once participants had finished the test, they were debriefed.

Physiological measurements, data treatment and analysis

Heart rate and skin conductance were measured with the same VU-AMS version 3.9 used in study 1 (de Geus et al., 1995). All data were treated similarly as in the previous study. Using the VU DAMS program (version 3.9), the data were extracted, visually inspected for artifacts and labelled. Analyses were done with the statistical software R version 3.2.2.

To test if the ANS responses differed between images, the image effect on heart rate and skin conductance was analyzed by means of a mixed model anova stating subject as random factor and the variables image, order of presentation of the images, as well as the interaction between the image and the order as fixed factors. Gender, age and BMI were only added to the model when their inclusion affected the main outcome. Permutation tests were carried out when the heart rate and skin conductance models did not fulfill the normality assumption. Post hoc analyses for image effects were performed using Tukey's HSD test for multiple comparisons.

2.3.2 Results

From our subsample of 40 participants, 24 were female (mean age=21.5±2.3, mean BMI=21.9±1.9 kg/m^2) and 16 were male (mean age=23.1±3.6, mean BMI=21.45±2.1 kg/m^2).

Effect of images on heart rate and skin conductance

The following section describes the results of the analysis done to assess if ANS responses differ upon exposure to images of different valence. This was done to determine if the effects found in Study 1 are a product of the image perception alone. Results for the mixed model analysis for the effect of ingredient images can be found in **Table 2.3**. The addition of gender, age and BMI did not affect the main outcome. As a result, the main model (see section "Physiological measurements, data treatment and analysis") was used.

Heart rate

The differences in delta heart rate between images were just marginally significant (p=0.06). There was no significant effect for the order of presentation between the images (p=0.087) nor for the interaction between image and order (p=0.789). This result indicates that heart rate did not change when observing images of different valence. This is different to what was seen in Study 1 and, therefore, in line with our expectations.

Skin conductance

The differences in delta skin conductance between images were significant ($p<0.001$). Post hoc tests revealed that the main differences were between the worms and the chocolate images and between the worms and the soy images, with the worms having the highest skin conductance response. We additionally found a significant effect for the order of presentation of the images ($p<0.001$) and for the interaction between the image and the order of presentation ($p=0.04$). The worms and soy images had a higher skin conductance when they were in the first position compared to the other three but this was not seen for the chocolate image. The results indicate that the changes in skin conductance for the negative image (worms) differed from those for the positive (chocolate) and neutral images (soy). In line with our expectations, these changes were different and smaller than what was found in Study 1. Moreover, they were dependent on the order in which the images were presented.

Table 2.3. Results of the mixed model anova considering image, order of presentation, and the interaction image: order (N=40).

	Image			**Order of presentation**			**Image* order**		
	df	F	*p*	df	F	*p*	df	F	*p*
Delta heart rate (bpm)	2	2.95	0.06	3	2.26	0.087	6	0.52	0.789
Skin conductance response (µs)	2	7.9	<0.001***	3	21.8	<0.001***	6	2.36	0.04**

**Significance at p<0.05.

***Significance at p<0.001.

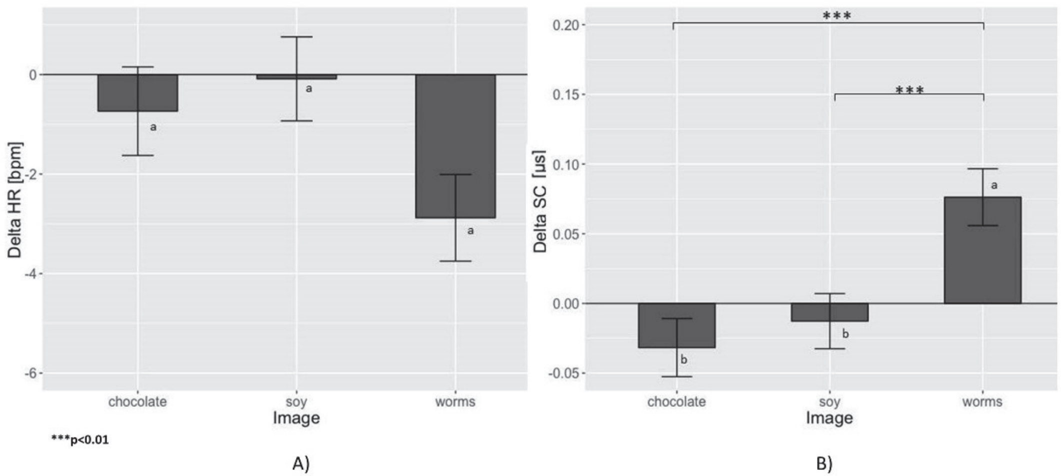


Figure 2.3. Adjusted mean (± SE) of (A) delta heart rate (bpm) and (B) delta skin conductance (µs) during the presentation of the images in Study 2 (N=40). Different letters indicate a significant difference between the group images (Tukey's HSD test, $p<0.05$).

2.4. General discussion

The aim of this work was to evaluate the ANS responses to images of different valence related to a product when expectations are created (presented before tasting) and when they are confirmed or disconfirmed (presented after tasting) and to further determine if the effects found are due to image perception alone.

An important finding of this work is that ANS responses when the images were presented before the tasting were similar to those when the images were presented after. The changes in heart rate and skin conductance between the two conditions did not reach statistical significance. Whenever similar results are found between two conditions, the common interpretation is that they share a common mechanism. However, in the case of our results, this interpretation may not be correct. It may be that the anticipatory reaction from seeing the image before tasting ("I am about to taste this!") had a magnitude similar to that of the confirmation and disconfirmation of the expectations created by the image once the sample had been tasted ("I just tasted this!"). Hence, both conditions (seeing the image before or after tasting) led to similar ANS response patterns even though they were driven by different behavioral mechanisms.

Our results show that the changes in ANS responses differed for heart rate and skin conductance. In the case of heart rate responses, the images with both a positive (chocolate) and a negative valence (worms) showed a larger cardiac deceleration than the neutral image (soy). An effect of valence on heart rate is commonly seen in studies, but what is usually found is that negative stimuli lead to a lower heart rate than positive stimuli. Consequently, negative stimuli should have led to a stronger cardiac deceleration (Brouwer, van Wouwe, Mühl, van Erp, & Toet, 2013). This is different from what we found in our study, which might lead to the belief that the ANS responses were able to measure the effect of the disconfirmation of expectations regardless of valence. However, according to Bradley and Lang (2007), the cardiac responses related to picture viewing seem to be more tightly linked to sensory processing. Heart rate deceleration, the usual response to the perceptions of visual stimuli, is a sign of attentional and incoming sensory information. Attention will more likely happen for stimuli that hold a significance/potential impact to the self than for neutral stimuli (Sánchez-Navarro, Martínez-Selva, Torrente, & Román, 2008). This goes in hand with Lacey and Lacey's theory, which states that deceleration accompanies the motivation to note and detect events while acceleration motivates ignoring certain events. When there is something that individuals find attractive or pleasant, attention is increased. Attention, however, can also be increased when individuals see something negative or aversive as this is a biologically advantageous mechanism (Lacey & Lacey, 2007). Hence, we need to consider that, compared to the neutral image, the images with a positive and a negative valence might have had the same effect on motivation and, in consequence, directed the attention of participants to the same extent for the incoming sensory information.

For the skin conductance responses, only the worms image led to a higher skin conductance than the soy image, while no differences between the responses to the soy and the chocolate image were observed. The measurement of skin conductance in psychophysiology is related to the responsiveness of the humane eccrine sweat glands to emotional stimuli. If a stimulus increases the defensive or appetitive motivation systems of a person, the skin conductance will increase. Whether a negative or a positive stimulus leads to a similar activation depends on the content and proportion of arousal of the stimulus (Bradley & Lang, 2007). The soy image triggered a low activation due to its neutral valence and congruence. Regarding the worms and chocolate image, it is possible that the worms image triggered a stronger defensive response than the appetitive response for the chocolate and therefore only the worms were significantly different from the soy. Food aversions might also have had an effect on the strong defense activation found for worms. Food aversions are usually linked to unpleasantness due to the nausea and vomiting that happen when a dangerous food is ingested (Midkiff & Bernstein, 1985; Profet, 1995). This link might have strengthened the response to the worms image.

Study 2 showed that presenting the same images without making participants believe that they will taste them led to changes in ANS responses that did not completely match those of Study 1. The worms image led to a stronger cardiac deceleration and a higher skin conductance than the chocolate and soy images. These changes, however, only reached statistical significance for skin conductance. Nevertheless, the patterns observed in Study 2 are in line with what has been found in previous research regarding visual stimuli (stronger cardiac deceleration for unpleasant stimuli and increased skin conductance for arousing stimuli) (Bradley, Moulder, & Lang, 2005; Brouwer et al., 2013; Hamm, Schupp, & Weike, 2002). Given that the responses in Study 1 and Study 2 differ, it is likely that the changes in ANS responses obtained in Study 1 are not fully due to visual perception.

It remains to be determined whether the size of the changes obtained in Study 2 for both heart rate and skin conductance could be considered as physiologically relevant. The highest mean difference obtained in skin conductance between the baseline and the image was 0.08 µs while skin conductance responses related to novel, unexpected, significant or aversive stimuli often range between 0.2 and 1.0 µs (Dawson et al., 2000). Likewise, the heart rate differences between the baseline and the image for the soy and chocolate images did not reach one beat. Published studies have found larger differences in heart rate and skin conductance when observing stimuli than the ones encountered in Study 2. For instance, Bradley et al. (2005) found changes between baseline and picture presentation of 1.1±0.69 bpm and of -0.24±0.10 µs, for heart rate and skin conductance respectively, while observing pleasant pictures that cue safety and of -1.2±0.63 bpm and-0.20±0.10 µs for unpleasant pictures that cue safety. Overduin and Jansen (1996) found that the changes in non-fasting subjects between baseline and the presentation of a food were of 3.6±4.1 bpm for heart rate and of 1.82±1.5 µs for skin conductance. Fasting subjects had heart rate changes of 6.8±4.9 bpm and skin conductance changes of 0.47±1.1 µs. It should be noted, however, that other factors from both study designs may have been playing a role in the magnitude of the responses. Bradley's study, for instance, used connotations linked with safety and threat and hence the responses to the images were more extreme. Likewise, the design of Overduin's study allowed other cues related to the exposure of each food (e.g., odor) to contribute in making the experience stronger.

The abovementioned differences pinpoint the importance of the whole multisensory experience and the context while testing the responses to food products. In Study 2, showing the images without any particular connotations might have led to a lack of engagement from participants. Noseworthy, Di Muro, and Murray (2014) had previously shown that participants that are bored or less interested (low aroused state) do not show differences in skin conductance when presented with images with incongruities. Participants from Study 2 may not have found the sensory experience relevant to their needs and, consequently, the mechanisms related to image perception were weak. In contrast, the responses of the participants from Study 1, where there was a tasting related to the images, were of a larger magnitude. It seems, therefore, that ensuring that participants find the sensory experience relevant (e.g., by telling them that they will taste what is shown) is of importance for the measurement of strong ANS responses to food products.

Our study has certain limitations. Even though heart rate and skin conductance responses were also measured at the moment of tasting in Study 1, the breathing patterns and movements of the participants were not fully controlled during this moment. As a consequence, the data during the moment of tasting contained noise and could not be additionally considered for this work. In addition to this, we did not ask participants to rate their liking of the sample immediately after each tasting. Such data could have allowed us to assess the link between expectations, liking and ANS responses. Regarding the responses found for the images in Study 1, further studies should be conducted to confirm our findings about what these responses represent. This may be challenging as the variety of ANS measures, units and procedures used in published studies make comparisons difficult. It is necessary to develop a standardized measurement procedure which should be followed by future studies in order to have comparable results.

The present work attempted to assess and complement Mandler´s theory by capturing the ANS responses when expectations are created and when they are confirmed and disconfirmed. The obtained results bring more insight into the validity of the use of ANS responses in the food domain and help understand the processes captured by ANS responses when presented to visual stimuli. Moreover, it considers that in some cases the differences in ANS responses might not be meaningful from a physiological point. Future research should look further into this topic considering that ANS responses are more insightful under a certain context of use. For example, the testing of novel foods that contain ingredients with a variety of scores in the arousal and pleasantness dimensions might be of interest. When designing such tests, however, it is necessary to consider the possibility that ANS responses may be more sensitive to relevant situations that show contrast (positive vs negative, safety vs threat) rather than similarity. In the case of novel foods, a potential contrast could be comparing the responses when first experiencing the product to those of subsequent tastings. The changes in ANS responses after continuous exposure to these foods might help capture the principles of acceptability.

2.5. Conclusion

The present work evaluated the changes in ANS responses related to observing images of different valence when expectations are created and when they are confirmed and disconfirmed. To the best of our knowledge, this is the first study to propose these two mechanisms together to help explain our physiological responses to food stimuli. The results of our research showed that the ANS responses obtained through the anticipatory responses related to the creation of expectations did not greatly differ from those measured during the confirmation and disconfirmation of expectations. In both cases, positive and negative images led to a stronger cardiac deceleration than neutral images. Moreover, the negative image led to a higher skin conductance than the positive and neutral image. The ANS responses obtained were a result of the sensory processing and defense mechanisms happening during the creation and (dis)confirmation of expectations. The second study confirmed that the effects were not fully due to visual processing. Hence, it seems that the context of use has an influence on the patterns and magnitude of the ANS responses to food cues. It is necessary for the individual to find the sensory experience relevant in order to have an effect large enough to be considered significant (both statistically and physiologically).

CHAPTER 3

Heart rate and skin conductance responses to taste, taste novelty and the disconfirmation of expectations

This chapter can be found as *Verastegui-Tena, L., van Trijp, H., & Piqueras-Fiszman, B. (2018). Heart rate and skin conductance responses to taste, taste novelty, and the (dis)confirmation of expectations. Food Quality and Preference, 65, 1-9*

Abstract

It remains unclear whether the responses of the autonomic nervous system (ANS) can measure how people respond to food. Results focused on emotional responses are contradictory. Hence, the focus has shifted to other components of emotion. Appraisals are components of emotion that are automatic and unconscious. The aim of this study was, therefore, to evaluate the differences in ANS responses related to appraisals; particularly taste novelty, valence, and the disconfirmation of expectations.

A hundred and fifty-five participants joined this study. They tasted samples of different valence (sweet and bitter) twice: the first time without knowing the taste and the second while being informed of the taste. After this first block, participants tasted two additional samples: one that confirmed expectations and one that disconfirmed them. Heart rate and skin conductance were measured. Results show that the second experience with a taste led to cardiac deceleration. Heart rate changes were only related to valence when participants' expectations were (dis)confirmed. Heart rate decreased for those tastes that disconfirmed expectations and increased for those that confirmed them and the sweet sample had larger increases in heart rate than the bitter. Skin conductance changed in regards to novelty and valence but not to the disconfirmation of expectations. It increased for the bitter sample, decreased for the sweet, and was always higher during the first experience than during the second. In conclusion, the results suggest that cardiac responses are more sensitive to novelty and the disconfirmation of expectations while skin conductance responses capture novelty and valence.

3.1. Introduction

The acceptance and rejection of food are influenced by a series of variables. While sensory liking plays a role, other variables such as environmental factors (ambiance and food varieties), the presence of others, or even the emotions felt are of importance. Emotions have been found to have a bidirectional effect with food: they influence food choice and the foods consumed can influence them (He, Boesveldt, de Graaf, & de Wijk, 2016). Food researchers have therefore developed an interest in the subject of emotional responses to food.

Researchers have been looking for a reliable way to measure how people instinctively respond to food products (Mojet et al., 2015). Explicit measurements such as self-reports are commonly used but have significant disadvantages. Variables such as social desirability may bias participants and make them enhance positive aspects or deny negative ones when reporting their reactions. Moreover, reporting one's emotional state may lead to reflective processes that can change the emotional experience (Bartoszek & Cervone, 2016). For these reasons, along with the fact that emotions are said to have a conscious and an unconscious component, the additional use of implicit measurements (non-verbal) is increasing. Implicit measurements reflect uncontrollable fast mechanisms that the person is unaware of (Lebens et al., 2011). They may provide a more holistic view regarding the emotional and motivational responses to food (Walsh, Duncan, Bell, O'Keefe, & Gallagher, 2017). Commonly used implicit measurements in food research include facial expressions, neuroimaging, or physiological measures such as those of the autonomic nervous system (ANS) (Spinelli & Niedziela, 2016).

The ANS is a component of the peripheral nervous system which is in charge of both the activation (sympathetic branch) and relaxation (parasympathetic branch) of the body (Mauss & Robinson, 2009). It functions without voluntary control or awareness of the person (McCorry, 2007). While the ANS is considered a major component of the emotional response, there are still more controversies than consensual views on the topic of ANS and emotion (Levenson, 2014). The ANS activity is not exclusively a function of emotional responding but encompasses a variety of other functions such as homeostasis, effort, and attention. This makes it difficult for researchers to determine whether the ANS is a good measure of emotional responses to food. Results regarding the link of ANS and emotion are inconsistent among studies. The focus has, therefore, shifted to the study of ANS and other dimensions or components of emotion that might lead to clearer results than emotions alone (Mauss & Robinson, 2009). One such component could be appraisals.

Appraisals, same as the ANS, are automatic and unconscious (Ellsworth & Scherer, 2002). They could be seen as applying a series of “checks” and “sub-checks” when perceiving a stimulus. The stimulus is defined as relevant by a series of dimensions, which are part of the main structure in which the evaluation takes place. Once the stimulus is seen as relevant, further appraisals occur. These appraisals eventually lead to an emotion and to ANS responses. Hence, the ANS responses created by emotions are intimately related to the appraisals that produced them (Derryberry & Reed, 2002; Frijda & Mesquita, 1998).

The most basic appraisals at the level of perception and of importance for food research are those of novelty and valence or intrinsic pleasantness. Novelty detection draws attention and leads to further processing to determine whether any action is required (Ellsworth & Scherer, 2002). Novelty can be extracted through perceptual processing. In general, situations that are novel are situations in which the person has not had any previous experience. Most situations, however, are not completely novel. Stimuli are compared to pre-existing ideas or knowledge (Lazarus & Folkman, 1984). In the case of food, for example, people already have preconceived ideas about the sensory characteristics of the product (taste, texture, among others) and how much they are going to like it (Tarancón et al., 2014). Hence, some appraisal theories do not consider novelty but instead include related concepts such as disconfirmation of expectations or unexpectedness to capture this incongruency between existing stimuli and the expectations of the person (Derryberry & Reed, 2002; Moors et al., 2013). The appraisal of valence determines which will be the reaction or response of the organism. These reactions could be approach (led by attraction and liking) or avoidance (led by aversion or disgust). The appraisal of valence usually happens quickly and, as a result, is difficult to separate from that of the experience of attention. Particularly in those cases in which the stimulus is seen as negative, other appraisals follow such as certainty, agency, and power (Ellsworth & Scherer, 2002).

Literature regarding ANS, novelty, and valence in food research have used different types of stimuli and shown different results. Rousmans et al. (2000) found that compared to baseline, a sucrose solution led to the smallest changes in heart rate (instantaneous heart rate) and electrodermal activity (skin resistance amplitude and ohmic perturbation duration index), while a bitter solution led to the largest changes. Danner et al. (2014) found that, compared to baseline, juices that were disliked (and therefore had a negative valence) were associated with higher skin conductance responses (SCR) than those that were liked (and therefore had a positive valence) but no differences were seen in HR. Likewise, de Wijk et al. (2012) found that disliked foods led to a decrease in SCR in adults compared to liked foods but to similar heart rate responses between liked and disliked foods. These studies, however, do not consider the differences in ANS when a stimulus is encountered for the first time, and consequently seen as novel. Worth mentioning is the study by Delplanque et al. (2009) which looks at the sequential unfolding of novelty and pleasantness in odors. The results of this study show that novel odors elicited greater skin conductance responses than odors that were repeated and that negative (unpleasant) odors led to greater skin conductance responses than positive (pleasant) odors. In contrast, novel odors led to a decrease in heart rate compared to repeated odors and positive odors led to a stronger cardiac deceleration than negative odors. Studies regarding ANS and the disconfirmation of expectations in food research are scarce. Data by de Wijk and Boesveldt (2016) show that when participants tasted a breakfast drink that was different from the one that was visually inspected, the ANS responses, particularly that of skin conductance, were increased. The first study in Chapter 2 (Verastegui-Tena, Schulte-Holierhoek, van Trijp, & Piqueras-Fiszman, 2017) showed that ingredient images that disconfirmed expectations from the tasted experience led to cardiac deceleration regardless of the valence of the image while in the case of skin conductance only the negative image led to an increase that differed from that of a neutral congruent image.

It seems that the use of ANS responses in food research, though a novel approach in the area, needs a deeper understanding in order to be used adequately. Disentangling the responses to basic appraisals can be the tool to a better understanding and interpretation. To the best of our knowledge, no study has looked at the ANS responses to novelty, valence, and the disconfirmation of expectations together in a systematic way. The aim of this study is, therefore, to discern the differences in ANS responses related to novelty (first encounter with a taste with no previous information), to the disconfirmation of expectations, and to valence. For this purpose, the experiment was divided in two blocks. In the first block, participants drank two times a sample with a positive taste and a sample with a negative taste (these tastes being pretested for valence). The first time without being told beforehand the taste of the sample and the second time with previous knowledge of the taste of the sample. The effect of novelty related to the first experience with a taste (henceforth referred to as "taste novelty") was measured by comparing the changes from baseline of the ANS responses of the first time participants tasted the sample to those of the second time. Valence (henceforth referred to as "taste" or "taste valence") was assessed by comparing the changes from baseline of the ANS responses associated to the positive taste to those of the negative one. For the second block of the study, participants tasted samples with a taste that confirmed their expectations (the taste matched the one they had been told beforehand and was, therefore, expected) and a taste that disconfirmed them (the taste was different to that they had been told beforehand and was, therefore, unexpected). The effect of the disconfirmation of expectations was assessed through the comparison of the changes from baseline of the ANS responses between the expected sample and the unexpected one.

Our hypotheses were that a) the novelty of the taste would engage the attention of the participant and, as a consequence, the first tasting would lead to larger increases in skin conductance and smaller decreases in heart rate than the second tasting; b) following the pattern of previous studies, the positive taste will lead to lower ANS responses than the negative taste; and c) samples that disconfirm expectations will activate a defensive response and, therefore, lead to higher increases in skin conductance and heart rate.

3.2. Materials and methods

Screening and selection of participants

A hundred and fifty-five participants ranging from 18 to 45 years of age were recruited from the Wageningen area and surroundings. Participants were recruited via flyers and advertisements in buildings and supermarkets close to Wageningen University. They were excluded if they had a BMI higher than 27.5 kg/m^2, had any known cardiac problems, were non–tasters for bitter, or were allergic to the ingredients of the stimuli used. In order to test if they were non-tasters for bitter, all potential participants were given a phenylthiocarbamide (P.T.C) test paper

(Selfcontrol CD 265 D 01, Bern, CH) and asked to chew it. Those who could not sense the bitterness of the P.T.C test paper were given a sample with quinine to confirm their taster status. Participants who could sense the bitterness were given a summary of the procedure that would be followed during the study and were asked to schedule an appointment if they fulfilled all requirements and agreed to participate. The Social Sciences Ethics Committee of Wageningen University approved the study.

Experiment session

The study took place in the Marketing and Consumer Research group premises from Wageningen University, the Netherlands. Participants were instructed to wear comfortable clothes for the study (sport clothes were recommended). The study took place in a well-lit white room where participants were seated in front of a computer with OpenSesame version 3.1.2 (Mathôt et al., 2012). Before starting the test, they were given an informed consent. Once the participant had signed the consent, the researcher started placing the sensor pads.

Seven sensor pads (Kendall^{TM} H98S8 60-mm micropore ECG electrodes) were placed on each participant. Five were on the chest and two on the back. For the measurement of skin conductance, two sensors were placed (Biopac® TSD 203 Electrodermal Response Transducer), one on the index finger and the other on the middle finger of the non-dominant hand. The researcher checked all signals to avoid any problems due to electrode misplacements. After all signals were checked, participants were asked to remain still, to close their eyes, and to breathe normally for one minute.

