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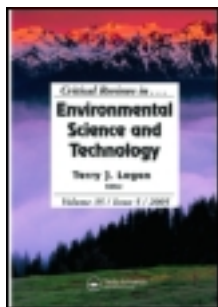
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Tastes and odors of water - Quantifying objective analyses: a review

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Usually, organoleptic criteria have to be fulfilled for tap water in order for it to be considered safe to drink. This can be achieved at three levels: chemical analysis, sensory analysis or through electrophysiology. While chemical analysis has been quite extensively discussed in the literature and as consumer perception is of vital importance, we propose a review of the latter two levels, namely sensory analysis and electrophysiology. We first recall some basics of perception and how it can be influenced by stimuli properties, human intrinsic factors and contextual factors, which are critical for an efficient measurement. Next, we present sensory analysis methods, as these are usually carried out in order to measure consumer appreciation of water. The drawbacks of such methods are then discussed before introducing the alternative of electrophysiological measurements. Some evidence that activity from the central and autonomic nervous systems can be measured in response to gustatory and olfactory stimuli in water is first described. Then, a review of objective physiological methods in the literature, developed to assess the emotional aspect of these reactions, is detailed. Finally, the possibility of correlating and predicting the quality, intensity and hedonic dimension of a stimulus in water with sensory self-report and nervous system responses is discussed.

Keywords: taste, odor, drinking water, central and autonomic nervous systems, electrophysiology, sensory analysis

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Introduction

The gustatory and olfactory appreciation of drinking water is very important in acceptance behavior. Drinking water must observe organoleptic criteria and have no off-flavors, which consumers associate with potential health risks. Many organic and inorganic compounds have been identified as responsible for after-tastes or after-odors in drinking water. The drinking water taste and odor wheel was developed by Suffet *et al.* (1999) and identifies the main tastes and odors in water, as well as some reference standards, reviewed by Suffet *et al.* (2004). This review has been used to develop a common language for odor and taste sensory panels and to present to the water industry the current knowledge about the identification of the “common” organoleptic characteristics found in drinking water. Taste and odor in drinking water are due to a complex mixture of molecules. Their concentrations are usually very weak, thus detecting and identifying them remains a difficult task. In this paper, it is proposed to review the different methodologies which enable the measurement of tastes and odors of water using humans as measuring instruments.

Figure 1 summarizes the overall elements that can be considered, from the stimulus itself to its final perception, and the possible measurements when addressing such a topic. It presents the influencing factors, such as the contextual factors influencing both the stimulus and the human subject, the human intrinsic factors influencing the perception chain, and three different types of measuring method which are available to obtain the characteristics of a stimulus at different levels of the chain.

There are purely objective methods to identify and/or quantify tastes and odors in water; chemical measurements can be made (link ① in Figure 1) directly on the stimulus, such as spectroscopy (Gowen *et al.*, 2012), solid-phase extraction (SPE), solid-phase microextraction with headspace (SPME-HS) or stir bar sorptive extraction (SBSE) coupled with liquid or gas chromatography mass spectrometry (LC/MS or GC/MS) analyses (Agus *et al.*, 2012; Maillet *et al.*, 2009; Sun *et al.*, 2012; Wu and Duirk, 2013). However, chemical measurements are not easy to correlate with human perceptions because the latter are the result of complex sensory and interpretation processes (Sáenz-Navajas *et al.*, 2010). Instrumental measurements can scarcely predict perceptions and are generally less sensitive than the human sensory system (Lawless and Heymann, 2010).

Several subjective methods have been developed to assess emotional reactions in response to olfactory or gustatory stimuli in water. Generally, the measurement of taste or odor performances of products is conducted by sensory panels using questionnaires about specific attributes or hedonic evaluation. Measurements obtained with sensory methodologies strive to be as objective as possible, although they are based on the individual's assessment (Lawless and Heymann, 2010; Meilgaard *et al.*, 2007). The self-report response recorded by sensory analysis (link ③ in Figure 1) is influenced by cognitive processing of the information and thus differs from the spontaneous emotional response before this integration. Moreover, the verbalization of feelings and perceptions is difficult and limited (Köster, 1990). How can we overcome these limitations and avoid the problem of subjectivity?