The study consisted of two blocks. The first block involved two tasting rounds of three samples and was used to test the effect of taste novelty and taste (**Figure 3.1**). For this part, participants were separated randomly in three groups. For all groups, the first sample of each round was water and was used as a baseline. The second sample was given without giving the participants any previous information of the taste. The third sample was identical to the previous one and was given after participants were told the taste. The second and third samples could be either sweet (positive valence) or bitter (negative valence). Participants in Group 1 were given bitter samples in the first round and sweet samples in the second round. For the participants in Group 2 the order was altered, they received the sweet samples in the first round and the bitter ones in the second. At the end of each tasting, participants were directed to a questionnaire in which they rated, by means of a 100-mm visual analogue scale (VAS), the pleasantness and taste intensity of the bitter and sweet samples. Group 3 was given water samples using a similar procedure but without asking participants to rate the pleasantness and taste intensity of the water. This was done to avoid any confusion or suspicion of the participants, as water is considered to be neutral. Group 1 and 2 were used for the main analysis of taste novelty and pleasantness. Due to the lack of pleasantness and taste intensity ratings, Group 3 could only be used for a separate analysis to have a general overview of how neutral samples compare to those with taste.

The second block of the study involved a tasting of four samples and was used to test the effect of the disconfirmation of expectations (**Figure 3.2**). For this part, participants were divided in five groups. For all groups, the first and third samples were water. The second and fourth samples varied according to the group. For Group 1, participants were told that the second and fourth samples were bitter, but in reality the fourth sample was sweet, an unexpected taste that disconfirmed expectations. For Group 2 the order was reversed, the unexpected sweet sample was given in the second position. Groups 3 and 4 were told that both the second and fourth samples were sweet. However, for Group 3 the fourth sample was in reality bitter, while for Group 4 the order was reversed and the unexpected bitter sample was given in the second position. All tastings where the opposite taste was given were covered as a mistake from the researcher giving the wrong sample. Finally, to be able to compare the taste samples with a neutral sample, Group 5 received water four times with no manipulation of the taste expectations of the samples.

Participants were asked to cleanse their palate with water after each sample and to make sure the taste was gone from their mouth before continuing with the next part of the test. After finishing the test, participants were debriefed. They were told the reason why they were given a sample different from the one they were told and the main objectives of the study.

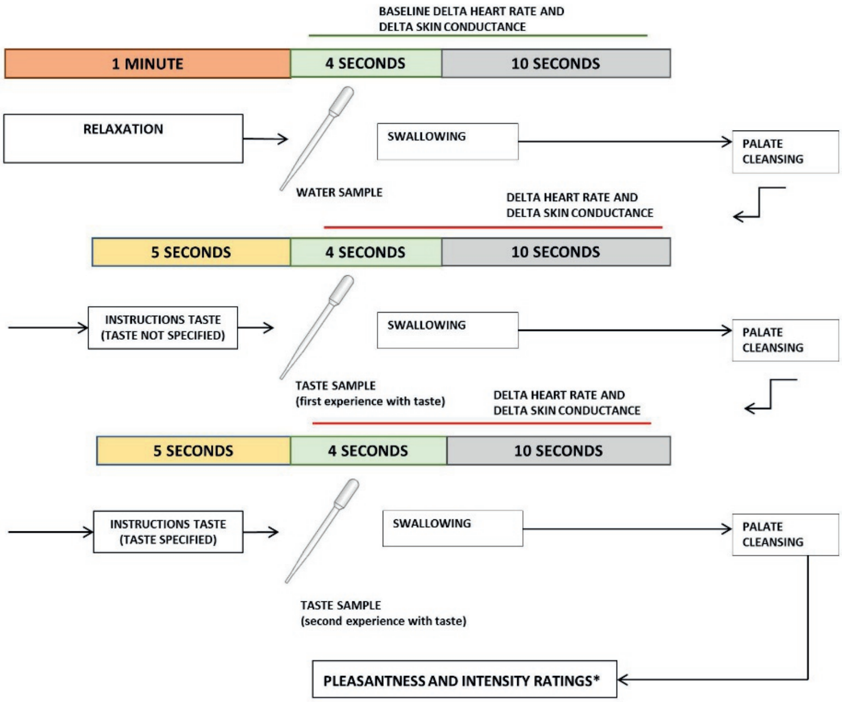


Figure 3.1. Procedure of the first block of the study (one round of three tastings). Heart rate and skin conductance were measured while participants tasted twice sweet and bitter samples. The first sample consisted of water and served as a baseline. The second and third samples contained the same taste. Participants tried the second sample without previous knowledge of the taste (taste not specified). For the third sample participants were told beforehand the taste (taste specified).

3

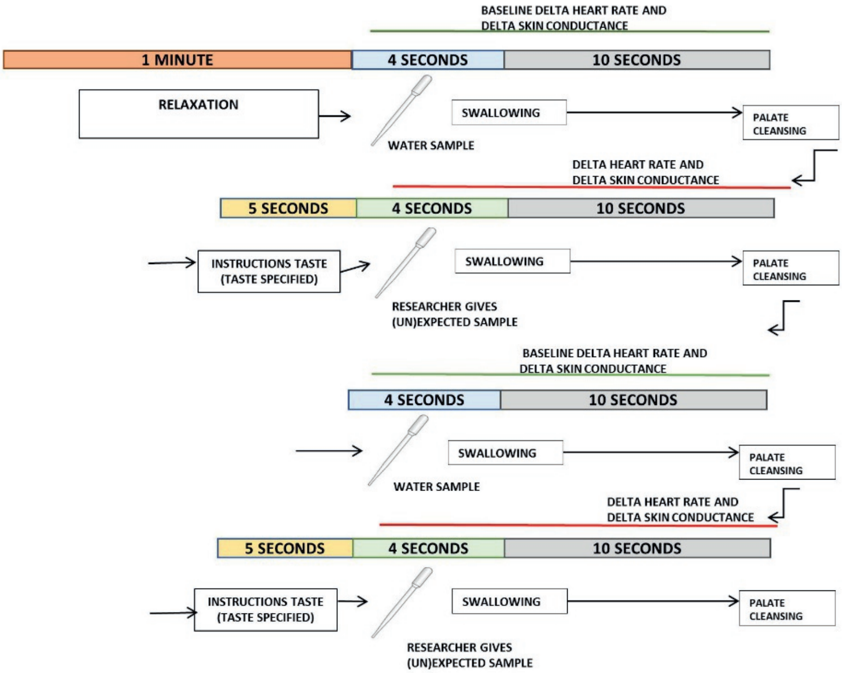


Figure 3.2. Procedure of the second block of the study. Heart rate and skin conductance were measured while participants tasted samples that confirmed (expected taste) and disconfirmed (unexpected taste) expectations. The first and third samples consisted of water. The second and fourth samples could have either an expected or unexpected taste, depending on the group the participant was assigned to.

Tasting samples

The samples used in this study were a sample with a positive valence consisting of sucrose (sweet) and a sample with a negative valence consisting of quinine (bitter). The sucrose sample had a molarity of $4.5x10^{-1}$ M. For its preparation, 154.033 g of table sugar (Van Gilse Krystal Suiker, Suiker Unie, Oud Gastel NL) were added in 1 L of water. The quinine sample had a molarity of $7.5x10^{-5}$ M. For its preparation 0.0297 g of quinine monohydrochloride dihydrate >95% FG (Sigma Aldrich Flavours & Fragrances, Milwaukee USA) were added in 1 L of water. The concentration of the samples was determined in preliminary tests done to ensure that the taste was detectable by participants and that the bitter sample was considered unpleasant (and hence, had a negative valence) in comparison to the sweet one (pleasantness ratings in preliminary tests of 58.4±18.0 in a 100-mm VAS scale for the sucrose solution and 23.4±15.1 for the bitter solution). All samples were presented at room temperature and given with a 3-ml pipette to avoid any movement from the participant. The volume given to each participant was 2 ml. This was chosen to imitate the normal swallow of saliva, which ranges from 1-2 ml, and to avoid big swallowing motions due to the size of the bolus (Levine, Ramchandani, & Rubesin, 2012).

Physiological measurements

Heart rate and skin conductance were measured throughout the study. For these measurements the VU-AMS version 3.9 (de Geus et al., 1995) was used. The ECG had a sampling rate of 1000 Hz and heart rate was obtained from the time between two adjacent R waves. Skin conductance was sampled at a rate of 10 Hz with a signal range between 0-95 µs. The signal was filtered both in forward and reverse direction with a low-pass filter with a cut-off frequency of 2 Hz.

Data treatment and analysis

The VU DAMS program (version 3.9) was used for the extraction of the heart rate and skin conductance responses. All data were inspected for artifacts and labelled. The deviation from the baseline for heart rate (delta heart rate) and skin conductance (delta skin conductance) of each tasting was calculated by subtracting the mean heart rate and skin conductance of the tasting of the water samples to the mean heart rate and skin conductance of the samples with taste. For the statistical analysis, the R software version 3.4.0 was used.

To test if taste and taste novelty had an effect on ANS responses, heart rate and skin conductance from the first block of the study were analyzed by means of a mixed model anova stating subject as random factor, the variables taste (sweet or bitter), taste novelty (first or second experience with the taste), and as well as the interactions between the taste and taste novelty as fixed factors. Taste intensity ratings were also included as a covariate. To confirm that the sweet and bitter samples differed in pleasantness, the pleasantness ratings of the sweet and bitter samples were compared with a paired-sample t-test.

To test if the disconfirmation of expectations had an effect on ANS responses, heart rate and skin conductance from the second part of the study were analyzed by means of a mixed model anova considering subject as random factor and the variables real taste (the actual taste of the sample), disconfirmation (confirmation or disconfirmation of the taste told to participants) as well as the interactions between the real taste and the disconfirmation as fixed factors.

Two supplementary analyses were done to corroborate the results found. The first analysis was done with the responses of the first block of the study. The ANS responses of the two tastings of the bitter, sweet, and water groups were assessed. A mixed model with subject as random factor and the variables taste (sweet, bitter or water), taste novelty (first or second experience with the taste) and the interaction between taste and taste novelty was done for each taste. As participants in the water group did not rate the pleasantness and taste intensity of the sample, the covariate taste intensity was not included in the model. The assumption of this analysis was that the effect of taste novelty will be present in all samples and that the effects of taste will make the responses stronger for the sweet and bitter samples than for the water samples. The second analysis was done with the responses of the samples of the second block of the study. The ANS responses for the samples given in the second position were used. Only the bitter and sweet samples that confirmed expectations and the water samples were analyzed (note that the group that received water were not deceived at any moment, hence the use of the confirmation trials for the taste samples). An anova with the variable real taste (actual taste of the sample) was used. The assumption was that the responses for the water samples should be of a smaller magnitude than the other samples with taste and that the responses between the samples with taste will be similar to those of the main analysis.

For all the models used in the analysis, the variables gender, age, and BMI were assessed in separate models and only added to the main one if their inclusion affected the general outcome of the model. Post hoc analyses were performed using Tukey’s honest significance test (Tukey’s HSD) for multiple comparisons. Additional permutation tests were carried out for the models that did not fulfill the normality assumption.

3.3. Results

3.3.1. Pleasantness ratings of sweet and bitter samples

The following section describes the results of the comparison of the VAS scale pleasantness ratings given by the participants for the sweet and bitter samples. The results of the paired sample t-test show that there was a significant difference in the pleasantness ratings between the sweet sample and the bitter sample (t (88) = -17.44, p<0.001) with the bitter sample having lower pleasantness ratings (mean pleasantness 20.73±16.30) than the sweet (mean pleasantness 70.28±19.25) one.

3.3.2. ANS responses to taste and taste novelty (first block)

A hundred and fifty-five participants completed the study. For this first block, the data of 22 participants were excluded from the analysis: 11 due to ectopic beats, two due to abnormal heart rhythms, one due to mistakes during the execution of the study, and eight participants due to movements during the measurement. In total, the data of 133 participants were included in the analysis. Forty-four were part of the water group used in a separate analysis. The remaining 89 participants were part of the analyses of taste novelty, taste, and the (dis)confirmation of expectations. From these, 49 were female (mean age=24.4±4.2, mean BMI=21.5±1.8) and 40 were male (mean age= 25.0±4.7, mean BMI= 22.5±2.4).

<u>Effect of taste novelty and taste on heart rate and skin conductance</u>

The following section describes the results of the analysis of the effect of taste (sweet vs bitter) and taste novelty (1st experience vs 2nd experience with a taste) on heart rate and skin conductance (see **Figure 3.3**). The data of Group 1 and Group 2 were joined together given that the arrangement of the samples (order of bitter or sweet rounds) did not alter the results. Gender, age, and BMI were checked but not added in the final models, as they did not affect the main outcome of the model when included.

Heart rate

There was a significant effect of taste novelty (1st vs 2nd experience with a taste) in delta heart rate (F(1,263)= 78.69, p<0.001). Post hoc tests revealed that the differences between the first and second experience were found for both sweet and bitter, with the second experience presenting a larger decrease in heart rate than the first (p<0.001). We did not find a main effect of taste (F(1,284)= 0.104, p=0.747) or of the interaction between taste and taste novelty (F(1,263)= 0.631, p=0.428). These results show that, regardless of the taste, the second experience with a taste leads to a lower cardiac response than the first experience.

Skin conductance

There was a significant effect of taste novelty (1st vs 2nd experience with a taste) in delta skin conductance (F(1,263)= 4.36, p=0.038). Likewise, we found an effect of taste in delta skin conductance (F(1,281)= 26.84, p<0.001). There was no effect of the interaction between taste and taste novelty (F(1,263)= 0.68, p=0.412). Post hoc tests revealed that the second experience led to a smaller delta skin conductance than the first and that the bitter sample led to a larger increase in delta skin conductance than the sweet sample, which presented a negative delta skin conductance during the second tasting (p<0.001).

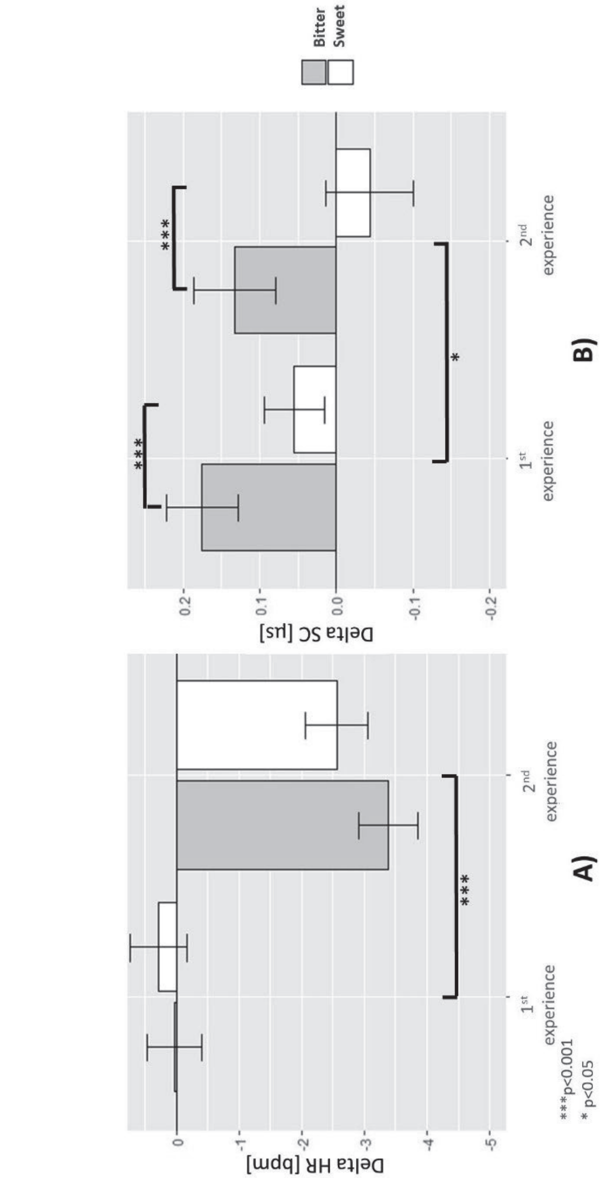


Figure 3.3. Mean (± SE) of (A) delta heart rate (beats per minute) and (B) delta skin conductance (μs) during the first and second experience with the sweet and bitter samples (N= 89).

Analysis with water as neutral stimulus

The following section describes the heart rate and skin conductance models assessing the novelty and taste effect for bitter, sweet, and water samples, done to complement the analysis of the first block of the study (see **Figure 3.4**).

Heart rate

There was a significant effect of taste novelty (1^{st} vs 2^{nd} experience with a taste) in delta heart rate ($F(1,398) = 68.388$, $p < 0.001$). Post hoc tests revealed the second experience presented a larger decrease in heart rate than the first. We did not find a main effect of taste ($F(2,197) = 0.99$, $p = 0.370$) or of the interaction between taste and taste novelty ($F(2,398) = 2.56$, $p = 0.078$). These results show that the differences between the first experience and the second experience are seen for all samples, including that of water, which had no taste.

Skin conductance

There was a significant effect of taste novelty (1^{st} vs 2^{nd} experience with a taste) in delta skin conductance ($F(1,398) = 8.43$, $p = 0.268$). Likewise, we found an effect of taste ($F(2,197) = 9.39$, $p < 0.001$). Post hoc tests revealed that the second experience led to smaller increases in delta skin conductance than the first. The sweet and bitter samples led to different changes in delta skin conductance, with the bitter sample leading to larger increases in skin conductance than those of the sweet. The delta skin conductance of the water sample, however, did not differ from those of the sweet and bitter samples. We did not find an effect of the interaction between taste and taste novelty ($F(2,398) = 0.463$, $p = 0.412$). These results show that the effect of taste novelty is also present in the water sample and that the skin conductance of the water samples remains similar to that of the sweet and bitter samples.

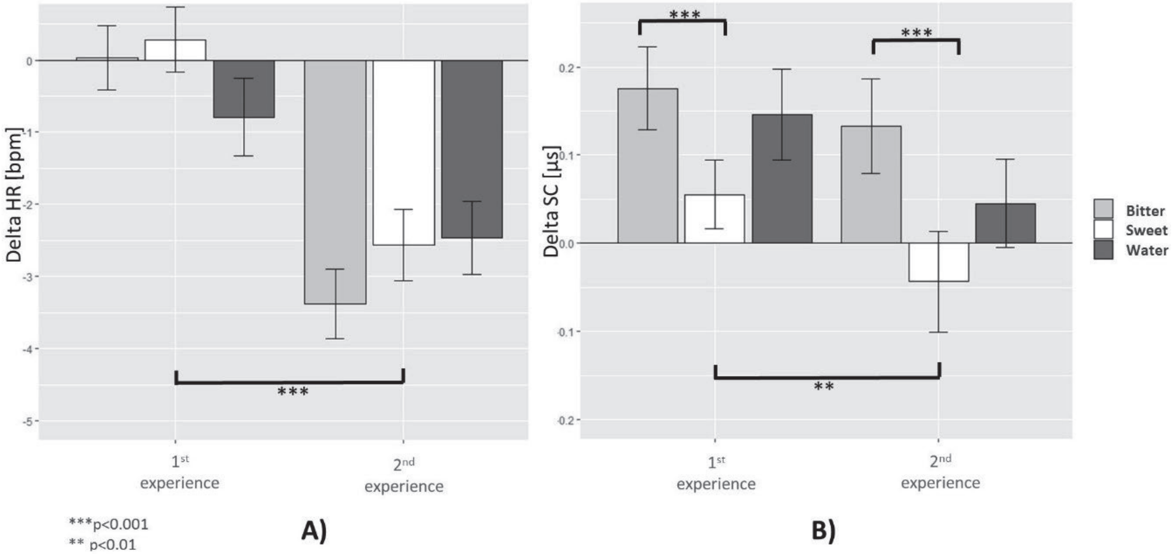


Figure 3.4. Mean (± SE) of (A) delta heart rate (beats per minute) and (B) delta skin conductance (µs) during the first and second experience of the bitter, sweet and water samples (N= 133).

3.3.3. Effect of the (dis)confirmation of expectations on ANS responses (second block)

From the 155 participants that completed the study, the data of 18 were excluded from the analysis of this second block. Eleven were excluded due to ectopic beats, two due to abnormal heart rhythms, one due to mistakes during the execution of the study, and four participants due to movements during the measurement. In total, the data of 137 participants were analyzed. From these, 26 were used for a separate analysis and given only water. The remaining 111 were used for the analysis of the disconfirmation of expectations. The demographic information is shown on **Table 3.1**.

Table 3.1. Demographics of the sample by divided by group (N=137).

	Bitter expected first	Bitter unexpected first	Sweet expected first	Sweet unexpected first	Water	*p*-value
N	24	28	29	30	26	-
Gender: Female(Male)	15(9)	16(12)	18(11)	15(15)	14(12)	0.865[a]
Age (years)	25.1 ±3.8	22.8 ±3.3	25.5 ±3.9	24.6 ±5.4	25.8 ±4.3	0.074[b]
BMI (kg/m^2)	22.2 ±2.3	21.6 ±2.0	21.4 ±1.7	22.4 ±1.9	21.9 ±2.4	0.341[b]

[a] *p*-value calculated with chi-square test.

[b] *p*-value calculated with anova.

Effect of (dis)confirmation of expectations on heart rate and skin conductance

The following section describes the results of the analysis of the effect of tasting samples with a taste that confirms (expected) and disconfirms expectations (unexpected) on heart rate and skin conductance (see **Figure 3.5**). The data of Group 1 and Group 2 and that of Group 3 and 4 were joined together given that the arrangement of the samples (confirmation or disconfirmation first) did not alter the results. Gender, age, and BMI were not added in the models as they did not affect the main outcome when included.

Heart rate

There was a significant effect of disconfirmation on delta heart rate ($F(1,218)= 8.75$, $p=0.003$). Likewise, the effect of the real taste was significant ($F(1,218)= 6.98$, $p=0.009$). Post hoc tests revealed that samples with tastes that disconfirmed expectations led to a decrease in heart rate and that the bitter sample led to a larger decrease in heart rate that the sweet sample. However, there was no effect of the interaction between real taste and disconfirmation ($F(1,218)= 2.76$, $p=0.098$).

Skin conductance

Disconfirmation did not have an effect on skin conductance ($F(1,218)= 0.01$, $p=0.977$). Likewise, the effects of taste and that of the interaction between taste and disconfirmation were not significant ($F(1,218)= 0.28$, $p=0.600$ and $F(1,218)= 0.31$, $p=0.580$ respectively). These results suggest that skin conductance does not change between tastes that confirm and disconfirm expectations as presented in this study.

Analyses including water as neutral stimulus

The following section describes the heart rate and skin conductance models used for the analysis used to corroborate the results of the second part of the study. As only the data of the samples that confirmed expectations and that were given in the second position were used, the results shown here relate to the responses of 79 participants (see **Figure 3.6**.).

Heart rate

There was a significant effect of real taste on delta heart rate ($F(2, 76)= 5.97$, $p=0.004$). The main differences were between the bitter taste and water, and between the sweet taste and water. These results show that the delta heart rate is similar for the sweet and bitter samples but is different from that of the water samples.

Skin conductance

The effect of real taste on delta skin conductance was not significant ($F(2, 76)= 0.34$, $p=0.711$). These results show that the delta skin conductance remains similar for the water samples and the samples with the bitter and sweet taste.

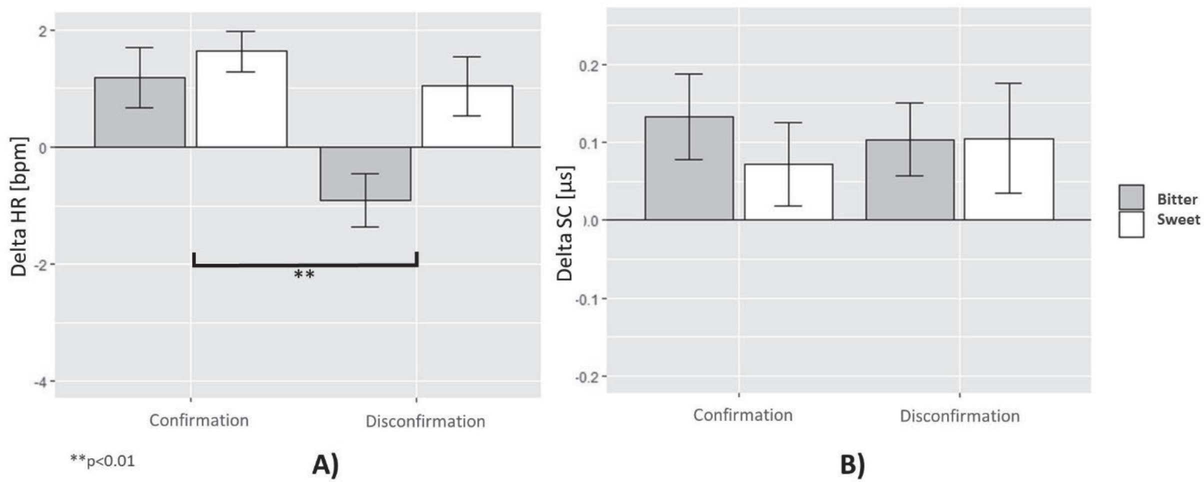


Figure 3.5. Mean (± SE) of (A) delta heart rate (beats per minute) and (B) delta skin conductance (µs) during the tasting of samples that confirm (expected) and disconfirm (unexpected) expectations (N= 111). The tastes shown on the graphs are the real tastes of the samples given to participants. Note that the effect of taste for heart rate was significant but not possible to represent in the graph.

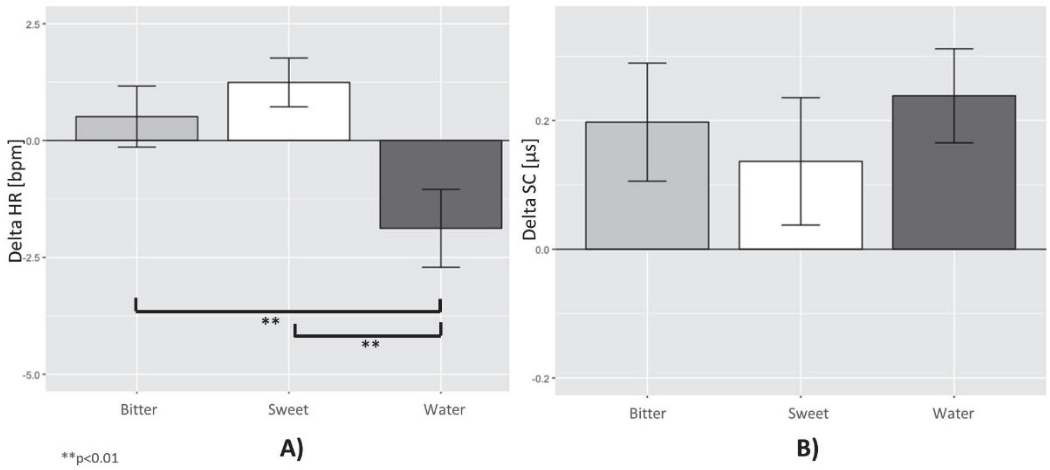


Figure 3.6. Mean (± SE) of (A) delta heart rate (beats per minute) and (B) delta skin conductance (µs) during the tasting of the first samples that confirm expectations (N=79).

3.4. Discussion

The aim of this study was to determine the differences in ANS responses associated with taste novelty (first experience with a taste), disconfirmation of expectations and the differences associated to valence (taste with a positive valence vs taste with a negative valence). One of the main findings of this study is that heart rate seems to be more sensitive to the differences related to taste novelty and the disconfirmation of expectations while skin conductance is sensitive to changes related to novelty and valence but not to the disconfirmation of expectations.

Taste novelty led to differences in heart rate, regardless of the valence of the taste. While heart rate remained similar to baseline for the first experience, the second experience with the samples led to a cardiac deceleration. Cardiac deceleration is a characteristic of the orientation response (OR). It occurs when dishabituation has taken place. Before such deceleration, however, the immediate response is that of arousal and is accompanied by a brief cardiac acceleration and a change in skin conductance. Longer lasting responses, signaled by cardiac deceleration, immediately follow and are associated to responses to attentional and sensory processing (Lacey & Lacey, 1970; Pribram, 1979). It seems likely that the heart rate responses during the first experience are a result of the combination of both arousal and attentional processing. The brief cardiac acceleration related to the arousal response to the novelty of the taste masked the expected deceleration of the OR and, as a result, the heart rate remained close to baseline. Only during the second experience with the samples, when the taste was no longer novel and the brief acceleration was no longer present, cardiac deceleration was the main response.

Skin conductance responses differed according to taste novelty and to valence. The first experience with a taste led to larger increases in skin conductance than the second experience. Stimuli that lead to higher skin conductance responses include novel, significant, or intense stimuli (Dindo & Fowles, 2008). Even for novel stimuli with moderate or low intensity, an increase in skin conductance after its presentation is interpreted as an attention or orientation response. This response indicates that the novel stimulus has been detected (Vila, 2004). Hence, the skin conductance changes to novelty are, same as the cardiac responses, related to the OR to the first encounter with the taste.

The skin conductance changes related to taste valence were stronger than those related to taste novelty. This might be because skin conductance primarily reflects the activation of the sympathetic nervous system. While an OR leads to a sympathetic-parasympathetic coactivation, effort mobilizing aspects, such as a defense mechanism, lead solely to sympathetic activation (Kreibig, 2016). In our study the bitter taste, which was negative, led to an increase in skin conductance while the sweet taste, which was positive, led to a decrease. The inner connotations of the bitter taste, constantly associated to noxious or toxic substances, might have triggered a strong defensive response which resulted in the increase in skin conductance (Rousmans et al., 2000). The exact mechanism why the sweet sample gives weaker responses is still unknown, Rousmans et al. (2000) mentions that it may be due to the fact that the sweet taste is familiar and frequently consumed and hence, the weak responses might be a product of habituation to the taste.

The results of the second block show that the disconfirmation of expectations was associated to changes in heart rate but not to skin conductance. Contrary to what we hypothesized, samples that disconfirmed expectations led to lower heart rates than those that confirmed expectations. This was seen particularly in the cases in which participants expected a positive taste (sweet) but got a negative one (bitter). It is possible that, instead of capturing the defense mechanism to the change in taste, the heart rate responses are again representing an orienting response to this change/disconfirmation ("this is not the taste I was told") as such change is deserving of attention (Graham & Clifton, 1966). An interesting finding was that heart rate responses were only sensitive to taste in the second part of the study but not in the first. This may be due to the tasting of samples that disconfirmed expectations included in the second part. The reactivity to the stimulus was heightened and the attention of the participants became stronger due to the significance of the change/disconfirmation in taste (Bradley, Keil, & Lang, 2012).

The analysis with water done with the data from the first block of the study confirms our interpretations regarding the ANS responses to taste novelty and taste. Heart rate and skin conductance responses were different between the first and the second tasting for all samples. Hence, we can confirm that novelty in itself leads to differences in these ANS responses. In the case of the effect of taste, the analysis with the water sample finds again that the heart rate responses do not differ in terms of taste. The skin conductance responses showed that the sweet taste had the lowest skin conductance while the bitter the highest. The changes in skin conductance related to water remained between those of the sweet and bitter samples. Only the difference between the sweet and bitter samples was statistically significant. It seems, therefore, that skin conductance can only differentiate between tastes of opposite valence. The analysis with water done with the data from the second block of the study shows that heart rate increased for the sweet and bitter samples while the water sample showed a low decrease. These heart rate and skin conductance responses were different from those of the first block of the study. Though in both cases the samples are congruent, the analyses performed are different as is the design and sample size. This may account for the differences found.

While the findings on ANS and taste novelty match our hypothesis, some of the results on valence were different than expected. The skin conductance responses to valence match what has been reported in other studies that use basic tastes (Horio, 2000; Robin et al., 2003; Rousmans et al., 2000), but the same does not apply for our results regarding heart rate. The findings of previous studies state that the tasting of a sample leads to an increase in heart rate, with quinine among those that give the strongest heart rate responses and sucrose the weakest. Our study found that the tasting of samples led to a cardiac deceleration and that this deceleration was similar for sweet and bitter samples. An increase was only seen in the second block of the study, where expectations were disconfirmed. However, in this part the heart rate responses to the sweet sample were stronger than those to the bitter one. Whether these differences were related again to the influence of the orientating response related to the disconfirmation (with bitter samples leading to a stronger orientating response and, as a consequence, a stronger cardiac deceleration) is still unclear.

The findings on ANS and the disconfirmation of expectations from this study present some similarities and differences from those of the first study in Chapter 2 (Verastegui-Tena et al., 2017), which also dealt with the topic of the disconfirmation of expectations. Both studies found an increase in skin conductance for the negative stimuli that disconfirmed expectations, and this increase did not differ significantly from that of the positive one. However, while in the study in Chapter 2 both the positive and negative stimuli that disconfirmed expectations led to a similar decrease in heart rate, in this study only the negative stimuli led to a decrease and it differed significantly from that of the positive. It is possible that this difference is due to the stimuli used in the studies. The stimuli in Chapter 2 were images while the present study used samples with different tastes. Moreover, the study in Chapter 2 did not separate the effect of novelty and valence from that of the disconfirmation of expectations while this study looks at these effects separately.

Our study has certain limitations. While we have data on perceived taste intensity for the first block of the study (effect of taste and taste novelty), we do not have this information for the second block (effect of the (dis)confirmation of expectations). This might have played a role in the absence of changes in skin conductance related to the disconfirmation of expectations as skin conductance has been said to be related to stimulus intensity (Bensafi et al., 2002; Dawson et al., 2000). Moreover, the first block of the study showed a significant effect of the taste intensity covariate on skin conductance. It is possible, therefore, that in order for skin conductance to discriminate among taste stimuli it is necessary to include the data about the perceived intensity. This is further strengthened by the results of the analysis with water of the first block. The participants that tasted only water were not asked to rate the pleasantness and taste intensity of the water sample as it was assumed to be neutral. Consequently, it was not possible to add these data in the analysis and we could only find differences between the extremes of the valence scale (sweet vs bitter samples). The data on taste intensity might have helped find differences between the water sample and the samples with taste. While an analysis on latency and amplitude may have also been helpful to find some differences, the short time intervals between stimuli may not allow it. Participants may not have gone back to baseline before being presented with another stimulus. Lastly, our study focuses on the effects of stimuli that are opposite in valence and congruity. Hence, we cannot know if the same effects would be found if such contrast were not present.

The present work brings more insight into the ANS responses to taste novelty, taste valence, and the disconfirmation of expectations. It looks at the effects of these appraisals on ANS responses and interprets their differences. Moreover, it shows that both heart rate and skin conductance capture changes related to novelty but that, while heart rate can also capture the differences related to the disconfirmations of expectations, the same does not apply for skin conductance. Likewise, it shows that skin conductance, though capable of capturing both novelty and valence, gives clearer results when measuring taste valence. The findings have implications for future research as they show that some ANS responses might be more useful for a particular design than for others. For example, skin conductance and other markers of sympathetic response could be used for products that contrast in particular dimensions such as valence and arousal while heart rate might be helpful to measure the habituation to novel products. Future studies should look further into the appropriate use of ANS responses for each design.

3.5. Conclusion

The aim of the present work was to evaluate the differences in heart rate and skin conductance related to taste novelty, valence and the disconfirmation of expectations. To the best of our knowledge, this is the first study to look separately at the effect of these appraisals on ANS responses. Our results show that cardiac responses differ in regards to novelty and the disconfirmation of expectations. Skin conductance responses differ in regards to novelty and valence but they do not change in regards to the disconfirmation of expectations. The changes in ANS responses in this study may be related to different mechanisms. Changes to novelty and the disconfirmation of expectations seem to be related to the orientation response while the changes to valence may be capturing defense mechanisms. Further research is, however, necessary to confirm these interpretations.

CHAPTER 4

Heart rate, skin conductance, and explicit responses to juice samples with varying levels of expectation (dis)confirmation

This chapter can be found as *Verastegui-Tena, L., van Trijp, H., & Piqueras-Fiszman, B. (2019). Heart rate, skin conductance, and explicit responses to juice samples with varying levels of expectation (dis)confirmation. Food Quality and Preference, 71, 320-331.*

Abstract

Disconfirmations between expectations and a product's actual properties can lead to different responses in individuals. Most researchers study these responses focusing on the final judgement of the product. However, looking at an individual's physiological responses like those of the autonomic nervous system (ANS) could help complement what is known about people's reactions and final response to disconfirmed expectations. This study evaluated how ANS responses change when tasting juice samples that were as expected, that differed slightly, or that differed greatly from manipulated expectations and whether these responses vary from those obtained when there is no manipulation of expectations.

Eighty-six participants tasted fruit and vegetable juices in two separate sessions. They were divided in two conditions. In Condition A, expectations were manipulated by showing participants the image of an ingredient and then providing them with a juice whose flavor was as expected, differed slightly, or differed greatly from that of the image. In Condition B, each juice was first tasted without explicit information shown beforehand and the image of the ingredient was shown afterwards. The images were the same as in Condition A. Heart rate and skin conductance were measured. To confirm that participants perceived confirmations and large and small disconfirmations when tasting the juices, they rated the samples in different sensory properties before and after tasting them. Results from most of the sensory ratings, except sourness and taste intensity, showed that participants perceived the designed confirmation and disconfirmation of expectations accordingly. Regarding ANS responses, heart rate had a larger increase during the second session than during the first. Skin conductance responses increased in Condition A but decreased in Condition B. In conclusion, our design managed to create confirmations and varying levels of disconfirmations. ANS responses did not capture them but seemed to capture factors like attention and the orientation response.

4.1. Introduction

Human perception of food can be largely influenced by external cues such as context effects and preconceived opinions about the food product (Si & Jiang, 2017). When interacting with food products, our brain interprets and integrates the previously stored information with the cues present in the food such as visual appearance, orthonasal olfactory cues, and auditory cues. This information creates expectations towards the food product about to be consumed. Our expectations may be related to what a food may taste like (sensory expectations) or related to how much we will like it (hedonic expectations). For both sensory and hedonic expectations, whether the experience confirms or not to what was expected can influence if the food is accepted or rejected (Spence, 2016).

Disconfirmations between what is expected and what is experienced can lead to different effects that affect the final judgement of a product. The main theories that have attempted to account for these effects include the assimilation, contrast, generalized negativity and the assimilation/contrast model. In assimilation, individuals adjust their perception to what was expected. This minimizes the difference between expectation and perception. Contrast occurs when this difference is maximized and hence expectation and perception go in opposite directions. Generalized negativity occurs when the individual rates the product negatively, regardless of whether it met or not their expectations. Lastly, the assimilation-contrast model states that the size of the differences between expectations and the perception of a food product will determine if assimilation or contrast occurs (Piqueras-Fiszman & Spence, 2015; Schifferstein, Kole & Mojet, 1999)

The assimilation-contrast model is used by researchers to account for the responses to foods that disconfirm expectations on different levels (Burgess, 2016). If the disconfirmation between the expected and perceived qualities of a food product is small, assimilation will likely occur. Individuals may not realize there is a disconfirmation and hence will evaluate the product quickly and according to their expectations. This process could also be described as incorporating the perceived food product into an existing schema (schema being a stored perception or interpretation of the world; Zhiqing, 2015). If the magnitude of the difference is large enough that individuals are aware of the disconfirmation, they are likely to do a more thorough analysis of the product and this close inspection could lead to accommodation and contrast (Geers & Lassiter, 2005; Schifferstein, 2013). This would relate to adjusting an old schema or building a new one in order to accommodate the perceived food product that does not match the schema (Zhiqing, 2015).

Most research regarding responses to different levels of disconfirmation that can lead to assimilation and contrast has focused on the final judgement of the product. Looking at other mechanisms derived from the disconfirmation of expectations would help broaden the knowledge on the responses that lead to that judgement. For example, situations that deal with disconfirmations of a large magnitude are believed to lead to strong reactions which take place immediately, unintentionally and automatically (Fulcher, Dean, & Trufil, 2016). In order to capture these reactions and their link to the magnitude of the disconfirmation, physiological measurements such as the responses of the autonomic nervous system (ANS) would be needed.

ANS responses are not under voluntary control (McCorry, 2007). Those commonly used in food research include heart rate and skin conductance responses. Heart rate responses reflect a combination of sympathetic (fight or flight reactions) and parasympathetic (resting) activity. Skin conductance relates only to the activity of the sympathetic nervous system (Mauss & Robinson, 2009). The use of ANS responses in food research presents challenges. A particular challenge is that ANS responses are linked to different functions in the body. They can be related to processes such as respiration, digestion and motor behavior. As a result, the ANS responses obtained in studies may be capturing more than just one physiological reaction to a stimulus (Mauss & Robinson, 2009).

Stimuli used in food research differ in various qualities such as taste, color, odor, and valence (measured generally as liking or pleasantness). Results have shown that the differentiation of ANS responses is clearer when the magnitude of the difference among stimuli is large. For example, Danner et al. (2014) measured the heart rate, skin temperature, and pulse volume amplitude to different juices and found clear skin conductance differences between fruit and vegetable juices. Compared to baseline, the disliked juices (fermented cabbage, mixed vegetables) presented larger increases in skin conductance responses than liked juices (orange, banana). Rousmans et al. (2000) found that, compared to baseline, the pleasant sweet taste had smaller increases in ANS responses, particularly heart rate and skin conductance, than the unpleasant tastes salty, sour, and bitter. Moreover, the strongest differences were between the sweet and bitter samples. De Wijk et al. (2012) found that disliked foods led to larger increases in skin conductance response than liked foods. Contradictory to these results are the ones from a study by Beyts et al. (2017) in which the heart rate and skin conductance responses of participants did not differ when sniffing beers with aromas that differed in liking. The authors concluded, however, that this finding could be because all aromas were related to beers. As the samples had the same carrier (beer), they were too similar to find physiological differences related to the assessment of aroma liking.

While the aforementioned studies have found changes related to large differences in liking/pleasantness, it is still unclear if small differences could induce changes in ANS responses. Moreover, there is little information about the changes in ANS related to the expectations prior to the consumption of these products, how their manipulation affects these results and whether the responses differ when a positive disconfirmation (something pleasant is perceived when an unpleasant taste is expected) or a negative disconfirmation (something unpleasant is perceived when a pleasant taste is expected) occur. According to Kahneman and Tversky's Prospect theory, the value of a gain should be perceived as smaller than that of a loss (Kahneman & Tversky, 1979). This would mean that the differences of a positive disconfirmation (gains) should be smaller than those of a negative disconfirmation (losses). Among the few studies with findings on ANS on this matter, the study in Chapter 3 (Verastegui-Tena, van Trijp, & Piqueras-Fiszman, 2018) showed that expectations that are disconfirmed seemed to be associated with changes in heart rate. There was a heart rate deceleration when participants expected something positive and received something generally perceived as negative. However, the participants in this study only tasted one sample with a taste that disconfirmed expectations and it is not clear whether the same results would be found if participants had tasted both samples with a positive and a negative disconfirmation.

To the best of our knowledge, there are still no studies that have focused on the use of ANS responses to disentangle the processes related to varying levels of positive and negative disconfirmations of expectations. Considering that the interpretation of a situation, rather than the situation itself is what determines the valence and intensity of a response (Siemer, Mauss, & Gross, 2007) and taking into account the various results showing changes in ANS responses with stimuli presenting large differences, ANS responses may present potential in capturing the processes related to the perception of small and large disconfirmations of different valences. The aim of this study is, therefore, to evaluate how ANS responses change in situations in which disconfirmations are small, and hence the individual interprets/perceives them as confirming their expectations and in situations in which these disconfirmations are large and hence the individual interprets/perceives it as a strong positive or negative deviation from their expectations. Moreover, it compares these responses to those obtained when there is no manipulation of expectations.

For this purpose, participants tasted samples from two juice types that were considered different in pleasantness (vegetable and fruit juices). The expectations of the participants were manipulated by showing them the supposed main ingredient of the sample before tasting it. Each juice group included one sample that confirmed the participants' expectations (the information given to them) and two that disconfirmed them. The incongruent samples disconfirmed expectations at different levels. In one, the level of disconfirmation was small while the other presented a larger level of disconfirmation. To confirm that the differences were indeed perceived as large and small disconfirmations, participants rated different sensory attributes and pleasantness before and after tasting each sample. Moreover, to evaluate if the ANS responses obtained when manipulating expectations differed from those obtained when expectations are not manipulated, the responses of these participants were compared to those of a separate set of participants that tasted the same samples before getting any ingredient information.

Our hypotheses are that a) samples with a small disconfirmation level will lead to similar ANS response patterns to those of samples that confirm expectations; b) samples in which the level of disconfirmation is large will lead to larger differences in ANS response patterns from those of samples that confirm expectations and those that have small disconfirmations. Moreover, following Kahneman and Tversky's Prospect theory, this difference will be stronger when the disconfirmation is negative than when the disconfirmation is positive; c) Following the assimilation-contrast theory, samples designed to confirm expectations and samples designed to create small disconfirmations will receive similar, assimilated, sensory ratings. Conversely, samples will be rated differently when designed to create a confirmation than when designed to create a large disconfirmation, reflecting a contrast response. This difference will be present for relevant attributes (pleasantness, sweetness, bitterness, and saltiness); d) samples will show different ANS responses when tasted before obtaining explicit information about the ingredient than when expectations are manipulated. ANS responses when tasting without explicit information will only reflect the difference in pleasantness of the samples.

4.2. Materials and methods

4.2.1. Screening and selection of participants

Eighty-six participants ranging from 18 to 45 years of age were recruited from the Wageningen area and surroundings. Participants were recruited via flyers and advertisements in buildings close to Wageningen University. They were excluded if they had a BMI higher than 24.9 kg/m^2, had any known cardiac or visual problems, or were allergic to any of the ingredients of the juices. Participants that were interested were given a summary of the procedure that would be followed during the study and were asked to schedule an appointment if they fulfilled all requirements and agreed to participate. The Social Sciences Ethics Committee of Wageningen University approved the study.

4.2.2 Tasting samples

The samples used in this study were commercially available fruit juices. A detailed sample description can be found in **Table 4.1**. All samples were stored in the fridge and taken out one hour before consumption. The samples were given with a 3-ml pipette. The researcher gave 2 ml of each juice sample to participants as this is among the usual range of a swallow of saliva (Levine et al., 2012). This volume was chosen to prevent any influence from movements or differences in breathing from bigger swallows (Arici et al., 2013).

Table 4.1. Names and description of the juices given in the study.

Type of juice	Name and brand	Ingredients	Producer
Pear	Appelsientje Hollandse Peren van 't land	Pear juice, vitamin C	Friesland Campina
Apple	Flevosap Appelsap	Apple juice (100%)	Flevosap BV
Orange	Appelsientje sinaasappelsap uit concentraat	Orange juice (100%)	Friesland Campina
Potato	Biotta Potato juice	Potato, fermented lactic acid, fennel, acidifying additive	Natur'Inov
Celery	Biotta Celery juice	Celery juice, fermented lactic acid	Natur'Inov
Carrot	Biosphere organic carrot juice	Carrot juice (99%), lemon juice (1%)	Bio Manufaktur Elm GmbH

4.2.3 Experiment session

The study took place in the Marketing and Consumer Research group premises from Wageningen University, the Netherlands. Participants were instructed to wear comfortable clothes for the study (sport clothes were recommended). The study took place in a well-lit white room where participants were seated in front of a computer with OpenSesame version 3.1.2 (Mathôt et al., 2012). Before starting the test, they were given an informed consent. Once the participant had signed the consent, the researcher started placing the sensor pads.

Seven sensor pads (Kendall™ H98S8 60-mm micropore ECG electrodes) were placed on each participant. Five were on the chest and two on the back. For the measurement of skin conductance, two sensors were placed (Biopac® TSD 203 Electrodermal Response Transducer), one on the index finger and the other on the middle finger of the non-dominant hand. The researcher checked all signals to avoid any problems due to electrode misplacements. After all signals were checked, participants started with the study.