An alternative way would be to access the involuntary and unconscious responses via the study of the nervous system responses to assess the emotional aspect of reactions to the tastes and odors of water (link ② in Figure 1). Central and autonomic nervous system activities have been studied in order to measure emotional reactivity in response to gustatory and olfactory stimuli. The perception of these stimuli induces physiological changes, particularly cardiovascular, visceral, or glandular responses (Boucsein, 1992) which can be continuously recorded. The interest of these methods would be the ability to go back up the perception chain and to record responses before the cognitive processing of the information. Moreover, the responses obtained have been poorly connected to chemical measurements or sensory judgments, although new technologies have made these measurements easier.

Chemical measurements have been extensively researched and are well described in the literature; this paper focuses on methods based exclusively on human measurements (links ② and ③ in Figure 1). The process of sensory perception is described to understand how it works and how it can be influenced by numerous parameters. These influencing factors are also detailed. Then, the main sensory methodologies to quantify tastes and odors in water are reviewed, as well as their limitations. In order to comprehend the unconscious mechanisms occurring at the nervous system level, the nervous system functions and organization are described. An inventory is made of the electrophysiological methodologies which could be used to go deeper into the perception process and record information before the cognitive processing of the information. Finally, we discuss the possibility of combining methodologies in order to

correlate chemical measurements, sensory judgments and physiological responses and to predict the characteristics of a stimulus in water.

Perception: a subjective concept

Perception process

In everyday language, sensations and perceptions are often confused. However, they are two processes which are both distinct and complementary. The data processing of information from a sensory stimulus can be divided into at least three steps. The stimulus comes into contact with the sensory organ and is then converted into a nervous signal sent to the brain. The physical stimulus is transformed into a sensation if the threshold is reached. Sensation may be defined as a passive process which brings information from the outside world to the body and the brain. It is passive because there is no conscious action to engage the sensory process. Then, the brain interprets, organizes and integrates the information, using past experiences and memories, and transforms the sensation into perception. Finally, a response is made from the subject's perception (Goldstein, 2009; Meilgaard *et al.*, 2007).

Taste and olfaction

The sense of taste enables the nutritional value or dangerousness of food to be evaluated. The taste system, also known as the gustatory system, consists of about 10,000 taste buds located in the oral and pharyngeal cavities. Each taste bud contains taste receptors innervated by the cranial nerves which bring information to the central nuclei and the cortical areas that process the information. The brain associates a positive hedonic value with nutrients, related to the pleasure

brought by the food tasted, and a negative value with poisons. Nutrient sources are sought after and reflexively ingested while poisons are reflexively expelled and their sources avoided. Humans experience a limited set of taste qualities. There are four basic tastes: sweet, salty, sour, and bitter (Delwiche, 1996). A fifth basic taste has been defined as the taste of monosodium glutamate (Barry and Frank, 2002). The olfactory sense is considered not very developed in humans; there are about 6 million receptors in the roof of the nasal cavity. Humans might recognize about 10,000 different odors with 1000 different receptor types. An odor can be provoked by numerous organic molecules which are quite volatile. The odor is the organoleptic property distinguished by the olfactory organ "sniffing" some volatile chemicals (AFNOR, 2006). Odors seem to have powerful connections with memories, especially those that are linked to emotions. In fact, olfactory organs have anatomical connections with parts of the brain involved in emotions and the memory process (Robin *et al.*, 1998; Willander and Larsson, 2007). The perception of flavors in foods and beverages is due to a combination of taste and olfactory senses.

When tasting a water sample, many different parameters influence the final perception and the associated response (Figure 1). Three types of factor may intervene: stimulus characteristics, human intrinsic factors and contextual factors. These are detailed below.