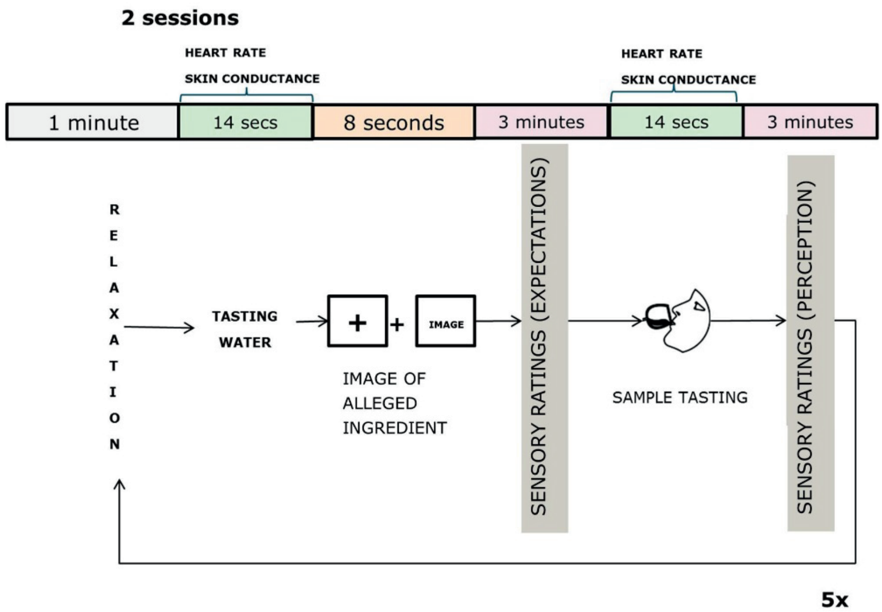


Figure 4.1. Study design for Condition A. The image corresponds to one tasting trial. The procedure shown was repeated for the five juices in each session.

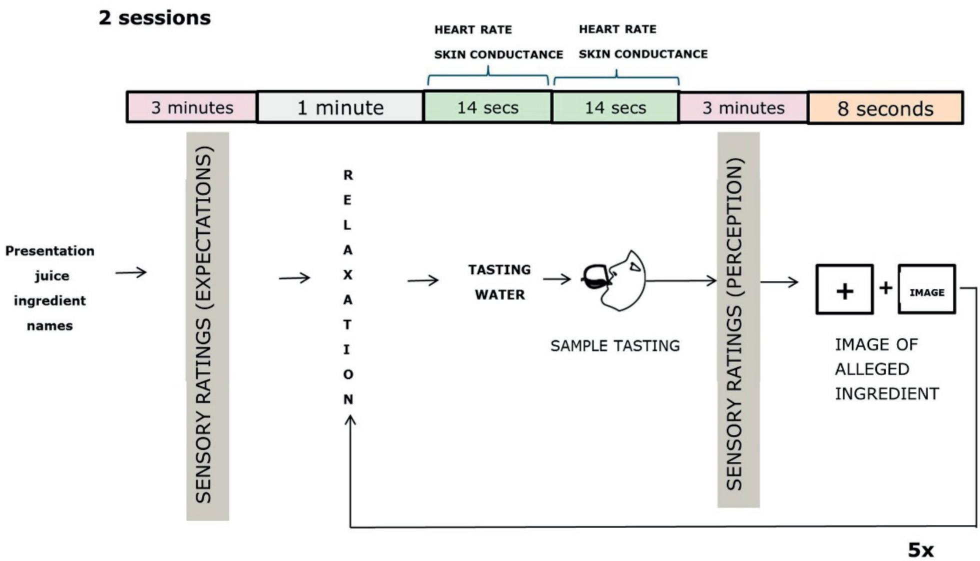


Figure 4.2. Study design for Condition B. The image corresponds to one tasting trial. The procedure shown was repeated for the five juices in each session.

The study consisted of tasting juice samples and observing the images of the alleged main ingredients of the juices. Participants were told that the aim of the research was to study the reactions to the juices. Participants were randomly assigned either to Condition A or to Condition B. For both conditions, participants attended two sessions: a Fruit Juice Session and a Vegetable Juice Session. These sessions were held on different days.

Each session consisted of tasting five juices and observing an image of the alleged main ingredient of the juice. Condition A and Condition B differed on the timing of the presentation of the images and juices (see **Figure 4.1** and **Figure 4.2**). In Condition A, participants tasted a water sample and thereafter were shown the image of the alleged main ingredient of the juice they were about to taste. After watching the image, participants were asked to fill out in 100-mm VAS scales their expectations about different sensory attributes of the juice, particularly the taste intensity, sweetness, bitterness, saltiness, sourness and pleasantness of the sample. Once participants finished the expectation ratings, they were given the juice sample and thereafter asked to rate in 100-mm VAS scales how they perceived the same sensory properties asked in the expectations ratings. An additional open-ended question was added in which they were asked to guess which was the real ingredient of the juice. This procedure was repeated for all five juices.

In Condition B, participants were presented first in text the name of various juice ingredients (including the five that would be given to taste) and asked to rate their expectations regarding the sensory properties of a juice with that ingredient, particularly those of pleasantness, taste intensity, sweetness, bitterness, saltiness, and sourness. This was done in order to have ratings performed in a similar situation as those of Condition A. That is, expectation ratings done before the consumption of the sample. The addition of names of other juice ingredients that would not be part of the tasting was done to prevent participants from guessing which would be the samples given to them to taste. After the expectation ratings, participants began with the tasting of the juices. Participants tasted a water sample and immediately after, they were given the juice sample. Once participants tasted the juice sample, they were asked to rate in 100-mm VAS scales how they perceived the same sensory properties asked in the expectations ratings and to guess which was the real ingredient of the sample. Thereafter, they were shown the image of the alleged main ingredient of the juice sample they had just tasted. This procedure was repeated for all five juices.

All images were presented for five seconds and preceded by a fixation cross that lasted three seconds. For both Condition A and Condition B, the images shown during the Fruit Juice Session were pears, apples, and oranges and the images shown during the Vegetable Juice Session were potatoes, celery, and carrot. The pears and potatoes images were shown three times in their corresponding session. To avoid any suspicion from these repeated images, participants were warned beforehand that they might see an image more than once because the study included juices of different brands. For each of these repeated images, participants then received the corresponding matching juice (pear or potato juice, depending on the session), a slightly different juice (apple or celery, respectively in order to create a small disconfirmation), or an extremely different juice (potato or pear, respectively in order to create a large disconfirmation). Two dummy tastings in each session involved showing orange and apple (in the fruit session), and carrots and celery (in the vegetable session) and receiving the corresponding matching juice (see **Table 4.2**).

Table 4.2. Juices given and ingredient images shown for each session and their level of disconfirmation.

Fruit juice session		
Ingredient image shown	**Real juice ingredient**	**Level of disconfirmation**
Pear	Pear	Confirmation
Orange	Orange	Confirmation (dummy)
Pear	Apple	Small disconfirmation
Apple	Apple	Confirmation (dummy)
Pear	Potato	Large disconfirmation
Vegetable juice session		
Ingredient image shown	**Real juice ingredient**	**Level of disconfirmation**
Potato	Potato	Confirmation
Carrot	Carrot	Confirmation (dummy)
Potato	Celery	Small disconfirmation
Celery	Celery	Confirmation (dummy)
Potato	Pear	Large disconfirmation

Before starting each juice tasting, participants were asked to remain still, to close their eyes, and to breathe normally for one minute. After tasting each juice, participants were asked to cleanse their palate with water and to make sure the taste of the juice was gone from their mouth before continuing with the next round. To ensure that they were familiar with the tasting procedure, participants practiced the tasting with a water sample before starting the rounds. After finishing the test, participants were debriefed. They were told the real nature of the samples given, why for some juices they were given a sample different from the one they were told and the main objectives of the study.

4.2.4. Physiological measurements

The VU-AMS version 3.9 (de Geus et al., 1995) was used for the heart rate and skin conductance measurements. The ECG had a sampling rate of 1000 Hz and heart rate was obtained from the time between two adjacent R waves. Skin conductance was sampled at a rate of 10 Hz with a signal range between 0-95 µs. The signal was filtered both in forward and reverse direction with a low-pass filter with a cut-off frequency of 2 Hz.

4.2.5. Data treatment and analysis

Heart rate and skin conductance responses were extracted with the VU DAMS program (version 3.9). These data were inspected for artifacts and labelled. For all juice samples tasted, the deviation from the baseline for heart rate (delta heart rate) and skin conductance (delta skin conductance) was calculated by subtracting the mean heart rate and skin conductance of the 14 seconds during the tasting of the water samples that preceded each juice to the mean heart rate and skin conductance of the 14 seconds during the tasting of the juice samples.

To test if the ANS responses capture the effects of a confirmation, small disconfirmation, and large disconfirmation of expectations and whether these depend on the type of juice tasted, a mixed model anova was performed on the data of Condition A considering subject as random factor, the variables juice tasted (fruit or vegetable), designed level of disconfirmation (confirmation [juice matches ingredient shown], small disconfirmation [juice has similar flavor to that of the ingredient shown], large disconfirmation [juice has an extremely different flavor to that of the ingredient shown]), attended session (first or second session), and the interaction between juice tasted and the level of (dis)confirmation as fixed factors. Taste intensity ratings from each juice were also included as a covariate as the intensity of a stimulus has been shown to have an effect in ANS responses, specifically skin conductance (Bensafi et al., 2002; Dawson et al., 2000; Verastegui-Tena et al., 2018).

To test if the ANS differed in Condition B (tasting without explicit information), a mixed model anova was performed stating subject as random factor and the variables juice tasted (fruit or vegetable) and attended session (first or second session) as fixed factors. Taste intensity ratings from each juice were also included as a covariate.

To test if ANS responses differed according to the condition, an additional analysis was performed in which the data of the juices that confirmed expectations and those that disconfirmed them in a small or large level in Condition A were merged to the data of the corresponding juice of Condition B. The delta heart rate and delta skin conductance from sessions 1 and 2 were analyzed together by means of a mixed model anova stating subject as random factor, the variables juice tasted (fruit or vegetable), condition (Condition A or Condition B) and the interaction between juice tasted and condition as fixed factors. Taste intensity ratings were also included as a covariate.

To test if the subjective ratings of the sensory properties capture the perception of a confirmation, small disconfirmation and large disconfirmation of expectations and whether the type of juice matters, from the data of Condition A the delta of each sensory property was calculated. For this delta, the expectation ratings were considered as a baseline and subtracted from the perception ratings. A mixed model anova for each sensory property was performed considering subject as random factor and the variables juice tasted (fruit or vegetable), designed level of (dis)confirmation (confirmation [juice matches ingredient shown], small disconfirmation [juice has similar flavor to that of the ingredient shown], large disconfirmation [juice has an extremely different flavor to that of the ingredient shown]) and the interaction between juice tasted and level of (dis)confirmation as fixed factors.

For all the models used in the analyses, the variables gender, age, and BMI were assessed in separate models and only added to the main one if their inclusion affected the general outcome of the model. Likewise, the variable guess (whether the ingredient was correctly or incorrectly guessed) was assessed and only added in the models when its effect was significant or when it affected the general outcome. Post hoc analyses were performed using Tukey's honest significance test (Tukey's HSD) for multiple comparisons.

4.3. Results

From the 86 participants recruited, two did not complete both sessions and had to be taken out of the study. From the 84 participants that completed the study, four were excluded due to missing data in their session files. One had to be excluded due to mistakes during the execution of study. Four participants presented a high number of ectopic beats in the labels used for analysis and were therefore excluded. In total, the data of 75 participants were used for the analysis, 41 females (mean age= 24.0 ±4.5, mean BMI=20.9±1.1) and 34 males (mean age= 24.8±4.0, mean BMI=22.0±1.8). The demographics divided by condition can be found in **Table 4.3**. No significant differences were observed between the participants in the two conditions.

Table 4.3. Demographics of the sample divided by group (N=75).

	Condition A	**Condition B**	***p*-value**
N	37	38	0.908 [a]
Gender: Female (Male)	20 (17)	21 (17)	0.999[a]
Age (years)	24.2 ±4.2	24.4 ±4.4	0.860[b]
BMI (kg/m^2)	21.2 ±1.4	21.6 ±1.7	0.310[b]

[a] *p*-value calculated with chi-square test.

[b] *p*-value calculated with Welch's t-test.

4.3.1. ANS responses when tasting samples designed to create a confirmation, small disconfirmation and large disconfirmation of expectations (manipulation of expectations)

The following section shows the results of the mixed model anova performed on the data of Condition A (manipulation of expectations) to test the effect of tasting samples designed to create a confirmation, small disconfirmation and large disconfirmation of expectations (see **Figure 4.3 A, B**).

Heart rate

There was no effect of the type of juice tasted (F(1,180)=3.32, *p*=0.070) nor of the designed level of (dis)confirmation (F(2,178)=1.18, *p*=0.341). Contrary to what was expected, the interaction between the type of juice tasted and the designed level of (dis)confirmation was not significant (F(2,178)=0.386, *p*=0.680). However, there was a significant effect of the attended session (F(1,178)=10.35, *p*=0.002). Post hoc tests revealed that the delta heart rate of participants was larger during the second session than the first.

Skin conductance

There was no effect of the type of juice tasted (F(1,181)=0.486, *p*=0.486) nor of the designed level of (dis)confirmation (F(2,178)=0.138, *p*=0.870). Likewise, there was no effect of the attended session (F(1,178)=0.214, *p*=0.643) and of the interaction between the type of juice tasted and the designed level of (dis)confirmation (F(2,178)=1.102, *p*=0.334). These results show that the delta skin conductance stays similar regardless of the juices tasted and the differences between the expectations raised and the actual juices.

4.3.2. ANS responses when tasting without explicit information

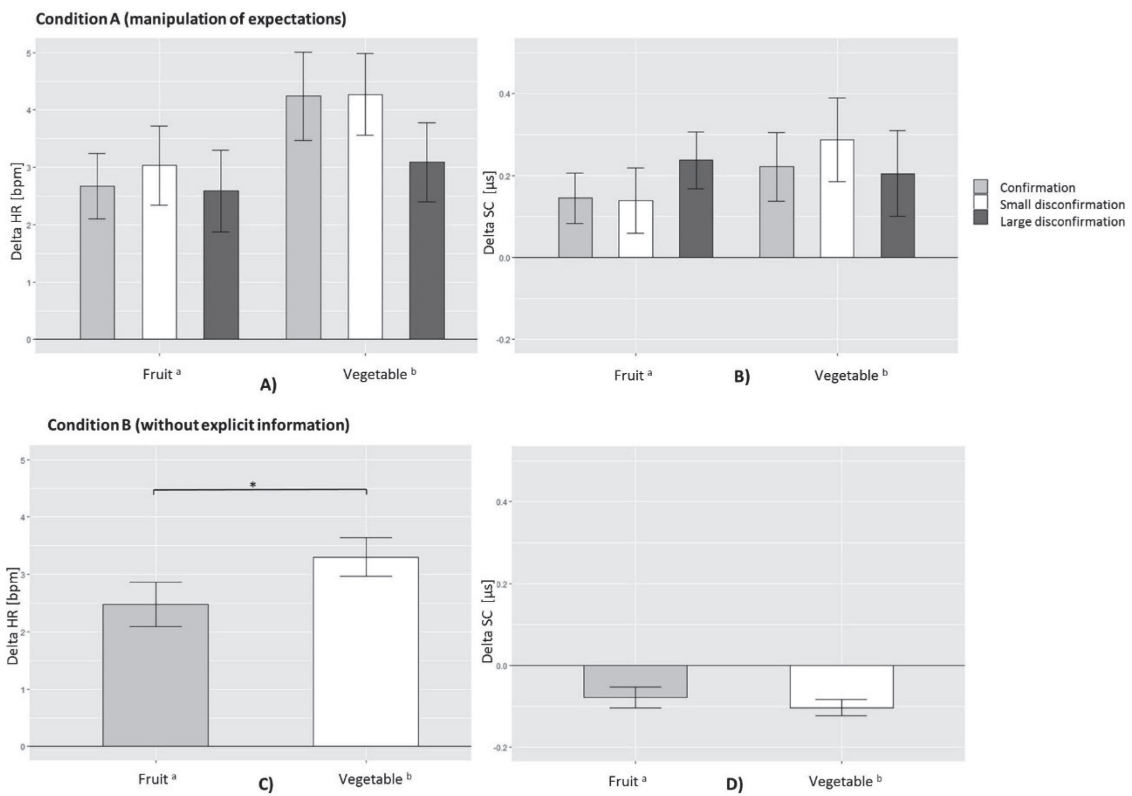
The following section shows the results of the mixed model anova performed on the data of Condition B. The purpose of this data is to show the changes in ANS responses when the juice samples are tasted without manipulating the expectations (see **Figure 4.3 C, D**).

Heart rate

There was an effect of the type of juice tasted (F(1,188)=4.16, *p*=0.043) but not of the attended session (F(1,187)=0.13, *p*=0.718). Post hoc tests revealed that the delta heart rate of participants was larger when tasting vegetable juices than when tasting fruit juices.

Skin conductance

There was no effect of the type of juice tasted (F(1,189)=0.886, *p*=0.347) nor of the attended session (F(1,188)=0.019, *p*=0.891). These results show that the delta skin conductance of participants stays the same regardless of the type of juice tasted.



[a] Combinations image shown-juice given: pear-pear (confirmation), pear-apple (small disconfirmation), pear-potato (large disconfirmation)

[b] Combinations image shown-juice given: potato-potato(confirmation), potato-celery (small disconfirmation), potato-potato (large disconfirmation)

Figure 4.3. Mean (± SE) of delta heart rate and skin conductance responses for Condition A (N=37; panels A and B respectively) and Condition B (N=38; panels C and D respectively).

4.3.3. Additional analysis: Differences in ANS responses depending on condition (tasting with manipulation of expectations vs. tasting without explicit information)

The following section shows the results of the mixed model anova for the effect of condition (manipulation of expectations vs. tasting without explicit information) on the ANS responses of each sample. A summary of the findings is stated in **Table 4.4**.

Tasting during confirmation of expectations vs. tasting without explicit information

Heart rate

There was an effect of juice tasted (F(1,76)=5.303, *p*=0.024) but no effect of condition (F(1,73)=0.563, *p*=0.455) nor of the interaction between the type of juice tasted and the condition (F(1,72)=0.192, *p*=0.662). Post hoc tests revealed that the delta heart rate of participants was higher for the vegetable juices than for the fruit ones.

Skin conductance

There was no effect of juice tasted (F(1,76)=0.051, *p*=0.821) but there was an effect for condition (F(1,74)=19.37, *p*<0.001). The interaction between the type of juice tasted and the condition (F(1,73)=1.047, *p*=0.310) was not significant. Post hoc tests revealed that the delta skin conductance of participants was higher during Condition A (tasting during confirmation of expectations) than during Condition B (tasting without explicit information).

Tasting during small disconfirmation of expectation vs. tasting without explicit information

Heart rate

There was no effect of juice tasted (F(1,72)=2.31, *p*=0.1322) nor of condition (F(1,75)=0.032, *p*=0.856). Likewise, the interaction between the type of juice tasted and the condition (F(1,72)=0.345, *p*=0.559) was not significant.

Skin conductance

There was no effect of juice tasted (F(1,73)=1.64, *p*=0.204) but there was an effect for condition (F(1,76)=15.86, *p*<0.001). The interaction between the type of juice tasted and the condition (F(1,73)=1.91, *p*=0.171) was not significant. Post hoc tests revealed that the delta skin conductance of participants was higher during Condition A (tasting during small disconfirmation of expectations) than during Condition B (tasting without explicit information).

Tasting during large disconfirmation of expectations vs. tasting without explicit information

Heart rate

There was no effect of juice tasted (F(1,79)=1.06, *p*=0.305) nor of condition (F(1,74)=0.162, *p*=0.6882). Likewise, the interaction between the type of juice tasted and the condition (F(1,73)=0.694, *p*=0.407) was not significant.

Skin conductance

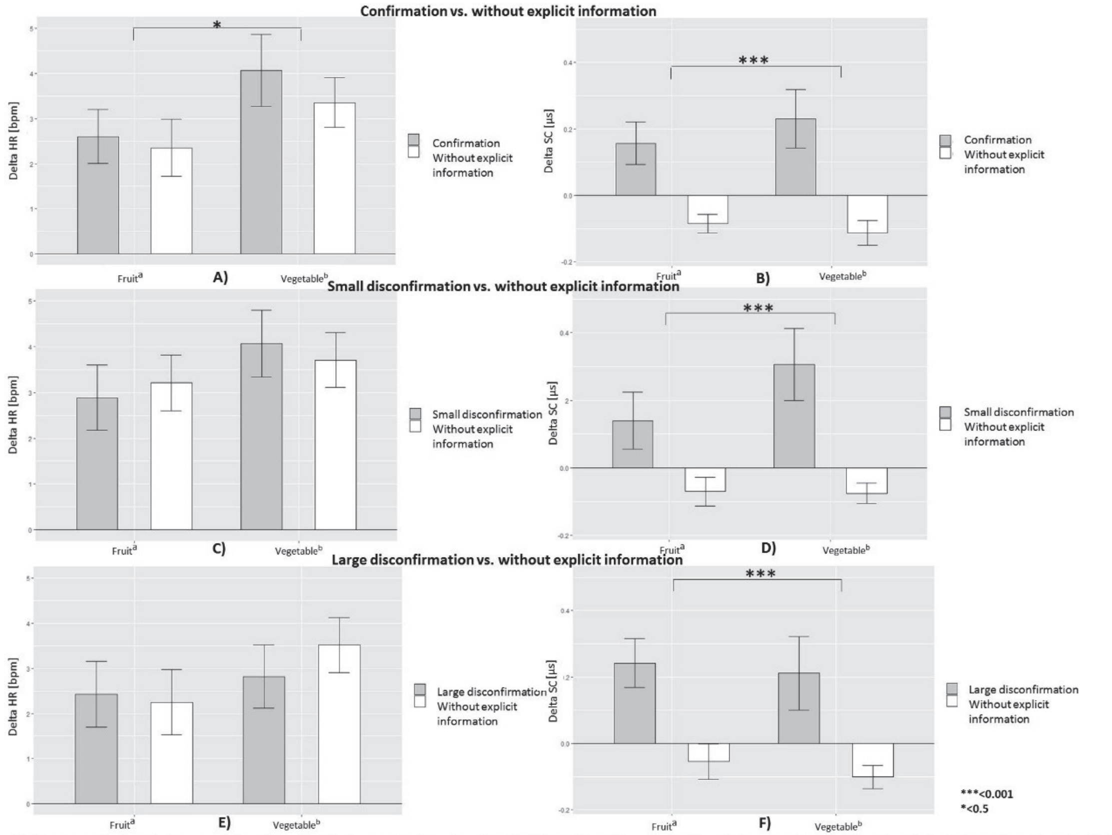
There was no effect of juice tasted (F(1,79)=1.52, *p*=0.22) but there was an effect for condition (F(1,74)=13.8, *p*<0.001). The interaction between the type of juice tasted and the condition (F(1,73)=0.001, *p*=0.969) was not significant. Post hoc tests revealed that the delta skin conductance of participants was higher during Condition A (tasting during large disconfirmation of expectations) than during Condition B (tasting without explicit information).

Table 4.4. Results of mixed model anova considering juice tasted, condition, and the interaction juice tasted: condition (N=75).

	Juice tasted			Condition			Juice tasted* condition		
	df	F	*p*	df	F	*p*	df	F	*p*
Confirmation vs without explicit information									
Delta heart rate (bpm)	1	5.303	0.024*	1	0.563	0.455	1	0.192	0.662
Delta Skin conductance (μs)	1	0.051	0.821	1	19.37	<0.001***	1	1.047	0.310
Small disconfirmation vs without explicit information									
Delta heart rate (bpm)	1	2.31	0.132	1	0.032	0.856	1	0.345	0.559
Delta Skin conductance (μs)	1	1.64	0.204	1	15.86	<0.001***	1	1.91	0.171
Large disconfirmation vs without explicit information									
Delta heart rate (bpm)	1	1.06	0.305	1	0.162	0.688	1	0.694	0.407
Delta Skin conductance (μs)	1	1.52	0.220	1	13.8	<0.001***	1	0.001	0.969

*Significance at *p*<0.05.

***Significance at *p*<0.001.



[a] Fruit: pear-pear (confirmation), pear-apple (small disconfirmation), pear-potato (large disconfirmation); [b] Vegetable: potato-potato (confirmation), potato-celery (small disconfirmation), potato-pear (large disconfirmation)

Figure 4.4. Mean (± SE) of delta heart rate and skin conductance responses when tasting samples designed to create a confirmation, small disconfirmation, and large disconfirmation of expectations vs. tasting without explicit information (N=75).

4.3.4. Subjective sensory ratings for samples designed to create a confirmation, small disconfirmation, and large disconfirmation of expectations

The following section shows the results for the mixed model anova done on the data of the sensory ratings in Condition A using the delta of each attribute. This delta consists of the difference between the sensory ratings derived from the expectations (ratings after seeing the image of the juice ingredient but before tasting the juice) and the sensory ratings after tasting each juice (see **Figure 4.5**). The purpose of this analysis is to verify whether the designed confirmation, small disconfirmation, and large disconfirmation were perceived and reported by participants and that the findings or lack thereof of the ANS responses can be explained in the terms of the manipulation of expectations. **Table 4.5-A** and **Table 4.5-B** show the mean ratings for each attribute given before (expected) and after (perceived) tasting each sample.

Bitterness

There was a significant main effect of the type of juice tasted ($F(1,180)=58.50$, $p<0.001$) and of the interaction between type of juice tasted and (dis)confirmation ($F(2,180)=31.02$, $p<0.001$). There was no main effect of the level of (dis)confirmation ($F(1,180)=2.22$, $p=0.110$). Post hoc tests revealed that the delta of the bitterness ratings for both fruit and vegetable juices were similar between juices designed to create a confirmation and the juice from the same group designed to create a small disconfirmation. In addition to this, the ratings for the juices designed to create a confirmation were significantly different from those for the same juice when designed to create a large disconfirmation.

Sweetness

There was a significant main effect of the type of juice tasted ($F(1,216)=191.07$, $p<0.001$) and of the interaction between type of juice tasted and type of (dis)confirmation ($F(2,216)=146.48$, $p<0.001$). There was no main effect of the level of (dis)confirmation ($F(1,216)=0.71$, $p=0.490$). Post hoc tests revealed that the delta of the sweetness ratings for both fruit and vegetable juices were similar between juices designed to create a confirmation and the juice from the same group designed to create a small disconfirmation. In addition to this, the ratings for juices creating a confirmation were significantly different from those for the same juice when designed to create a large disconfirmation.

Saltiness

There was a significant main effect of the type of juice tasted (F(1,180)=97.47, *p*<0.001) and of the interaction between type of juice tasted and (dis)confirmation (F(2,180)=22.84, *p*<0.001). There was no main effect of the level of (dis)confirmation (F(1,180)=1.46, *p*=0.233). Post hoc tests revealed that the delta of the saltiness ratings for both fruit and vegetable juices were similar between juices designed to create a confirmation and the juice from the same group designed to create a small disconfirmation of expectations. In addition to this, juices designed to create a confirmation were significantly different from those for the same juice when designed to create a large disconfirmation.

Sourness

There was a significant main effect of the type of juice tasted (F(1,180)=35.17, *p*<0.001) and of the interaction between type of juice tasted and (dis)confirmation (F(2,180)=6.336 *p*=0.002). Likewise, there was an effect of the level of (dis)confirmation (F(2,180)=3.92, *p*=0.021). Post hoc tests revealed that most of the differences were between fruit and vegetable juices but that these differences did not follow a logical pattern related to the level of disconfirmation. Ratings for both fruit and vegetable juices were similar between juices designed to create a confirmation and the juice from the same group designed to create a small disconfirmation of expectations. However, the same was true for the juices designed to create a confirmation compared to when they were designed to make a large disconfirmation. Hence, participants were not able to report clear differences in sourness related to the level of disconfirmation.

Pleasantness

There was a significant main effect of the type of juice tasted (F(1,216)=135.42, *p*<0.001) and of the interaction between type of juice tasted and disconfirmation (F(2,216)=175.54, *p*<0.001). There was no main effect of the level of disconfirmation (F(2,216)=1.71, *p*=0.170). Post hoc tests revealed that the delta of the pleasantness ratings for both fruit and vegetable juices were similar between juices creating congruency and assimilation. In addition to this, juices designed to create a confirmation were significantly different from those for the same juice when designed to create a large disconfirmation.

Taste intensity

There was a significant effect of the type of juice tasted (F(1,180)=17.54, *p*<0.001). There was no effect of the interaction between type of juice tasted and (dis)confirmation (F(2,180)=1.62, *p*=1.199) nor of the level of (dis)confirmation (F(2,216)=2.51, *p*=0.08). Post hoc tests revealed that the delta of the intensity ratings was larger for the vegetable juices than for the fruit juices. This shows that the taste intensity ratings can only capture the perceived differences between fruit and vegetable juices but none related to the level of disconfirmation.

Table 4.5-A. Mean expected and perceived sensory ratings for the attributes bitterness, sweetness, saltiness, sourness, pleasantness, and taste intensity for fruit juices with varying levels of disconfirmation in Condition A (N=37).

Fruit juice session							
Attributes**		**Confirmation (pear-pear)**	***p*-value***	**Small disconfirmation (pear-apple)**	***p*-value***	**Large disconfirmation (pear-potato)**	***p*-value***
Bitterness	Expected	7.7±12.8	0.999	10.8±18.7	0.999	7.5±12.6	<0.001
	Perceived	7.4±15.9		8.2±17.5		47.0±30.7	
Sweetness	Expected	71.7±18.5	0.999	67.5±20.6	0.964	76.2±13.8	<0.001
	Perceived	69.3±20.6		71.7±20.2		12.5±13.3	
Saltiness	Expected	6.1±12.5	0.999	4.0±6.5	0.999	7.6±12.2	<0.001
	Perceived	4.3±9.1		5.9±14.7		45.8±29.1	
Sourness	Expected	19.4±20.5	0.999	18.1±19.4	0.027	24.0±23.4	0.788
	Perceived	23.8±24.7		37.2±26.6		50.8±25.6	
Pleasantness	Expected	80.0±15.0	0.844	76.3 ±17.5	0.999	77.8±15.5	<0.001
	Perceived	70.8±21.2		71.6±21.5		14.2±17.2	
Taste intensity	Expected	60.8±22.7	0.999	61.3±24.0	0.865	66.0±19.3	0.999
	Perceived	61.6±24.9		68.4±20.4		75.9±19.3	

**p*-values obtained through Tukey's HSD test.

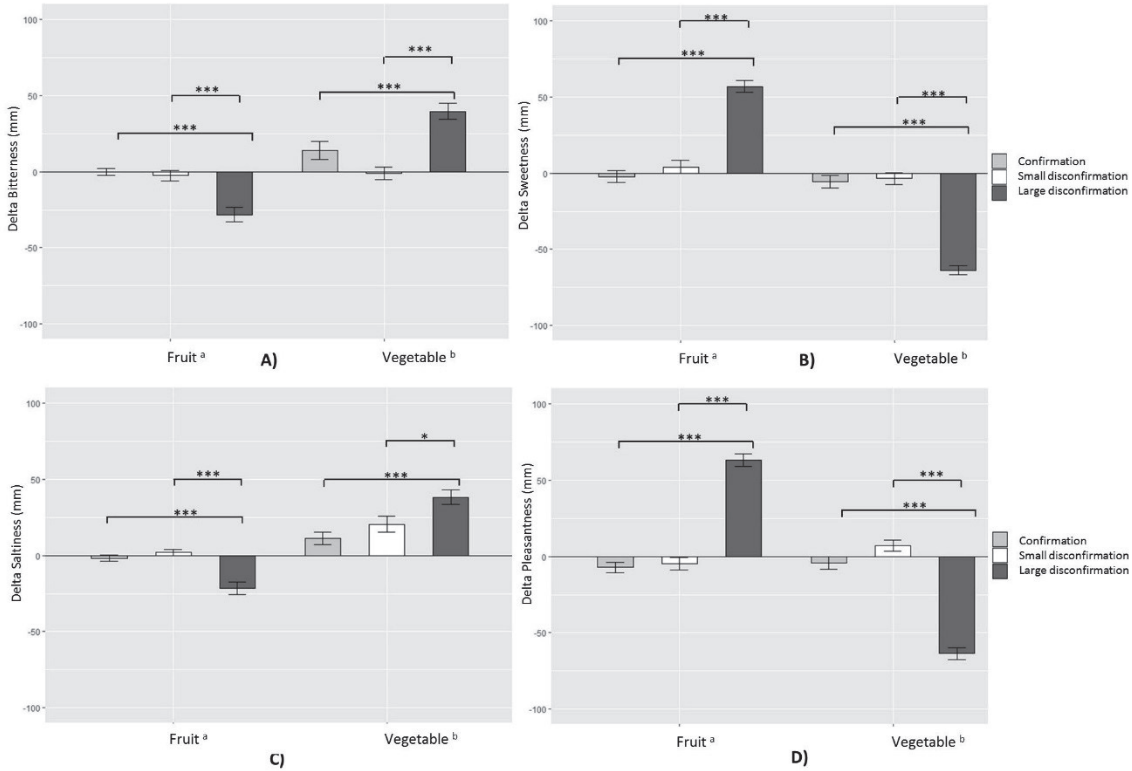
**Attributes were rated in 100-mm VAS scales.

Table 4.5-B. Mean expected and perceived sensory ratings for the attributes bitterness, sweetness, saltiness, sourness, pleasantness, and taste intensity for vegetable juices with varying levels of disconfirmation in Condition A (N=37).

Vegetable juice session							
Attributes**		**Confirmation (potato-potato)**	***p*-value***	**Small disconfirmation (potato-celery)**	***p*-value***	**Large disconfirmation (potato-pear)**	***p*-value***
Bitterness	Expected	31.14±27.5	0.039	34.6±24.8	0.999	34.2±28.0	<0.001
	Perceived	45.18±31.72		33.6±28.33		5.9±7.9	
Sweetness	Expected	19.8±22.0	0.825	20.2±19.2	0.999	21.8±20.9	<0.001
	Perceived	14.2±22.2		16.7±18.8		78.6±15.2	
Saltiness	Expected	28.8±23.3	0.044	25.1±23.0	<0.001	29.9±24.0	<0.001
	Perceived	40.0±24.6		45.6±29.9		8.2±11.3	
Sourness	Expected	22.4±25.0	<0.001	29.1±29.6	<0.001	35.5±31.2	<0.001
	Perceived	56.6±30.6		52.0±29.3		25.8±25.5	
Pleasantness	Expected	19.3±19.7	0.912	16.1±16.4	0.777	16.9±21.7	<0.001
	Perceived	14.9±20.8		23.3+21.1		79.9+14.8	
Taste intensity	Expected	48.6±28.5	<0.001	49.5±25.9	<0.001	56.0±30.0	0.663
	Perceived	74.87±18.7		71.9±18.9		60.6±24.9	

**p*-values obtained through Tukey's HSD test.

**Attributes were rated in 100-mm VAS scales.



[a] Combinations image shown-juice given: pear-pear (confirmation), pear-apple (small disconfirmation), pear-potato (large disconfirmation)

[b] Combinations image shown-juice given: potato-potato(confirmation), potato-celery (small disconfirmation), potato-potato (large disconfirmation)

Figure 4.5. Mean (± SE) of the delta of the bitterness (A), sweetness (B), saltiness (C), and pleasantness (D) ratings for samples designed to create a confirmation, small disconfirmation, and large disconfirmation (N=37). The labels in the x axis show the type of juice tasted.

4.3.5. Complementary information: Percentage of correct, assimilated incorrect, and incorrect guesses during the manipulation of expectations

Table 4.6 shows the percentage of correct, assimilated (participants report the flavor of the image shown when tasting the sample with a small disconfirmation), and incorrect guesses for each level of designed (dis)confirmation. Participants guessed the majority of the times the ingredient correctly when there was no disconfirmation between the taste of the juice and the image of the ingredient. In the case of a large disconfirmation, a higher percentage of participants guessed the ingredient incorrectly for the vegetable juices while for the fruit juices the proportion of correct and incorrect guesses were similar. Likewise, in the case of a small disconfirmation for the fruit juices, a higher percentage of participants had assimilated guesses while for the vegetable juices there were more incorrect guesses. This shows that vegetable juices were more difficult to recognize than fruit juices.

Table 4.6. Percentage of correct and incorrect guesses of the juice ingredients and designed level of disconfirmation. Data of Condition A (N=37).

Session	Designed level of disconfirmation	Correct guesses (real flavor)	Assimilated guesses (flavor of image)	Incorrect guesses	*p*-value*
Fruit session	Taste matches image (pear-pear)	91.9%	0.00%	8.1%	<0.001
	Small disconfirmation between taste and image (pear-apple)	29.7%	64.7%	5.4%	0.04
	Large disconfirmation between taste and image (pear-potato)	46.0%	8.0%	46.0%	0.005
Vegetable session	Taste matches image (potato-potato)	83.8%	0.00%	16.2%	<0.001
	Small disconfirmation between taste and image (potato-celery)	27.0%	29.7%	43.3%	0.432
	Large disconfirmation between taste and image (potato-pear)	35.1%	5.4%	59.5%	<0.001

**p*-values calculated with chi-square test.

4.4. Discussion

The aim of the present study was to evaluate how ANS responses changed when tasting samples that create a confirmation, small disconfirmation and large disconfirmation of expectations and whether these ANS responses were different from those when there is no manipulation of expectations. In addition to this, we checked whether the perception of the different levels of disconfirmation was reported subjectively through sensory ratings collected before and after tasting each sample. For this purpose, ingredients were shown to create specific expectations, and juices that matched or mismatched the ingredient information were provided to taste. The results obtained from this study could not confirm our initial hypotheses regarding ANS responses. The main finding in this study is that, while the design was able to create a confirmation, small disconfirmation, and large disconfirmation of both sensory and hedonic expectations according to the self-reported ratings, the effects captured by ANS responses seem to be related to other factors. We further discuss these findings in the paragraphs below.

When expectations were explicitly manipulated (Condition A), heart rate responses were not able to capture a direct effect related to the level of (dis)confirmation, as had been expected. These responses, however, showed differences related to the session. The increase in delta heart rate was larger during the participants' second session than during the first. It could be that during the first session there was a cardiac deceleration involved that made the increase smaller. Heart rate deceleration points to the orienting response (OR) and sensory intake (Bradley, 2009). When the OR occurs, the sensitivity to stimulation is enhanced. The OR can be related to both novelty or to reactive involuntary attention to a change (DeGangi, 2012). In this case, the deceleration and subsequent smaller increase in the first session might be pointing to participants dealing with more new factors than in the second. While both sessions dealt with the taste of the sample and its (dis)confirmation with the ingredient mentioned before, the first session also dealt with the novelty of the design (first experience with the design).

When expectations were not explicitly manipulated (Condition B), heart rate responses differed according to the type of juice tasted (fruit vs vegetable). The same effect was found when comparing directly the heart rate responses in situations in which there is no explicit information to those of the same juices when designed to create a confirmation. This is most likely because the focus in both lay solely on the taste of the sample as there was no disconfirmation to be processed. Therefore, it is likely that a disconfirmation in the design, such as the one found in most samples of Condition A, requires participants to use more resources to enhance the sensory processing. This prevents any heart rate differences related to other factors to be captured.

Skin conductance responses remained similar across juice samples in both Condition A and Condition B but differed only according to the condition in which participants were placed. The skin conductance responses in Condition A showed an increase from baseline while those in Condition B showed a decrease. An increase in skin conductance is linked to arousal and higher attentiveness. Attention in itself could also be seen as a state similar to arousal such that when subjects pay attention they respond to more stimuli (Frith & Allen, 1983). It is possible that the skin conductance responses are capturing a state of heightened arousal related to the increase in attention necessary for processing the information related to the (dis)confirmation of expectations of Condition A.

Our findings on skin conductance in Condition B differ from those of previous studies. The studies by Rousmans et al. (2000), Danner et al. (2014), and de Wijk et al. (2012) found changes in skin conductance between stimuli that differed in liking while in our case they remained similar. However, in the case of the study of de Wijk et al., the stimuli were solid foods and hence were different from those used in our study. Our findings on heart rate match those from Rousmans et al. (2000). Both studies found lower heart rates for the pleasant solutions. However, there is still a difference in stimuli as Rousmans's study used solutions with basic tastes and ours used juices. The study by Danner et al. (2014) used, same as in our study, fruit and vegetable juices. However, our results do not match those of Danner's study. They found differences in skin conductance but not in heart rate responses. However, there are differences in the design that may account for these dissimilarities. Danner et al. (2014) did not use a water sample as a baseline measurement and the juice stimuli used included different hedonic ranges within the same category (mean liking on a 9-point hedonic scale for banana: 7.41±0.18, grapefruit: 3.19±0.24, orange: 6.43±0.20, mixed vegetable: 3.32±0.26, fermented cabbage: 2.62+0.24), while in our study the pleasant juices were the fruit juices (mean liking in a 100-mm VAS scale for pear: 70.8±21.2 , apple: 71.6±21.5) and the unpleasant juices were the vegetable ones (mean liking in a 100-mm VAS scale for potato: 14.9±20.8, celery: 23.27+21.1).

We did not find any differences in the magnitude of the heart rate and skin conductance responses related to whether the disconfirmation was positive or negative. Likewise, we did not find an asymmetry in the sensory ratings related to the type of disconfirmation. The magnitude of the differences in our study was similar in both deltas for all sensory ratings and only the direction changed. This means that both the positive and negative disconfirmation were reported as equally sensed by the participants. Therefore, as the positive disconfirmations (gains) did not give smaller responses than negative disconfirmations (losses), we were not able to confirm the processes described in Kahneman and Tversky's Prospect theory through the explicit (sensory ratings) and implicit (ANS responses) measurements used in this study (Kahneman & Tversky, 1979).

Most sensory ratings in this study show that participants perceived the disconfirmations accordingly. Moreover, these ratings seem to point to assimilation and contrast as a product of the designed levels of disconfirmation. The bitterness, sweetness, saltiness, and pleasantness ratings were similar for both fruit and vegetable juices that were designed to confirm expectations and those that created small disconfirmations. Hence it is possible that there was assimilation as the level of disconfirmation was small enough for participants to ignore this deviation and rate the juices similarly to what had been expected from the image ingredient (Schifferstein, 2013). An apparent contrast can be found through the significant differences between fruit and vegetable juices when designed to create a confirmation and when designed to create a large disconfirmation. Even though the fruit juices were identical, they were rated as sweeter, less salty, less bitter, and more pleasant when a vegetable taste was expected (positive disconfirmation) than when the matching fruit taste was expected. Likewise, vegetable juices were rated as less sweet, more salty, more bitter, and less pleasant when a fruit taste was expected (negative disconfirmation) than when the matching vegetable taste was expected (see **Table 4.5-A** and **Table 4.5-B**). This shows that in large disconfirmation situations, the expectations and perception ratings go in different directions as is the case when contrast occurs (Piqueras-Fiszman & Spence, 2015).

Sourness and taste intensity ratings showed different patterns from those of the attributes mentioned in the previous paragraph. This was already expected as fruit and vegetable juices are opposites in bitterness, sweetness, saltiness, and pleasantness but differences in sourness and taste intensity might not be as easy to perceive and report and could have been influenced by other factors of the design. The delta ratings of taste intensity when tasting samples that created large disconfirmations were similar to those of the same samples when they confirmed expectations. It might be that the incongruency of the juice made participants adapt their taste intensity ratings to that of their expectations ("the vegetable taste is as intense as I expected the fruit taste to be"). The sourness ratings were similar for both fruit and vegetable juices when the same juices were tasted when creating a confirmation and a large disconfirmation of expectations. Fruit juices are generally acidic (Birkhed, 1984). Likewise, the vegetable juices used in this study were produced through lactic fermentation. Lactic acid fermentation decreases the pH of a product which results in an increase in sourness (McFeeters, 2004). It is possible that in both cases, the deltas reflect the difference between the sourness of an expected fresh product (shown in the image) and that of the perceived processed juice and hence no difference related to the mismatch from the group expected (fruit or vegetable) was seen.

There are certain factors in our study that might have influenced our results. Due to the design of the study, we preferred not to use the same participants for Condition A and Condition B. As the physiological responses differ across subjects, it is difficult to know whether the same results would be found if all participants had been subjected to the tasting without explicit information and the tasting during the manipulation of expectations. Secondly, while the sensory ratings seem to point to assimilation and contrast, we cannot confirm with certainty that the designed disconfirmations led indeed to these processes. This applies particularly to the case of the juices designed to create a small disconfirmation of expectations (and which could lead to assimilation). The juices were similar to those designed to create a confirmation of expectations in various attributes, including the ones rated. It is difficult to know whether the lack of differences in the subjective ratings between these juices is due to the general similarities or due to assimilation itself. Lastly, we did not ask participants to rate the familiarity of the juices. Given that we have participants from different nationalities, it is possible that the fact that certain fruits and vegetables are more often consumed in certain countries could have influenced our results. For example, unfamiliar foods have been found to be perceived as riskier than familiar foods (Fischer & Frewer, 2009). Hence, if participants found one of the ingredients as unfamiliar their response is likely to be influenced by the arousal caused by the perceived risk.

The findings of this study provide more information on how the effects of confirmation and different levels of disconfirmations affect ANS responses. The ANS responses in this study seem to capture the effects related to the reaction to the general situation (disconfirmation=enhanced attention) rather than to the level of disconfirmation in the stimuli itself. Further research is necessary to discern how to use ANS measurements in food research. For example, it might be that ANS responses can only capture effects when a stimulus is extremely unfamiliar or poses a threat, as attention will more likely happen if a stimuli is significant to the self (Lacey & Lacey, 2007). Moreover, such stimuli could enhance defensive responses which can be captured by skin conductance (Bradley & Lang, 2007).

4.5. Conclusion

The present work evaluated for the first time the potential of ANS responses to capture the effects of tasting samples designed to create a confirmation, small disconfirmation, and large disconfirmation of expectations. It additionally evaluated whether these responses differ to those obtained when expectations are not manipulated. Our results point to differences in heart rate and skin conductance that seem to be related to the orientation response and attention but no differences were found according to the level of disconfirmation between stimuli. Further research is necessary to confirm these findings and to determine the effects within a study related to food research that ANS are capable of measuring.

CHAPTER 5

Heart rate and skin conductance responses during the creation and (dis)confirmation of food expectations: Replicability and the link with arousal and attention.

This chapter has been submitted as *Verastegui-Tena, L., van Trijp, H., & Piqueras-Fiszman, B. (2019). Heart rate and skin conductance responses during the creation and disconfirmation of expectations: Replicability and the link with arousal and attention.*

Abstract

Expectations are important for the information and sensory processing of food products. Their creation and (dis)confirmation may lead to heightened attention and arousal, which enhances the perceptual processing of a product. The responses of the autonomic nervous system (ANS) could be used to study this interaction. Cardiac deceleration may capture attention while skin conductance increases may capture attention or arousal. Our aim was, therefore, to evaluate if the creation and (dis)confirmation of expectations lead to ANS responses related to attention and arousal and whether they can be replicated.

Seventy-three participants tasted five samples, three of which were identical. Participants were shown the main ingredient of the sample either before or after tasting. For the identical samples, three images were shown: worms, chocolate, and soy. After the tasting, a manipulation check was done in which participants saw images from the International Affective Picture System (IAPS). Finally, to evaluate if images that attracted attention matched those with different ANS responses, participants did a dot probe task with the ingredient images. Heart rate and skin conductance were measured. The dot probe task showed no changes in attention between images. The creation and (dis)confirmation of expectations when observing ingredient images led to similar ANS responses. Cardiac deceleration was similar for all images. Skin conductance had a significantly different increase for the worms than for the soy and chocolate. Heart rate remained similar during the tasting of the samples but skin conductance increases were larger when participants believed the sample had worms. The observation of IAPS images showed that skin conductance discriminated images differing in arousal.