Factors influencing sensory perception***Influence of stimulus characteristics*****Concentration**

The increasing flavor perception intensity of a solution is positively correlated with its concentration of sapid and odorous components. However, it is recognized that the relationship between these two parameters, perceived intensity and concentration, is not linear (Berglund *et al.*, 1971; Moskowitz and Arabie, 1970) but has a sigmoid shape (Figure 2). When the concentration of the stimulus is below the liminal region, i.e. in the infraliminal region, the taster cannot isolate the sensation caused by the substance from the background noise of the sensory system. The liminal zone corresponds to the region where the threshold values of the subject are found. Beyond this zone, i.e. in the supraliminal region, using suprathreshold stimuli, the perception becomes stable and its intensity increases with the concentration of sapid or odorous substances. In the supraliminal region, the relationship between the intensity of the perception and the concentration of the stimulus may be a power function (Stevens, 1969) or a logarithmic function according to the Weber-Fechner law proposed in 1831 by Weber (Fechner, 1860; Weber, 1995). Beyond a certain concentration, the saturation zone is reached and the perceived intensity stays almost stable despite an increasing concentration (Ramalho, 1999).

Moreover, for some chemicals, their concentration influences the quality of the perceived taste or odor. For example, the smell of 2-chlorophenol has been described as "musty, sweet, flowery" at

low concentrations and mainly "chemical disinfectant" at higher concentrations (Young *et al.*, 1996).

Temperature

The temperature of the tasted sample affects the perception of flavor intensity (Moskowitz, 1973; Pangborn *et al.*, 1970). Whelton and Dietrich (2004) studied the effect of two temperatures commonly encountered by consumers of tap water, 25°C and 45°C, on different odorous compounds in water. They showed that, at equal concentrations, the odor intensity of geosmin, methylisoborneol (MIB) and chlorine was higher at 45°C than at 25°C. For other compounds, such as isobutanol, the water temperature does not influence odor perception. The French standard (AFNOR, 2006) advises maintaining the samples at a controlled temperature of $23 \pm 2^\circ\text{C}$ during tastings.

Molecular structure

Some structural properties give a substance a sapid or odorous power. The compound should have a moderate molecular weight to be sufficiently volatile, low polarity, some solubility in water, and therefore in the saliva or mucus, a high vapor pressure (the higher the vapor pressure is, the faster it evaporates) and some lipophilic properties (in order to cross the cell membrane made of lipids). However, the substance does not need to possess particular functional groups or be chemically reactive (Meierhenrich *et al.*, 2005). The relationship between the structure of substances and their activity has already been studied and has yielded results. Blum *et al.* (1994) estimated the Quantitative Structure-Activity Relationships (QSAR) of 363 chemicals while

Brasquet *et al.* (1999) correlated the adsorption capacity of 55 organic compounds with their molecular structure using the QSAR method.

Regarding the relationship between the structure of an odorous molecule and its perceived odor, there are still many details to be clarified. The first difficulty is that the "response" is not a physical property or a measurable biological activity but a perception described qualitatively by a human subject. Moreover, the qualitative study of these relationships has shown that, in some cases, molecules with similar structures have similar but distinguishable smells, but that a small structural change can be sufficient to induce totally different olfactory perceptions. On the contrary, very different structures can have similar odors (Meierhenrich *et al.*, 2005).

Interactions in mixtures

When food is masticated, or when a drink is put into the mouth, the perceived flavor is a complex mixture of different odorous and sapid compounds. Recent studies have shown that a human subject is able to perceive up to three compounds in mixtures of tastes (Marshall *et al.*, 2005) or four in the case of odors (Livermore and Laing, 1998). Furthermore, identified odors or tastes are generally complex mixtures of odors or tastes themselves; they do not usually have the smell or taste quality and/or intensity of the mixture when sampled separately (Frijters, 1987). These qualitative and quantitative differences are due to several phenomena which may occur in odorant and taste mixtures at different levels: chemical, oral and cognitive (Keast and Breslin, 2003). They can be (i) a suppressive phenomenon, when the mixture intensity evaluation is lower than that for the stronger component, (ii) synergistic, when the mixture intensity evaluation is higher than the intensity response to one single component at the mixture concentration, (iii)

hypoadditive, when the mixture intensity evaluation is equal to that for the stronger component or (iv) averaging, when the mixture intensity evaluation is determined by the average effect of all the individual components. The effect of one component can be different depending on its concentration. Keast and Breslin (2003) reviewed the taste-taste interaction effect and highlighted a general behavior in which taste stimuli lead to an enhanced response at low intensity concentration mixtures, to additivity at medium intensity concentrations, and to suppression at high intensity concentrations.