In conclusion, skin conductance captures differences in arousal when expectations are created or disconfirmed and has good replicability. It was not possible to evaluate changes in attention as heart rate responses and attentional bias scores remained similar between images.

5.1. Introduction

Expectations are an important factor in the human-food interaction (van der Laan et al., 2011). They can evoke a variety of responses in people which can have an influence in the subsequent judgement of foods (Piqueras-Fiszman & Spence, 2015). These responses can be physiological or cognitive and are indices of attention and orienting organized by defensive and appetitive systems. Defense and appetitive systems initiate sensory processes such as the enhancement of perceptual processing but also motor processes such as the preparation of action. They serve as the basis for the pleasant or aversive responses towards foods (Bradley, 2009). Expectations are, therefore, important for the information and sensory processing and the subsequent acceptance or rejection of a food product (Sinke, Forkmann, Schmidt, Wiech, & Bingel, 2016).

Expectations (both its creation and (dis)confirmation) can lead to attention, which is one of the fundamental aspects for the in-depth processing of sensory information. The creation of expectations involves the anticipation of events, which leads to attention for perceptual preparation but also to arousal for motor preparation (Balkenius, Förster, Johansson, & Thorsteinsdottir, 2008). The disconfirmation of expectations leads to reactive attention (Balkenius et al., 2008). The disconfirmation is seen as a signal towards a relevant stimulus and helps the stimulus be detected more rapidly and accurately and perceived more vividly (Summerfield & Egner, 2009; Thigpen, Gruss, Garcia, Herring, & Keil, 2018). Both positive and negative disconfirmations have the potential to attract attention. However, extreme disconfirmations are usually more attention-grabbing especially if they point to a threat (Starzomska, 2017). Extreme disconfirmations will also lead to stronger responses from individuals. The difficulty of resolving the incongruity will stimulate arousal, which will be higher than in situations in which the disconfirmations are moderate or low. This same arousal is believed to motivate individuals into engaging more with the stimulus and pay more attention (Noseworthy et al., 2014). Attention and arousal, therefore, interact closely during the creation and (dis)confirmation of expectations.

Attention can be measured through different tools such as attention tasks, physiological and neurological responses. Among the tasks intended to measure attention, the dot probe task is deemed a superior and direct test. It is considered more effective than other available tests such as the Stroop color naming task (Starzomska, 2017). Physiological responses for the measurement of attention include responses of the autonomic nervous system (ANS) such as pupil size, muscular tension, and electrodermal and cardiac responses (Cohen & O' Donnell, 2013). Heart rate deceleration in particular has been found to be linked to stimuli that attract attention while heart rate acceleration is linked to rejection of stimuli (Lacey & Lacey, 2007). Skin conductance is considered the best index of modulation of the states of bodily arousal in emotional, cognitive and behavioral situations. Skin conductance has also been linked to attention. An increase in skin conductance has been suggested to reflect the processing of information. The more attention given to a particular stimuli the higher the skin conductance (Frith & Allen, 1983). Skin conductance increase, however, could also be a marker of rejection triggered by a defensive response. In order to correctly discriminate both responses it is recommended to examine the heart rate response: if there is a cardiac deceleration then the skin conductance is related to orientation, attention and sensory processing while if the increase is accompanied by a cardiac acceleration it will point to a defense response (Verschuere, Crombez, Smolders, & De Clercq, 2009).

ANS responses may have the potential to capture the changes in attention and arousal related to the creation and (dis)confirmation of food expectations. Studies that show the changes in ANS responses from the processing of information when subjects are exposed to food or food cues have shown various results. De Wijk et al (2012) found increases in skin conductance when observing liked foods compared to when observing disliked foods. Heart rate responses first increased and then gradually decreased but the pattern remained similar for both liked and disliked foods. Drobes et al. (2001) found that observing pleasant, unpleasant, and food visual cues led to a decrease in heart rate compared to baseline but that the difference in the deceleration between valenced pictures and food pictures was significantly less when subjects had been food deprived for 24 hrs. Skin conductance increased for all pictures with the pleasant and unpleasant pictures leading to the highest increases; however, subjects that were food deprived showed no differences in skin conductance. Overduin and Jansen (1996) found there was a slight increase from baseline of heart rate and skin conductance responses when smelling and observing foods but that these responses did not differ from those when smelling and observing soap. It is still not clear if the responses obtained in these studies actually represent attention and arousal. Most studies link them with dimensions such as valence. Furthermore, they do not consider whether the expectations regarding the foods shown might have affected the sensory processing and the subsequent ANS responses obtained. The few studies dealing with food expectations and ANS responses have found that the creation and disconfirmation of expectations led to stronger heart rate decelerations when observing visual stimuli (Verastegui-Tena et al., 2017). Likewise, the tasting of incongruent stimuli led to a cardiac deceleration. These results might point to attentional resources being activated by the anticipation and disconfirmation. Skin conductance increases have only been seen for the creation and disconfirmation of negative visual stimuli (Verastegui-Tena et al., 2017; Verastegui-Tena et al., 2018). All these findings, however, have not yet been tested for their replicability. Results on ANS responses have been found to be contradictory across studies which may also explain the lack of clarity regarding the processes captured by them (Cacioppo et al., 2000).

To the best of our knowledge, studies have not looked further at the link between attention, arousal, and the ANS responses derived from expectations. Furthermore, it remains yet to be seen whether the attention and arousal from a disconfirmation can make identical stimuli lead to different ANS responses. Given that attention to specific properties can affect the consumption experience of a food, it is possible that enhanced attention derived from a disconfirmation can lead to the same effect (van Rijn, de Graaf, & Smeets, 2018). For this purpose, the aim of this study is to evaluate whether stimuli that (dis)confirm expectations lead to ANS responses that point to attention and whether these same stimuli match the stimuli to which attention is allocated during an attention task and to the stimuli with the highest skin conductance increases. Likewise, we examine if the creation of expectations evokes the same attention and arousal as the (dis)confirmation of expectations. Moreover, we examine if ANS responses when tasting identical samples change if they are coupled with visual stimuli of different valence that manipulate taste expectations. We additionally assess how these responses differ from ANS responses when taste expectations are not manipulated. Finally, we examine if ANS responses are reproducible by comparing the results obtained here to those of the first study in Chapter 2, which used the same stimuli.

Our hypotheses are that a) visual stimuli that disconfirm expectations will lead to higher attention and sensory processing and, as a result, stronger cardiac decelerations and faster reaction times in an attention task; b) visual stimuli that show stronger cardiac deceleration will also show higher increases in skin conductance; c) Identical samples will lead to different ANS responses when participants taste them while believing that the taste matches what was depicted by the visual stimuli. If the corresponding visual stimulus–sample taste combination disconfirmed expectations, there will be a stronger cardiac deceleration and skin conductance increase than when the expectations are confirmed; d) identical samples will lead to similar ANS responses when expectations are not manipulated; e) ANS responses related to visual stimuli will match those from the first study in Chapter 2 in which the same stimuli were used.

5.2. Materials and methods

5.2.1. Recruitment and selection of participants

Ninety-five people between 18 and 45 years old were recruited from the Wageningen area and surroundings. Participants were recruited through flyers in the university area and dorm rooms as well as through advertisements in social media. Participants were excluded if they smoked, had a BMI higher than 24.9 kg/m2, had any attention or visual impairments, if they were allergic to any of the ingredients of the samples, and if they were vegan or consumed frequently plant-based dairy substitutes. Participants coming from Asian countries were also excluded, as they might be more familiar to the taste of the samples. Participants were given a summary of the procedure of the study and asked to schedule an appointment if they were interested in participating. The Social Sciences Ethics Committee of Wageningen University approved the study.

5.2.2. Experiment procedure

The study took place in the premises of the Marketing and Consumer Behaviour group of Wageningen University in a well-lit room with a computer that contained the software Open Sesame version 3.2 (Mathôt et al., 2012). Participants were asked to abstain from eating and drinking (except for water) one hour before taking part in the study and to wear comfortable clothes. Upon arrival, participants were given an informed consent and, after this was signed, the researcher began placing the sensor pads.

Seven sensor pads were placed for the measurement of the cardiac responses. Five on the chest and two on the back. For the measurement of skin conductance two sensors were placed in the index and middle finger of the non-dominant hand. After placing all electrodes, participants were asked to sit in front of a computer where the researcher checked all signals and where the computer tasks would take place.

The study consisted of three parts. The first part was a tasting session that followed a design similar to the one from the first study in Chapter 2 (Verastegui-Tena et al., 2017). Participants were divided in two conditions (see **Figure 5.1** and **Figure 5.2**). For both conditions, participants were told that they would taste blindly five samples with similar flavors but derived from different main ingredients. An image of the supposed main ingredient would be shown to them in the computer. Participants in Condition 1 saw the ingredient image before tasting the sample. This condition corresponded to the creation of expectations by the visual stimuli and to the subsequent ingredient-taste (dis)confirmation of expectations when tasting the sample. Participants in Condition 2 saw the ingredient image after tasting the sample. This condition corresponded to a tasting with no manipulation of expectations and to a disconfirmation taste-ingredient by the visual stimuli. All participants tasted before each sample a water sample which was considered as a baseline. This meant that each sample had its own baseline. After each tasting participants rated in 100-mm VAS scales the pleasantness and taste intensity of the samples. They cleansed their palate after each sample by eating a water cracker (Carr's Original table water, Carr's of Carlisle, UK) and a sip of water. Before starting, participants did a practice block which consisted of two parts. In the first, they tasted a water sample and a soy drink sample. No image was shown for this part. In the second, participants practiced just with two water samples and an image of water bottles was shown. All images were shown for five seconds and preceded by a fixation cross which was shown for three seconds.

The five samples consisted of three identical soy samples (also identical to the soy sample given in the practice block) and two dummy samples. The images of the supposed main ingredients for the identical soy samples consisted of two images that disconfirmed expectations: one negative (worms), one positive (chocolate), and a neutral image that confirmed expectations (soy). The images of the dummy samples consisted of an image of rice and an image of oats. The dummy samples were added to prevent participants from realizing that three samples were identical and were given in the second and fifth position. The worms, chocolate, and soy image were randomly assigned to the first, third and fourth position. To further prevent participants from guessing, a break was added in which participants had to read a neutral text about Roman architecture. This break happened between the samples three and four.

After the tasting, participants started the second part of the design. This part was added as a manipulation check for the skin conductance and arousal ratings measurements. Participants were shown two blocks of five images and asked to look at them and rate their pleasantness and arousal on a 100-mm VAS scale. For the first block, participants were shown the same images shown during the first part of the study and asked to rate them considering how they felt the first time they saw them during the tasting. For the second block participants were shown a set of five images of the International Affective Picture System (IAPS) with varying levels of valence and arousal and asked to rate how pleasant and arousing they found them (Lang, Bradley, & Cuthbert, 2008). All images were shown for five seconds and preceded by a fixation cross which was shown for three seconds. Once they had finished, participants were disconnected and given five minutes of rest before starting with the last task.

The last part of the study consisted of a dot probe task. The task followed a similar design to that of di Pellegrino et al (2011). Participants were shown on the screen two images at opposite sides. The presentation of these images was followed by a probe (*) which appeared randomly in the location of one of the images. Participants were asked to specify as fast as possible with the correct key on their keyboard whether the probe appeared left (key "z") or right (key "m"). The complete task consisted of six repetitions of 24 trials. Twelve were main trials consisting of the factorial combination of the three main images of the tasting session (soy, chocolate or worms) x 2 (location of the probe: left or right) x 2 (ingredient location: left or right). The ingredient images were compared against an image of water bottles, which was used as a neutral. The remaining 12 trials were fillers consisting of pairs of dummy ingredient images (the original dummy images from the tasting) compared to the neutral image. Two of these fillers were additionally buffers and therefore shown at the beginning of the task. All images were shown for 500 ms. The probe was shown for a maximum of one second. A break of three seconds was added in between to allow participants to rest. All ingredient images were shown the same amount of times during the task. To ensure that the task was still related to expectations, participants were told that after the task they would be asked some questions and that from their answer they would receive a final sample to taste.

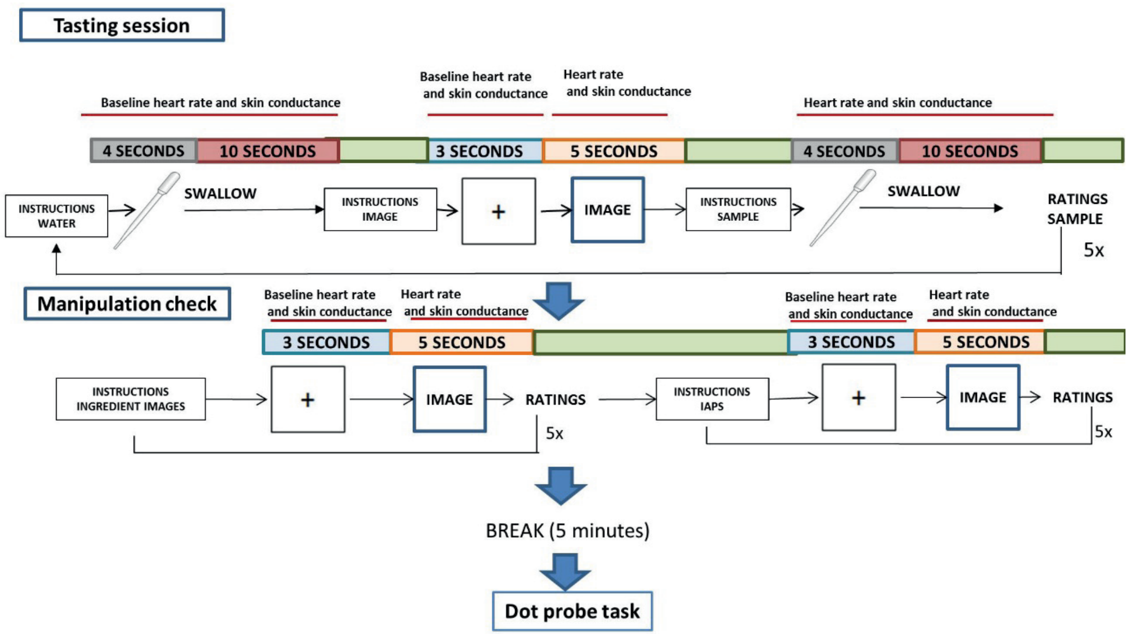


Figure 5.1. Procedure for Condition 1. In this condition, participants saw the ingredient images before tasting the samples. The (+) represents the fixation cross that was used as a baseline for each image.

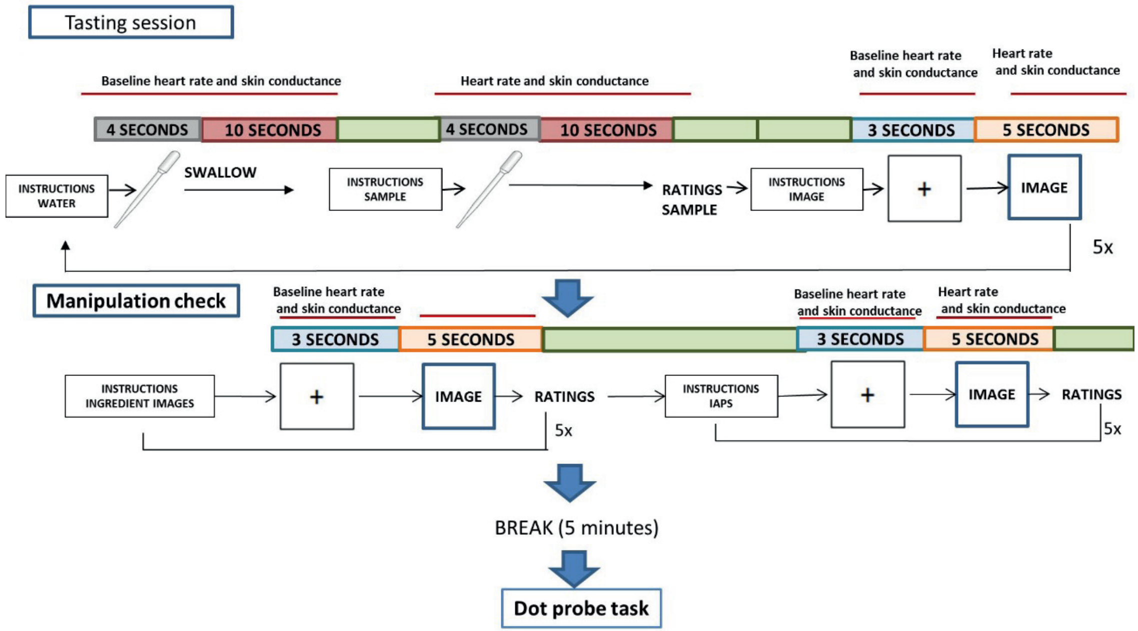


Figure 5.2. Procedure for Condition 2. In this condition, participants saw the ingredient images after they had tasted the samples. The (+) represents the fixation cross that was used as a baseline for each image.

5.2.3. Stimuli

Visual stimuli

All visual stimuli shown were standardized to a 450x60, 96 dpi resolution and presented on a white background.

Ingredient images

The same images used in Chapter 2 were shown. The positive incongruent image consisted of a picture of dark chocolate, the negative incongruent image consisted of a picture of worms, and the congruent image consisted of a picture of soy. Dummy images were a picture of rice and a picture of oats. The water image consisted of a picture of five water bottles.

IAPS images

The set of five IAPS images shown consisted of one image with high arousal and positive valence (HAPV), one with high arousal and negative valence (HANV), one with low arousal and positive valence (LAPV), one high one with medium valence and medium arousal (MAMV), and one with low arousal and medium valence (LAMV). The LAMV was used because it was not possible to find an image with negative valence and low arousal. The ratings and images depicted can be found in **Table 5.1**.

Table 5.1. Valence and arousal ratings of IAPS images.

	Picture depicted	IAPS slide number	Valence ratings Mean (SD)*	Arousal ratings Mean (SD)*
MAMV	Dogs race	1505	4.13(1.66)	4.73(1.83)
HANV	Burn victim	3053	1.3(0.97)	6.91(2.57)
HAPV	Erotic couple	4668	6.67(1.69)	7.13(1.62)
LAPV	Flower	5000	7.08(1.77)	2.67(1.99)
LAMV	Office	7700	4.25(1.45)	2.95(2.17)

* Ratings were given using a 9-point Self-Assessment Manikin scale, by a sample of 100 college students.

5.2.4. Tasting samples

A total of 2 ml of each sample (the equivalent to one swallow of saliva) was given to participants through a pipette (Levine et al., 2012). The samples given matched those from the first study in Chapter 2. The identical samples consisted of an unsweetened soy drink (AH zachte soja drink ongezoet, Zaandam NL) in which one pill of sweetener was added (Natrena zoetjes DE Master Blenders, Amsterdam the Netherlands). The sweetener was diluted in 5 ml of hot water and added to 250 ml of the soy drink.

The dummy samples were a rice drink and an oat drink. They consisted of a combination of 200 ml of the drink (rice (Rice Dream Original, Hain Europe NV, Aalter Belgium) or oats (Oats Dream Original, Hain Europe NV, Aalter Belgium)) and 100 ml of the unsweetened soy drink.

5.2.5. Physiological measurements

Heart rate and skin conductance responses were measured using the VU-AMS version 3.9 (de Geus et al., 1995). The sampling rate of the ECG was 1000 Hz. Heart rate was obtained using the time between adjacent R waves. Skin conductance was sampled at a rate of 10 Hz. The signal range was 0 to 95 µs. The signal was filtered both in forward and reverse direction with a low-pass filter with a cut-off frequency of 2 Hz.

5.2.6. Data treatment and analysis

ANS responses were analyzed using the VU-DAMS program (version 3.9). For each image and sample, the deviation from the baseline for heart rate and skin conductance was calculated. The mean heart rate and skin conductance of the fixation cross was considered the baseline for the images. The delta heart rate and skin conductance was calculated by subtracting the corresponding baseline from the mean heart rate and skin conductance of the image presentation.

The mean heart rate and skin conductance during the water tasting preceding each sample was considered the baseline for the samples. The delta heart rate and skin conductance for the tasting was therefore calculated by subtracting this baseline from the mean heart rate and skin conductance during the tasting of each sample.

For the dot probe task, the attentional bias score for each image was used. For its calculation, the mean reaction time for each of the ingredient images was obtained and subtracted from the mean reaction time for the water image.

In order to analyze the changes in heart rate responses related to the observation of visual stimuli, a mixed model anova was performed with the delta heart rate as dependent variable and the variables image (chocolate, soy or worms), condition (image presented before tasting or after tasting), order of presentation and the interactions between image and condition and image and order of presentation as fixed factors and subject as random factor. Likewise, in order to analyze the changes in skin conductance, a similar model was used with delta skin conductance as dependent variable. These models were chosen in order to correctly compare the results with those from the study in Chapter 2.

For the analysis of the changes of ANS responses during the tasting, the data from the two conditions were divided. For each condition, a mixed model anova was performed with delta heart rate as dependent variable and the variables sample tasted, order of presentation and the interaction between the sample tasted and the order of presentation as fixed factors and subject as random factor. The pleasantness and taste intensity ratings were included as covariates. For the analysis of the changes in skin conductance, a similar model was used with delta skin conductance as dependent variable.

For the manipulation check, the heart rate and skin conductance responses when observing the IAPS images were analyzed by means of a mixed model anova with image as fixed factor, pleasantness and arousal ratings as covariates and subject as random factor.

Finally, for the changes in attentional bias in the dot probe task, the differences in attentional bias scores were analyzed by means of a mixed model anova with attentional bias as dependent variable, image as fixed factor, pleasantness and arousal as covariates and subject as random factor.

All statistical analysis were done with R software (version 3.4.). Outlier observations were only taken out when they presented a Cook's D higher than 4/n and their elimination affected the results of the model (Nieuwenhuis, Grotenhuis te, & Pelzer, 2012). Gender, BMI and age were only added to the models if they had a significant effect and changed the overall results of the models.

5.3. Results

Ninety-five participants completed the study. Nine guessed the aim of the study and were excluded from the analysis. The data of two participants could not be used: one due to flu-like symptoms and the other due to vision problems. Ten participants had to be excluded due to artefacts in the ANS responses, two due to ectopic beats and eight due to movement artifacts in the skin conductance response. The data files of one participant were not saved and hence could not be added to the analysis. In total, the data of 73 participants were included in the analysis, 41 females (mean age=23.6±4.3, mean BMI=21.3±1.8) and 32 males (mean age=27.2±5.5, mean BM1=21.7±1.6). The demographics of the sample can be found in **Table 5.2**.

Table 5.2. Demographics of study sample (N=73).

	Image before tasting (C1)	Image after tasting (C2)	*p*-value
N	37	36	-
Female	20	21	0.906*
Male	17	15	
Age (years)	26.6±6.0	23.8±3.6	0.020**
BMI (kg/m^2)	22.1±1.8	20.9±1.5	0.001**

**p*-value calculated with Welch's t-test.

***p*-value calculated with chi-square test.

5.3.1. Effect of observing images that create and (dis)confirm expectations on heart rate and skin conductance

The following section describes the results obtained from the analysis of the effect of the observation of the three ingredient images on heart rate and skin conductance when expectations are created (image before tasting) and when they are (dis)confirmed (image after tasting) (see **Figure 5.3**). The variables gender, BMI and age were not added to the final model given that their inclusion did not affect the results. For the skin conductance model, three outlier observations were taken out of the analysis.

3.1.1. Heart rate

Delta heart rate was not significantly different between images ($F(2,141)=0.17$, $p=0.844$). There was no effect of order ($F(2,141)=0.28$, $p=0.756$) and condition ($F(1,70)=0.30$, $p=0.590$). Likewise the interactions between image and order and image and condition were not significant ($F(4,184)=0.38$, $p=0.819$ and $F(2,139)=0.77$, $p=0.464$ respectively). These results show that the observation of the images led to a similar cardiac deceleration and that this did not change due to the order of the presentation of the images or whether the image was presented before or after the tasting.

3.1.2. Skin conductance

There was an effect of image on delta skin conductance ($F(2,139)=6.98$, $p=0.001$). Post hoc tests revealed that the worms image led to a higher increase in delta skin conductance than the soy image ($p<0.001$) but was not significantly different from that of the chocolate ($p=0.110$). The effect of order did not reach significance ($F(2,139)=2.80$, $p=0.064$). There was no effect of condition ($F(1,70)=0.08$, $p=0.778$) nor of the interaction between image and order ($F(4,196)=1.19$, $p=0.318$) and image and condition ($F(2,137)=0.68$, $p=0.507$). These results show that regardless of whether the image was presented before or after the tasting, the observation of the worms image led to a significantly different increase in skin conductance than the chocolate and the soy.

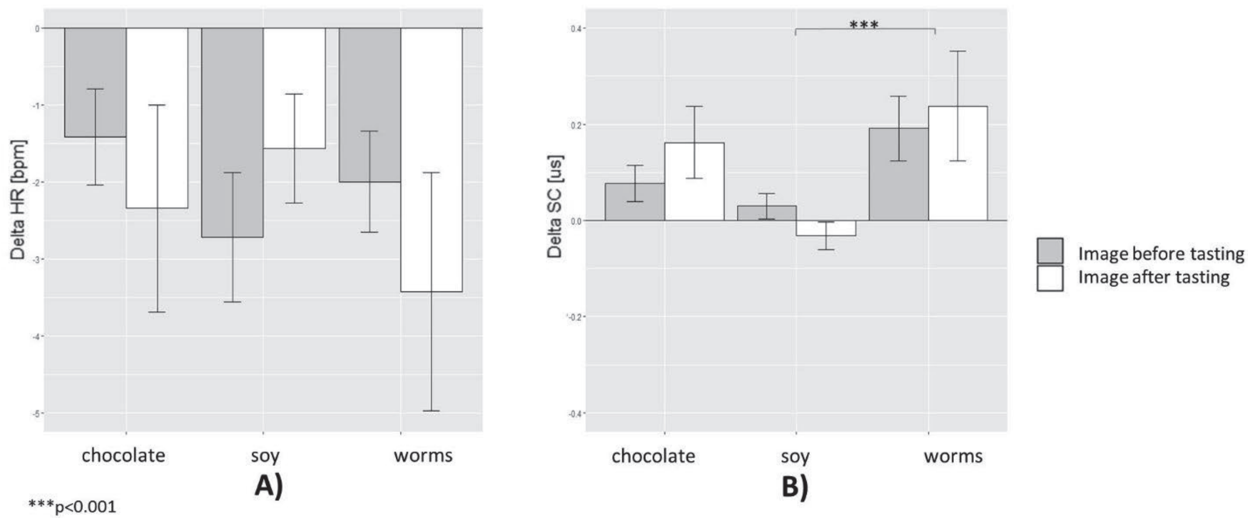


Figure 5.3. Mean (±SE) delta heart rate (A) and delta skin conductance (B) for the observation of the ingredient images (N=73).

5.3.2. Attentional bias when observing neutral, incongruent pleasant and incongruent unpleasant ingredient images

The following section describes the results of the analysis done to assess the differences in the attentional bias scores when performing a dot probe task with the three ingredient images (see **Figure 5.4**).

There was no significant difference between the attentional bias scores for the images (F(2,177)=1.86, p=0.160). Likewise the effect of the subjective pleasantness ratings was not significant (F(1,204)=1.82, p=0.179) nor of the arousal ratings (F(1,211)=2.04, p=0.324). These results show that attention did not differ when observing the three ingredient images.

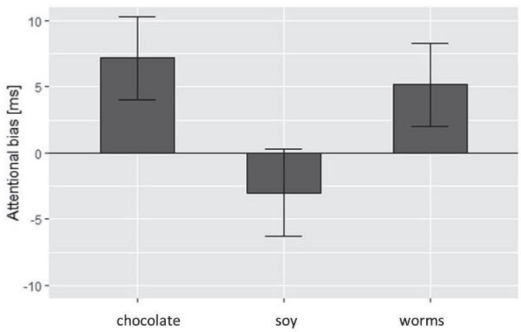


Figure 5.4. Mean (±SE) attentional bias scores for the three ingredients.

5.3.3. Complementary information: Pleasantness and arousal ratings of the three ingredient images

Table 5.3 shows the mean pleasantness and arousal ratings for the three ingredient images. The means presented are not separated by condition given that this factor did not have an effect in the models. The chocolate image was rated as positive (high pleasantness) and with high arousal while the worms image was rated as negative (low pleasantness) and with high arousal.

Table 5.3. Mean pleasantness and arousal ratings (rated with a 100-mm VAS scale) of the three ingredient images (N=73).

	Chocolate	Soy	Worms	*p*-value*
Pleasantness	79.8±16.6[a]	60.8±19.8[b]	12.9±17.7[c]	<0.001
Arousal	63.73±21.8[d]	33.1±21.9[e]	70.7±25.4[d]	<0.001

**p*-value obtained with univariate anova. Different letters mark a significant difference between the images (Tukey's HSD test $p<0.05$).

5.3.4. Manipulation check: Effect of observing images with different levels of arousal and valence on heart rate and skin conductance

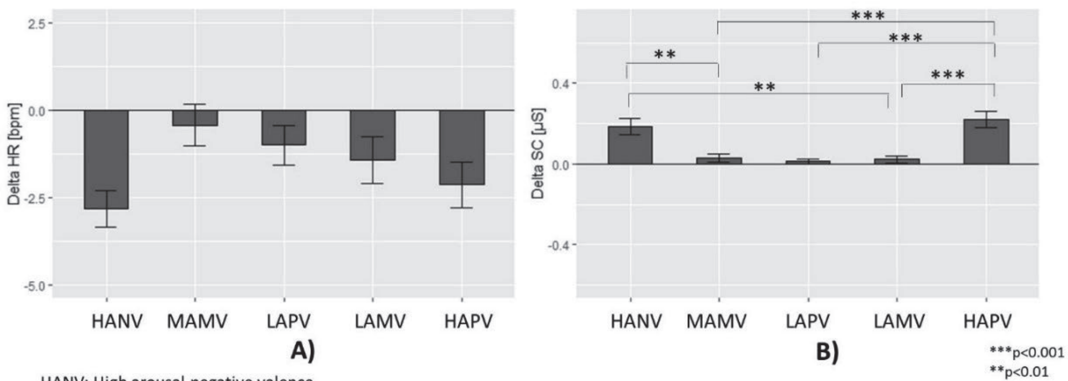
The following section describes the results of the analysis done to assess the effect of observing IAPS images with different degrees of arousal and valence on ANS responses (see **Figure 5.5**). The addition of the variables gender, BMI and age did not affect the results and were therefore not added to the final model.

5.3.4.1. Heart rate

Delta heart rate did not differ significantly between images ($F(4,310)=2.23$, $p=0.065$). Likewise there was no effect of the pleasantness and arousal subjective ratings ($F(1,346)=0.12$, $p=0.730$ and $F(1,342)=0.25$, $p=0.620$ respectively). These results show that heart rate remained similar across images of different valence and arousal.

5.3.4.2. Skin conductance

Delta skin conductance was significantly different among images ($F(4,299)=7.46$, $p<0.001$). There was no effect of self-reported pleasantness nor of arousal ($F(1,323)=0.03$, $p=0.861$ and $F(1,356)=0.71$, $p=0.400$ respectively). Post hoc tests reveal that the skin conductance was higher for the high arousal positive valence (HAPV) image than for the low arousal positive valence (LAPV), the low arousal medium valence image (LAMV) and the medium arousal medium valence image (MAMV) ($p<0.001$ for all three). Skin conductance was also higher for the high arousal and negative valence (HANV) image than for the LAMV and the MAMV ($p=0.030$ and $p=0.001$ respectively). The difference between the HANV and the LAPV could not reach significance ($p=0.080$).



HANV: High arousal negative valence
MAMV: Medium arousal medium valence
LAPV: Low arousal positive valence
LAMV: Low arousal medium valence
HAPV: High arousal positive valence

Figure 5.5. Mean (±SE) delta heart rate (A) and delta skin conductance (B) for the IAPS images with different degrees of valence and arousal.

5.3.5. Effect of tasting identical samples before and after observing the ingredient images on heart rate and skin conductance

The following section describes the results of the analysis done to assess the effect of tasting identical samples on ANS responses. The effect is assessed when an image is seen before the tasting of each sample (and hence there is a (dis)confirmation ingredient-taste) and when the image is seen after tasting the sample (and hence there is no manipulation of expectations at the time of the tasting) (see **Figure 5.6**). The variables gender, BMI and age were not added in the models given that their inclusion did not affect the results obtained.

5.3.5.1. Heart rate and skin conductance responses for the tasting of identical samples when ingredient images are seen before the tasting ((dis)confirmation ingredient-taste)

5.3.5.1.1. Heart rate

Delta heart rate did not differ significantly after tasting the three identical samples ($F(2,68)=0.25$, $p=0.778$). There was no effect of order ($F(2,68)=0.42$, $p=0.660$). The interactions between sample and order remained likewise not significant ($F(4,96)=0.87$, $p=0.486$). There was also no effect of pleasantness or taste intensity ($F(1,90)=0.46$, $p=0.501$ and $F(1,98)=0.24$, $p=0.625$ respectively). These results show that delta heart rate was similar for all samples when the image ingredient was presented before the tasting.

5.3.5.1.2. Skin conductance

Delta skin conductance was significantly different between the three identical samples ($F(2,68)=12.06$, $p<0.001$). The effect of order was not significant ($F(2,68)=1.49$, $p=0.233$). Likewise the interaction between sample and order was not significant ($F(4,99)=0.39$, $p=0.814$). There was also no effect of pleasantness or taste intensity ($F(1,76)=0.29$, $p=0.591$ and $F(1,99)=3.27$, $p=0.073$ respectively). Post hoc tests revealed that when observing the ingredient images before tasting the identical samples, the sample corresponding to the worms image led to a higher delta skin conductance than those given after observing the chocolate ($p<0.001$) and the soy image ($p<0.001$).

5.3.5.2. Heart rate and skin conductance responses for the tasting of identical samples when ingredient images are seen after the tasting (no manipulation of expectations)

5.3.5.2.1. Heart rate

Delta heart rate did not differ significantly after tasting the three identical samples ($F(2,66)=0.55$, $p=0.578$). There was no effect of order ($F(2,67)=0.68$, $p=0.510$). The interactions between sample and order remained likewise not significant ($F(4,92)=0.70$, $p=0.594$). There was also no effect of pleasantness or taste intensity ($F(1,91)=2.07$, $p=0.153$ and $F(1,96)=0.96$, $p=0.328$ respectively). These results show that delta heart rate was similar for all samples when participants tasted the sample when there was no manipulation of expectations.

5.3.5.2.2. Skin conductance

Delta skin conductance did not differ significantly after tasting the three identical samples ($F(2,66)=0.29$, $p=0.750$). There was no effect of order ($F(2,67)=0.35$, $p=0.710$). The interactions between sample and order remained likewise not significant ($F(4,95)=0.62$, $p=0.648$). There was an effect of pleasantness ($F(1,69)=4.37$, $p=0.04$) but no effect of taste intensity ($F(1,78)=2.20$, $p=0.142$). These results show that delta skin conductance was similar for all samples when participants tasted the sample when there was no manipulation of expectations.

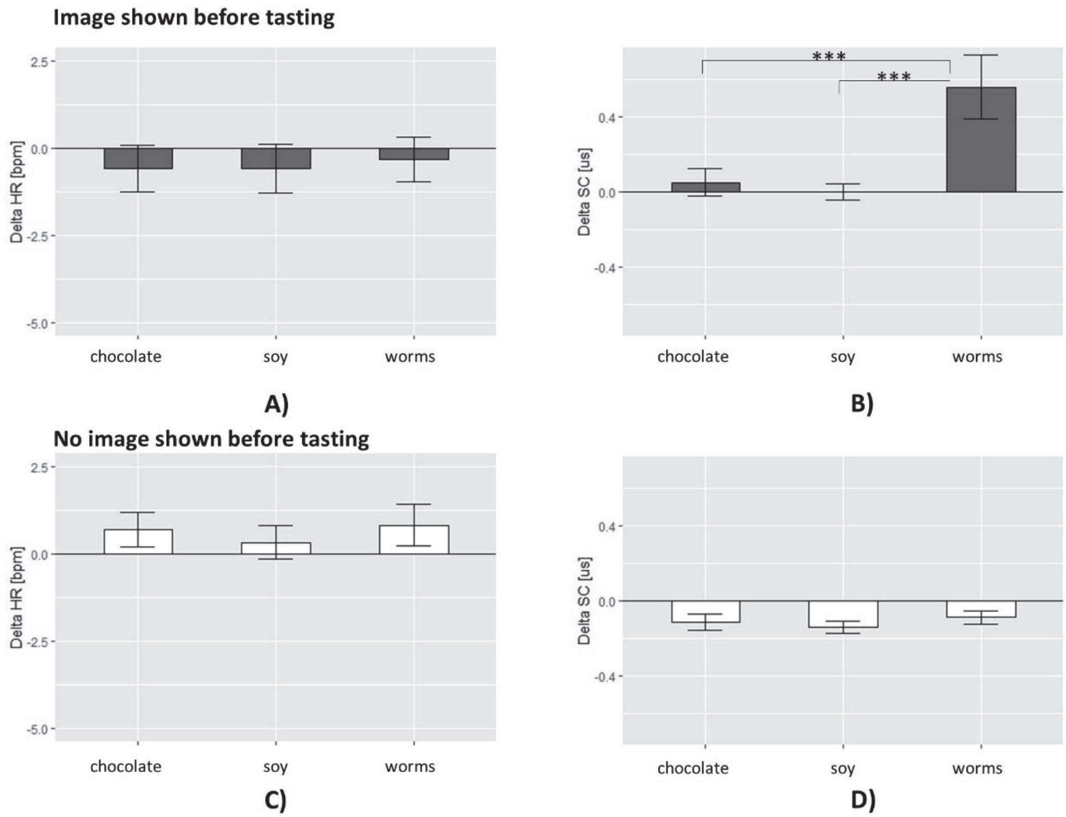


Figure 5.6. Mean (±SE) delta heart rate and delta skin conductance for tasting identical samples when an ingredient image was seen before tasting the sample (A and B) and when there was no manipulation of expectations (C and D).

5.3.6 Complementary information: Pleasantness and taste intensity ratings for the three identical samples before and after observing the ingredient images

To further evaluate if the identical samples that showed differences in ANS responses were perceived differently, **Table 5.4** shows the pleasantness and taste intensity ratings given by participants for the three identical samples during the two conditions. Participants rated the samples as less intense and less pleasant when the supposedly corresponding image was shown before the tasting. Likewise, the taste intensity ratings were lower in this condition.

Table 5.4. Mean pleasantness and intensity ratings for the three identical soy samples before and after seeing the ingredient images (N=73).

Pleasantness**				
	Chocolate	**Soy**	**Worms**	***p*-value***
Image shown before tasting (disconfirmation of expectations ingredient-taste)	44.9±23.7 [a]	49.9±21.9 [a]	39.7±23.4 [a]	0.166
Image shown after tasting(no manipulation of expectations)	56.0±19.3 [b]	52.4±20.0 [b]	52.8±18.9 [b]	0.771
Taste intensity**				
Image shown before tasting (disconfirmation of expectations ingredient-taste)	39.3±22.1[a]	38.0±20.6[a]	40.4±20.4[a]	0.882
Image shown after tasting (no manipulation of expectations)	47.1±18.2[b]	47.1±16.5[b]	45.4±18.5[b]	0.892

**p*-value obtained through an univariate anova. Different letters mark a significant difference between the images (Tukey's HSD test $p<0.05$).
**Attributes rated in a 100-mm VAS scale.

5.4. Discussion

The aim of this study was to evaluate whether heart rate and skin conductance responses during the creation and (dis)confirmation of expectations are related to attention and arousal and whether the attention and arousal of a disconfirmation can lead to different ANS responses when tasting identical stimuli. We additionally examined the replicability of ANS responses by comparing the results from this study with one in which the same stimuli were used. The results obtained in this study could not let us evaluate whether the ANS responses are linked to attention. However, one of the main findings is that heart rate responses are difficult to replicate. Skin conductance responses seem more stable and capture changes related to arousal when expectations are created and (dis)confirmed.

The observation of the three ingredient images led to a cardiac deceleration. The deceleration was similar between the two conditions (creation or (dis)confirmation of expectations). Interestingly, while the subjective arousal and pleasantness ratings showed that the images differed in valence and arousal, the cardiac deceleration was similar for all images. As cardiac deceleration is related to attention and sensory processing, it seems that attention did not vary when observing the images. The results from the dot probe task support this assumption as they showed that there were no differences in the attentional bias between images. This result is different to what was obtained in the first study in Chapter 2 where the chocolate and worms image had a stronger cardiac deceleration than the soy image in both conditions (Verastegui-Tena et al., 2017). The main difference between the aforementioned study and this study is the characteristics of the sample. The current study is done on an international student sample while the other was a Dutch sample. It is possible that individual characteristics play a role in the attention given to the images. Attention has been related to individual differences, which may be in part related to cognitive control. These include processes related to sensory detection, perception, appraisal, memory, among others (Corr, 2010). People differ in terms of the dimensions that attract their attention. The most salient dimension is dependent on what the person finds most useful and makes more sense in their particular situation (Rollinson, 2005). It is possible then that the Dutch sample and the student sample focused on different dimensions when observing the images.

Contrary to the heart rate responses, the results from the skin conductance responses when observing the images during the creation and (dis)confirmation of expectations are similar to the ones from Chapter 2. The only difference found was that while the first study in Chapter 2 found an effect of order, the current study did not. This may be because in the current study, we used already an image (water) during the practice and hence the novelty of observing an image was gone. Nevertheless, the findings regarding the effect of the ingredient images from both studies match. The worms image, which was rated as highly arousing and with a negative valence, led to a higher increase in skin conductance than the chocolate and the soy image. This was seen during both the creation and (dis) confirmation of expectations. Increases in skin conductance coupled with heart deceleration are said to be related to attention (Verschuere et al., 2009). However, the fact that there are differences in skin conductance between images but not in heart rate responses may mean that the process captured by skin conductance is the acute stress response to the worms and not attention. Moreover, the fact that the skin conductance responses match those of a previous study while the heart rate responses do not may be also due to the nature of this stress response. The acute stress response or fight or flight reaction is one of the first and most primitive responses. It is shaped by natural selection and is the most immediate response to stressor exposure (Nesse, Bhatnagar, Ellis, & Fink, 2016; Ulrich-Lai & Herman, 2009). This entails that, being an innate first reaction; it would not be affected by as many factors as attention would.

The results from the manipulation check showed that heart rate responses could not differentiate among images of different valence and arousal. Skin conductance responses showed that the high arousal images led to a higher increase in skin conductance than those with low arousal. Our results have some similarities but also some differences with those of Bradley and Lang (2007). Both studies found that skin conductance differentiates only with the most arousing stimuli. However, Bradley and Lang found that unpleasant stimuli led to stronger cardiac decelerations. This difference may again be due to individual differences between samples. However, it might be also related to the measurement used as Bradley and Lang looked at the cardiac pattern while we looked at deltas.

The results from the tasting of the identical samples showed that heart rate responses did not change when tasting the samples, regardless of whether they had seen before the ingredient image or not. Skin conductance responses, however, did show a difference when the ingredient images were seen before tasting the samples. Skin conductance increase was larger when participants drank the sample thinking it contained worms than when they believed the sample had chocolate or soy. The differences while tasting the samples were more marked than when observing the ingredient images. This may be related to a stronger defense mechanism. Eating possesses always the risk of ingesting harmful bacteria or parasites (Reed & Knaapila, 2010). Particularly in the case of insect-based products or similar, some societies avoid eating them as they are seen as transmitters of disease (House, 2016). It is possible that the participants in our sample had a similar conception and hence the defense reaction was enhanced during the consumption of the sample.

Our study has certain limitations. While the dot probe task showed similar results as the heart rate responses in regards to attention, the lack of differences in attentional bias scores could also be due to the design of the study. Participants stayed for an hour and the dot probe task was the last part of the study. Attention is usually facilitated by task engagement. However, fatigue states such as monotony or boredom can affect task engagement (Matthews, Warm, Reinerman, Langheim, & Saxby, 2010). In the case of this study, it is possible that participants were bored and no longer engaged in the dot probe task due to the duration of the study and, as a result, no differences were seen. On a similar matter, it is possible that it was the combination of the small differences in the design, rather than just the attentional differences among participants, that prevented us from replicating the heart rate responses from a previous study. It is, therefore, difficult to pinpoint one single factor captured by the heart rate response. A final limitation is that, while we excluded participants that may be used to the soy and soy taste such as vegan, Asian, or participants that consumed frequently plant-based dairy substitutes, we did not ask participants how familiar they were with all the ingredients. High familiarity might have led to weak ANS responses, while a lack of familiarity might have led to stronger ones because the ingredients might have been perceived as riskier (Fischer & Frewer, 2009).

The findings of this study help clarify the usefulness of ANS responses in food research. Our findings show that heart rate responses are difficult to replicate. Skin conductance shows more concrete and reproducible results. Skin conductance captures arousal when expectations are created and (dis)confirmed but its capacity to differentiate is dependent on the degree of arousal that is created. It may be necessary to look for other ANS responses that may be more helpful in food research. For example, event-related potentials are also said to capture arousal and eye-tracking is also used for attention. The measurement of the activity in the corrugator muscle may also be of use as it captures valence (Bradley & Lang, 2007; Duc, Bays, & Husain, 2008). Further research, however, needs to be done in order to assess if these measurements are able to capture these dimensions better than heart rate and skin conductance or if they present the same limitations.

5.5. Conclusion

The present study evaluated whether the ANS responses when expectations are created or (dis)confirmed are linked to attention and arousal and whether heart rate and skin conductance responses in these situations can be replicated. The results from our research show that skin conductance had a good replicability and captured arousal during the creation and disconfirmation of expectations. Heart rate responses could not be replicated and showed no changes. Therefore, it was not possible to evaluate their link with attention.

CHAPTER 6

General Discussion

General discussion

In order to better understand how individuals perceive and react to food in their environment, this thesis aimed to assess how the (dis)confirmation of food expectations and the components of relevance, valence, arousal, and attention are reflected in ANS responses. To achieve this, the following topics were addressed I) *ANS responses elicited by stimuli of different valence when expectations are created and (dis)confirmed and when stimuli are presented in situations of different relevance* **(Chapter 2)**, II) *ANS responses related to novelty (first experience with a stimulus), valence (pleasant or unpleasant stimulus) and disconfirmations of expectations* **(Chapter 3)**, III) *ANS responses related to the degree of the expectation disconfirmation* **(Chapter 4)**, IV) *Influence of attention and arousal in ANS responses to expectation (dis)confirmation* **(Chapter 5)**. This chapter describes the findings on each topic and discusses the scientific and practical implications of the use of ANS responses when measuring responses to food.

Overview of main findings

ANS responses elicited by stimuli of different valence when expectations are created and (dis)confirmed and when stimuli are presented in situations of different relevance

To assess the changes in ANS responses related to the creation and (dis)confirmation of expectations, stimuli of different valence (one positive, one neutral and one negative) were shown before (creation/anticipation) and after ((dis)confirmation) tasting identical samples. The effect of relevance (meaning how significant something is deemed for an individual's well-being) was assessed by changing the situation in which the stimuli were shown (just visual presentation vs. tasting involved). Chapter 2 shows that the changes in ANS responses seem to be related to more than just expectations. Compared to baseline levels, all stimuli led to a decrease in heart rate. However, stimuli that disconfirmed expectations led to larger heart rate decreases than stimuli that confirmed expectations, regardless of valence. Even though this result could be due to the disconfirmation created by the stimuli, decreases in heart rate are normally considered a sign of attentional processes and incoming sensory information (Sánchez-Navarro et al., 2008). Moreover, the same heart rate patterns were seen for the creation of expectations. Similar to this, skin conductance responses presented similar patterns for the creation and the (dis)confirmation of expectations. Skin conductance showed significantly larger increases for the negative stimulus than for the positive and neutral stimuli. Increases in skin conductance have been described in the literature as an indicator of arousal and defense responses, which are normally present with negative or threating stimuli (Nesse et al., 2016; Rosebrock et al., 2016). Chapter 2 additionally shows that presenting stimuli in a situation that is not deemed relevant by individuals (such as observing stimuli with the knowledge that they do not have to ingest what is shown) led to a lack of engagement and weaker ANS responses.

This chapter's contribution lies in the findings that ANS responses seem to capture more than just (dis)confirmations of expectations. The responses obtained could be related to attention and defense reactions. In addition to this, relevance plays a role in the intensity of the response.

Novelty, valence, and the disconfirmation of expectations

Chapter 3 disentangles the effects of novelty, valence, and the disconfirmation of expectations on ANS responses. Novelty was measured by evaluating the differences between the ANS responses when tasting for the first time a sample (without any previous knowledge about the taste) and the ANS responses when tasting it immediately after for the second time (already having knowledge and experience on the taste). Valence was measured by evaluating the differences between neutral, negative and positive taste samples. Disconfirmations were measured by comparing the ANS responses when tasting samples with a taste that matched the one informed to subjects to the ANS responses when tasting samples with a different taste from that informed to subjects. Chapter 3 shows that novelty evokes changes in heart rate and skin conductance. Heart rate remained similar to baseline levels during the first experience with a taste. However, the second experience led to a decrease in heart rate. Skin conductance increases from baseline levels were larger for the first experience with a taste than for the second experience. These changes could be a combination of arousal and attentional responses related to an initial orientation response (Leclercq & Zimmermann, 2004). Skin conductance increases have been usually seen for arousing stimulus and after the presentation of new stimuli (Dindo & Fowles, 2008). In the case of the cardiac responses, heart rate could be first briefly accelerating (which may reflect a heightened arousal induced by the new stimulus) and then decelerating due to an orientation response. This would explain why during the first experience there were almost no changes from baseline levels while during the second there was a marked deceleration.

This chapter also showed that skin conductance changed according to valence, with negative stimuli leading to larger increases in skin conductance than positive or neutral stimuli. This may be, same as in Chapter 2, once again related to a defense response. Finally, this chapter shows that the effect of disconfirmations seems to only be captured by heart rate. Compared to baseline levels, heart rate decelerated when there was a negative taste disconfirmation. This may point to attention processes to the change/disconfirmation.

Degree of disconfirmation and ANS responses

Chapter 4 assesses how the degree of the disconfirmation alters ANS responses. ANS responses to tastes were assessed when there was no information given about the taste and when there were taste confirmations and large and small taste disconfirmations (expectation manipulation). Self-reports were used to confirm that the different degrees of disconfirmation were perceived accordingly. This chapter shows that, heart rate and skin conductance responses were not able to capture the differences in the level of disconfirmation between samples. However, heart rate responses showed a smaller increase from baseline levels during the first tasting session than during the second. It is still not clear what led to this result. It may be that, as the design was new to participants, an orientation response created a deceleration in heart rate that made the increase smaller. Skin conductance responses, on the other hand, showed larger increases when participants had no information about the taste than when confirmations and disconfirmations occurred. Once again, the reason behind these results is not clear. It may be that due to the manipulation of expectations, participants were more aroused and needed to pay more attention to the (dis)confirmations. As a result, skin conductance increased. Heart rate and skin conductance responses among samples were different when no information about the taste was given. Heart rate responses reflected differences between samples of different valence, with a larger increase for samples deemed unpleasant. Skin conductance responses remained similar between samples. This chapter, therefore, shows that ANS responses are not affected by the degree of the disconfirmations. However, ANS responses could be again reflecting the orientation response related to the novelty of the design and heightened arousal and attention related to the processing of the manipulation of expectations.

Attention and arousal

Chapter 5 considers the findings of the previous chapters and aims to determine whether the responses obtained when there is a (dis)confirmation of expectations are indeed mainly related to the components of attention and arousal. In addition to this, the chapter assesses whether the findings on ANS responses are replicable. In order to do this, this chapter uses a similar design and identical stimuli to those of the first study in Chapter 2. The main finding of Chapter 5 is that heart rate responses did not show the same patterns as in Chapter 2. The small differences in the design may have played a role on this. Skin conductance responses, on the other hand, exhibited the same patterns found in previous chapters. They were able to differentiate between stimuli that contrasted in arousal, with larger skin conductance increases from baseline levels for stimuli high in arousal than for stimuli low in arousal.

This chapter contributes to the literature of ANS responses by showing that heart rate responses are difficult to replicate. This may be due to attentional differences among participants. However, it is also possible that heart rate captures a combination of components from the design. As a result, it is difficult to pinpoint just one main component reflected by the response. Skin conductance responses show more stability than heart rate responses but can only differentiate between the most arousing stimuli.

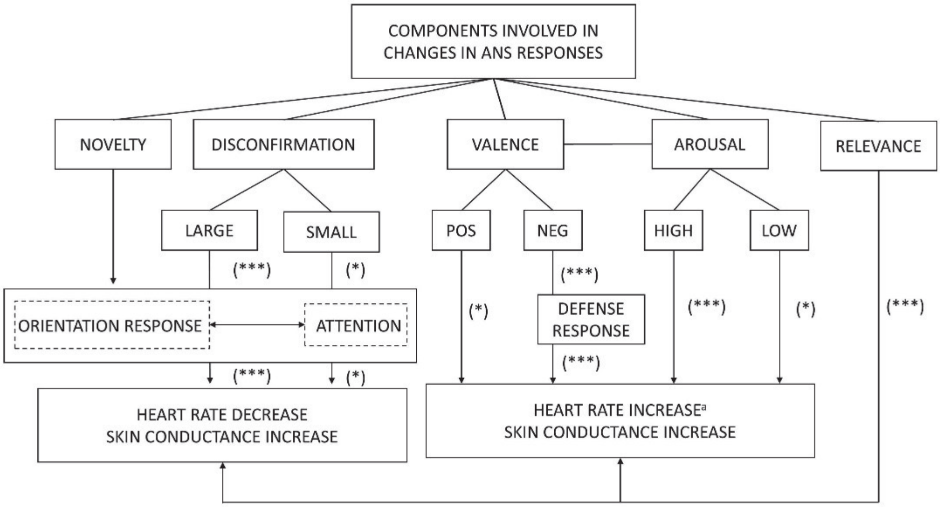
Scientific implications

This thesis used as a starting basis Mandler's schema incongruity theory to assess the effect of disconfirmations and other components such as relevance, valence, attention and arousal on ANS responses. The chapters on this thesis show that, as proposed by Mandler, disconfirmations can lead to changes in ANS responses. Large disconfirmations lead mostly to ANS responses of larger magnitude than confirmations or small disconfirmations. However, Mandler's reasoning behind this effect may not be complete. Mandler proposed that in cases of large disconfirmations, accommodation (changing the schema to include the new information) is what leads to the ANS responses of larger magnitude (Mandler, 1982). This thesis, however, shows that ANS responses are more likely related to orienting and attentional processes coming from the initial perception of the stimulus and the processing of the disconfirmation as well as some innate immediate reactions, such as defense responses. The novelty and valence of the stimulus, as well as how arousing the individual found it, influence these processes. Moreover, the more relevant the stimulus is to the individual, the more intense the ANS response is.

The sequence in which the components lead to ANS responses during the disconfirmation of expectations could be as follows. The novelty of a stimulus leads to an orienting response and subsequent attentional and sensory processing from the subject. Relevance here already takes a part, as relevant stimuli will be seen as more worthy of attention. The incoming information from the sensory and attentional processing includes the valence of the stimulus and how arousing it is for the individual (depending on its saliency, part of these characteristics might have already been detected by the individual and have led to the initial novelty detection). Valence and arousal are sometimes linked (i.e. the more negative a stimulus, the higher the arousal) and can already lead to innate reactions such as defense responses. With the information that has already been processed, individuals check how similar (confirmation) or dissimilar (disconfirmation) the stimulus is from their expectations. In order to do this, individuals use again attentional processes. If after this process disconfirmations are deemed negative or a threat, they can lead to defense responses.

The sequence described above presents similarities with what had been stated by appraisal theories. The basic assumption of these theories is that the organism's evaluation of particular components in their surroundings is determined by the intensity and quality of the physiological and affective response (Moors et al., 2013). Similar to these theories, our findings show that components such as novelty, valence and relevance can already lead to changes in ANS responses. Most appraisal theories however, consider arousal and disconfirmations as part of the novelty detection (Ellsworth & Scherer, 2002). This thesis shows that, although they do play a role, there is also some further processing of these components after the step of novelty detection.

Another contribution of this thesis is the finding that heart rate and skin conductance responses may not capture the same components. Changes in heart rate responses can be evoked mainly by novel stimuli or stimuli that disconfirm expectations. These changes may be related to the orientation response and attention. Both the orientation response and attention lead to a cardiac deceleration. Novel stimuli or stimuli with a valence (particularly negative) lead to large increases in skin conductance. These changes may be linked to arousal and defense responses (especially in the case of negative stimuli) (**Figure 6.1**).



[a] This result was only found in one study.

Figure 6.1. Summary of results. The (*) sign refers to weak responses. The (***) sign refers to strong responses.

Finally, this thesis shows that skin conductance results can be replicated and have, therefore, a high reliability. Furthermore, the findings on the effects of the arousal related to novelty, valence and defense responses support what had been stated and found in previous research (Dawson et al., 2011; Robin et al., 2003; Rousmans et al., 2000). Heart rate responses, on the other hand, cannot be replicated easily. This could also explain why some of our findings do not match what has been seen in previous research. This is particularly the case when looking at the effect of valence. Heart rate changes have been associated with valence (Hamm et al., 2002). Heart rate decrease from baseline levels is larger for negative visual stimuli than positive visual stimuli (Pollatos, Herbert, Matthias, & Schandry, 2007). Likewise, negative taste stimuli lead to larger heart rates increases than positive taste stimuli (Robin et al., 2003; Rousmans et al., 2000). In this thesis, only one study could find an effect of valence. Hence, it was not possible to make an association of heart rate with this component.

Practical relevance

The findings on this thesis have implications on the future use of ANS for the measurement of the reactions to food. ANS responses had been previously proposed as tools to measure the unconscious motives and associations that lead to the liking or disliking of certain foods (de Wijk & Boesveldt, 2016). This thesis shows that the components captured by ANS may not serve this practical purpose. As such, they might not be the best tool for areas such as food research. ANS responses may only be of use when study designs include highly relevant, novel, arousing or contrasting disconfirmations or stimuli. However, the characteristics that would be captured by ANS would already be noticeable and could be better measured by cheaper methods.

The use of ANS responses in this thesis still provides some insight on how different components during the perception of disconfirmation lead to changes in ANS responses. Due to the similarities that were found with appraisal theories, a potential use of ANS could be assessing the most salient appraisal in different food contexts. Currently theorists still differ on the appraisals they consider more important (Ellsworth & Scherer, 2002). The use of ANS responses could help clarify this topic. Moreover, their use could go further than that of the food context.

Limitations and future research

One of the main limitations of this thesis is the measurement of short-term ANS responses to food. This entailed that certain physiological variables that need longer time intervals (such as heart rate variability) could not be used. Moreover, responses might change over longer periods of time. For example Brouwer et al. (2013) found that the effects on heart rate and skin conductance responses from visual, auditory and bimodal (visual and auditory) stimuli changed over time. Heart rate responses to arousal and valence habituated but skin conductance responses to arousal heightened (Brouwer et al., 2013).

The characteristics of the study designs in this thesis present another limitation. Electrodes had to be placed on participants and movement was limited while connected to the device. Moreover, the samples were always administered through a pipette by the researcher. This might have made participants uncomfortable and influenced our results. Moreover, the fact that the studies were performed in a laboratory setting affects the generalizability of the results to real-life situations.

The measurements used in this thesis could only measure basic components during the disconfirmation of expectations. Future studies can evaluate whether other ANS responses such as pupil dilation, blood pressure or heart rate variability can provide a broader view of what happens during this process. Another option could be, as mentioned above, switching the focus to that of appraisals.

Final conclusion

The research from this thesis assessed the effect of expectation (dis)confirmation and the components related to it (novelty, valence, attention and arousal) in heart rate and skin conductance. The findings on this thesis show that, compared to baseline levels, disconfirmations lead to larger heart rate decreases and larger increases in skin conductance. These responses could be reflecting changes in orientation and attention. Novelty and valence (particularly negative) lead to large increases in skin conductance but these responses are linked to the arousal of the stimuli. Relevance influences the intensity of these ANS responses by making them stronger when the stimuli are deemed important or significant by individuals.

Finally, this thesis shows that skin conductance responses are less variable than heart rate responses, as they can be easily replicated. Results with heart rate responses should, therefore, be interpreted with caution. Although the ANS responses obtained in this thesis provide some insight into the reactions that occur during the human-food interaction, other measurements might be more helpful in answering the current questions of food research.

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SUMMARY

The interaction with food involves a series of unexplored reactions that may help understand how individuals perceive the food in their environment. Among these reactions, the responses of the autonomic nervous system (ANS) are believed to reveal affective states, motivation, and preferences that people may not be able to articulate when experiencing food. An individual's experience with food can be affected when expectations are disconfirmed. Expectations provide an idea (based on previous experiences, pre-set ideas, beliefs or even the context of consumption) of what the food product should look, smell, feel, and taste like. The novelty, valence, and relevance of the food product along with how arousing it is for the individual can also contribute in the responses related to expectations. The present thesis aimed to assess if ANS responses (particularly heart rate and skin conductance) capture the processes related to the (dis)confirmation of expectations when individuals are presented to food products and the effect that novelty, arousal, valence, relevance, and attention might have in these responses. This thesis addressed four topics in order to achieve this objective: I) ANS responses elicited by food stimuli of different valence when expectations are created and (dis)confirmed and when food stimuli are presented in situations of different relevance, II) ANS responses related to novelty (first experience with a stimulus), valence (pleasant or unpleasant stimulus) and (dis)confirmations of expectations, III) ANS responses related to the degree of the expectation disconfirmation, IV) Influence of attention and arousal in ANS responses to expectation (dis)confirmation.

The comparison of ANS responses obtained when expectations were created and when they were (dis)confirmed showed that ANS responses captured more than the effect of expectations (Chapter 2). The creation and (dis)confirmation of expectations led to similar ANS response patterns. The responses obtained (heart rate decrease from baseline and skin conductance increase from baseline) seemed to be related to enhanced attention for the positive and negative stimuli and to defense reactions for the negative stimulus. Relevance was found to intensify all the ANS responses to stimuli. Looking separately at the patterns of ANS responses related to novelty, valence and disconfirmations showed that novelty evokes changes in heart rate (small increase followed by a decrease from baseline) and skin conductance (increase from baseline) that could be a combination of arousal and attentional responses related to an initial orientation response (Chapter 3). Skin conductance changed according to valence, with negative stimuli leading to larger increases in skin conductance than positive or neutral stimuli. The effect of disconfirmations seemed

to only be captured by heart rate and may be related attention processes to the change/disconfirmation.

ANS responses were not affected by the degree of the disconfirmations (Chapter 4). A study design in which expectations were manipulated to create small and large disconfirmations only found ANS responses that could be again reflecting the orientation response and attention (heart rate decrease from baseline) related to the novelty of the design and heightened arousal and attention (increase in skin conductance) related to the processing of the manipulation of expectations. Evaluating whether the ANS responses obtained in all studies indeed reflected attention and arousal showed that skin conductance was a stable measure for arousal (Chapter 5). However, it could only differentiate between the most arousing stimuli. It was not possible to confirm whether ANS responses reflect attention due to the lack of replicability of heart rate responses.

Overall, the chapters of this thesis show that disconfirmations lead to ANS responses (particularly heart rate decreases and skin conductance increases from baseline) that could be reflecting changes in orientation and attention. Novelty and valence (particularly negative) lead to large increases in skin conductance but these responses are linked to the arousal of the stimuli. Relevance influences the intensity of these ANS responses by making them stronger when the stimuli are deemed important or significant by individuals. The findings of this thesis show that the components captured by ANS responses may not be helpful for studying the reactions to food. Research areas such as food research could only use ANS responses in study designs that include highly relevant, novel, arousing or contrasting disconfirmations or stimuli. However, the characteristics that would be captured by ANS would already be noticeable and could be better measured by cheaper methods.

AKNOWLEDGEMENTS

While the fact that a thesis is not the work of a single person is common knowledge, I found myself having quite the humbling experience when I started writing this section. The list of people that have made this thesis possible is long. I am grateful to you all and I would like to let the world know how amazing you have been by dedicating a few words to you.

Mom, you have supported my career choices since the beginning. You never doubted me or tried to stop me. I have only heard words of encouragement from you, even when I said I would leave my home country to pursue my career. Thank you, I see now how difficult that must have been for you. Dad, I do not know if I have ever told you this but I admire you. You have always inspired me to follow my dreams. "It doesn't hurt to try" you have always said. I try to follow your motto every day. Thank you for being present for all the important events in my life, even now that there is a whole ocean separating us. Bárbara, thank you for being here. I know life and work are hectic and you still changed your vacation plans in order to be present at my defense. Thank you for always being my best friend and for helping me even when I was being too proud to ask for help.

Special thanks to my supervisor Dr. Betina Piqueras Fiszman and to my promotor Prof. Hans van Trijp who have guided me during these 4 years. Betina, I am glad you chose me as your first PhD. You were not only my supervisor but also a great friend. You were strict when necessary but you always stayed empathic. I hope you have enjoyed working with me as much as I enjoyed working with you. Hans, even though you had the weight of the whole MCB on your shoulders, you always read my work and gave the necessary feedback. You were always objective and professional during our meetings and looked for the best options for the project. Thank you for dedicating time to me and my project and for always giving quality advice.

I would also like to thank my professors and supervisors in Mexico. Ana Bertha Pérez Lizaur, MSc, Dr. Héctor Bourges Rodríguez, and Dr. Elizabeth Tejero Barrera. It was thanks to them that I looked into the option of coming to the Netherlands (and was eventually able to come here). Thank you for your support and advice and for encouraging me to continue my education after I finished my bachelor's.

Thank you MCB for all your help! Thank you Lisanne, Naomi, Giulia, Robert, Nigel, Kunalai, Kristina, Xin, Bastian, Lenka, and all PhDs for the talks, jokes, advice, and for daring to taste the weirdest samples! Naomi, we are proud members of Betina's Latino team. Although we did not share an office, we definitely talked as if we did! We are living proof that it is dangerous to put two Latinas together. Giulia, my office mate, I am glad you came along. It was becoming a little bit boring in that office all by myself. I would also like to thank Ivo, Arnout, Erica, Ellen van Kleef, Ilona, Paul, and all the professors at MCB for their support and useful feedback. Special thanks to Monica Szalma for answering my questions about the English language. Finally, but not least, Ellen Vossen, Liesbeth, and Marloes for always helping me with a big smile on their faces and for answering my questions about how things worked at MCB.

There are also quite a number of people outside my group that helped during the development of the project. Marie and Eduardo, the nicest power couple on Earth who were always there when I needed help. Thank you Jos, Muriel, Eira, Moni, Dominique, Lennart, Evelina, Valentina, Rob, Carlos, Francisco, Benjamin, Ismaouil, Marco, Phillip, Monica Aguayo, Chema, Álvaro, Sarahí, Moisés, Sandra, Marlou, and Pol for all the tastings, for spreading the word, and for always sending me willing participants. Aurelia, thank you for always asking the right questions. Your assistance and your feedback were of incredible help for this project. Tiffany, even though you are far away, you were always willing to read my work, which shows what an amazing person you are. Priya and Eline, thank you for helping me during the development of the first and last study of this thesis.

Thanks to my paranymphs Pol and Lisanne. I know I had already mentioned you but I believe you deserve a second thanks for agreeing to sit for more than 45 minutes in the middle of a room without moving. It is difficult to keep a straight face when everyone is looking at you. You agreed without hesitation to all of this. You are amazing friends.

Martha Mondragón and Jorch. I think of you as my extended Mexican family in the Netherlands. Martha, you and Frans have taken care of me ever since I arrived to the Netherlands. Both my family and I are grateful for that. Jorch, you have always been there willing to help me and Chris (and now Antonio). You do so many things without expecting anything in return. I hope I can one day help you as much as you have helped me.

Jenny, Kees, Laura, and Gino. Thank you for accepting me in your family. Jenny and Kees, I am also grateful for all your help with Antonio. He enjoys every day that he stays with oma and opa. I can see the excitement in his face every time you walk in the room. I know he is in good hands and that I have no reason to worry when he is with you. Without your help I might not have been able to finish this PhD.

I would like to finish by thanking my little boy, Antonio, and my wonderful husband, Chris. You two make my life whole. Antonio, you have brought us so much happiness. Chris, you have been incredibly supportive. You have believed in me even when I did not believe in myself. You have stayed with me through good and bad and helped me in all ways possible. I love you sweetie.

ABOUT THE AUTHOR

Luz María Verástegui Tena was born on November 7th 1988 in Mexico City. She received a Bsc. in Nutrition and Food Science from Universidad Iberoamericana in 2012 and a Msc. in Nutrition and Health in 2014 from Wageningen University. In December 2014, she started her PhD at the Marketing and Consumer Behaviour group from Wageningen University. Luz has worked in projects related to Nutrigenomics, Sensory Science, Eating Behaviour and Psychophysiology. She also did social work in Chicago Illinois, giving nutrition advice and designing nutritional workshops and classes for immigrants in the United States.

Publications

Verastegui-Tena, L., Schulte-Holierhoek, A., van Trijp, H., & Piqueras-Fiszman, B. (2017). Beyond expectations: The responses of the autonomic nervous system to visual food cues. Physiology & Behavior, 179, 478-486.

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In review

Verastegui-Tena, L., van Trijp, H., & Piqueras-Fiszman, B. (2019). Heart rate and skin conductance responses during the creation and disconfirmation of expectations: Replicability and the link with arousal and attention.

Luz Maria Verastegui Tena
Wageningen School of Social Sciences (WASS)
Completed Training and Supervision Plan

Name of the learning activity	Department/Institute	Year	ECTS*
A) Project related competences			
PhD research proposal writing	WUR	2015	6
Techniques for writing and presenting a scientific paper	WGS	2016	1.2
Advanced sensory methods and sensometrics, MCB-32806	WUR	2015	6
Quantitative Data Analysis: Multivariate Techniques, YRM-50806	WUR/YRM	2017	6
B) General research related competences			
Introduction to R for people who use Statistics	Qi statistics, UK	2015	1
WASS introduction course	WASS	2015	1
Reviewing a scientific paper	WGS	2018	0.1
"Measuring Up to Expectations: A Food-related Study about ANS Responses and Expectations"	Conference Measuring Behaviour	2015	1
PhD lunch colloquia series	MCB	2014-2015	1
Psychology of health and environmental behaviour. Priming	WASS	2015	0.5
PhD carousel	WGS	2016-2017	0.6
Presenting with impact	Wageningen in to Languages	2017	1.0
Efficient writing strategies	Wageningen in to Languages	2018	1.3
Project and time management	WASS	2018	1.5
Scientific artwork vector graphic and images	WGS	2018	0.6
C) Career related competences			
Teaching assistant Sensory Perception and Consumer Preference	MCB	2015	1
Teaching assistant Creating Frameworks for Marketing and Consumer Behaviour	MCB	2019	1
Competence assessment	WASS	2018	0.3
Guest lecture Nutritional Neuroscience	HNE	2017-2019	1
Student thesis supervision	MCB	2016-2017	1
WIAS course survival guide to peer review	WIAS	2017	0.3
Brain training	WGS	2018	0.3
Paper Review	Physiology and Behavior	2018	1
Career assessment	WGS	2018	0.3
Career orientation	WGS	2019	1.2
Total			36.2

*One credit according to ECTS is on average equivalent to 28 hours of study load

The second chapter of this thesis was part of the FOCOM project which is supported by the European Regional Development Fund and the Dutch province Gelderland and Overijssel (Grant number 2011P017004).

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design by Sanne Houwing and Luz M. Verastegui Tena.

Printed by Proefschriftmaken ll www.proefschriftmaken.nl