Kim (2010) studied the masking phenomenon in competing odorant mixtures and showed that the threshold level of the mixtures was dependent on a single predominant compound and differed from the sum or the average intensity of all the components individually. Using chemicals with similar properties, they identified an averaging effect of all components on the intensity of the mixtures (Kim, 2011). Stevens (1996) gave an estimation of the degree of masking of each taste in a binary mixture and measured higher threshold levels when tastes were mixed. It was shown that sweet was the strongest suppressant of the other tastes in a mixture and the least masked by the other tastes (Green *et al.*, 2010). Münch *et al.* (2013) observed hypoadditivity between odorants on a particular receptor site in flies, due to competitive interactions between the ligands that bind to the same recognition site. Synergy between fruity odorants was observed (Ishii *et al.*, 2008) as well as between additives in meat (Aliani *et al.*, 2013).

Thus, even if the stimulus is controlled and similar from one subject to another, human subjects are used as measuring instruments in sensory analysis. And yet, humans are all different from each other, both intrinsically and temporally.

Human intrinsic factors

Thresholds

Parameters concerning the subjects can be studied in order to characterize them and assess their taste and olfactory functions. The threshold notion has been widely discussed and studied (Swets, 1961). Several types have been defined. The lowest concentration of a chemical substance that can be reliably detected is called its detection or absolute threshold (Bi and Ennis, 1998; Peng *et al.*, 2012). At very low concentrations, no taste or odor quality can be discerned, only that something is present that differs from the blanks. A second threshold type is the recognition threshold, defined as the lowest concentration at which odor quality is reliably discerned. The last type of threshold is the difference threshold, corresponding to the smallest amount by which a stimulus must be changed to make it perceptibly stronger or weaker. Lorig (2012) even discussed the existence of an unconscious effect of odors below sensory thresholds on the behavior. The French standard (AFNOR, 2012) gives a methodology for the measurement of individual thresholds on the basic tastes in water. Taste and odor concentration thresholds of potential drinking water contaminants have been measured in air and in water, including individual chemical types and material types (Table 1).

Age

The judgment of a taste and olfactory stimulus has been proved to lessen with age. Sensitivity at the detection threshold also decreases with increasing age (Koskinen and Tuorila, 2005; Murphy and Gilmore, 1989), but this decline has been found to be modest (Bitnes *et al.*, 2007a; Mojet *et al.*, 2001). Relative perception (intensity discrimination of one sample from another) does not seem to be affected by age while the absolute perception (intensity rating for one sample) decreases (Mojet *et al.*, 2003). The decline in sensitivity with age is believed to be greater in olfaction than in tasting (Kaneda *et al.*, 2000).

Gender

Odor and flavor perception abilities are affected by the gender of the subjects. Women are considered more sensitive to tastes and odors than men (Doty and Cameron, 2009), in terms of both detection and identification. Dalton *et al.* (2002) showed that sensitivity to odorants after repeated exposure increased faster and in a more efficient way in women than in men, i.e. their detection threshold was lower after repeated exposure. These results suggest that the feminine olfactory system could be associated with reproductive behavior, such as skin recognition and pair bonding. Taste perception also changes during pregnancy and the menstrual cycle (Kuga *et al.*, 1999).

Genetics

Genetics also determine sensitivity to taste and olfactory stimuli. Several studies have shown that some people are phenylthiocarbamide non-tasters, due to Mendelian recessive characteristics, or, on the contrary, supertasters (Bartoshuk, 2000). Recently, the same discovery was made for 6-n-

propylthiouracil (Reed *et al.*, 1999), which also has a bitter taste. Verhagen (2007) explains that there are neurophysiological convergences between a stimulus and individual intrinsic factors which are based on an anatomical reality. The phenomenon of perception may involve different areas, some of which could be directly related to the affective or hedonic dimensions, or to identity.

Experience

Bende and Nordin (1997) and Parr *et al.* (2002) showed that professional wine tasters had detection thresholds similar to novices, but they pointed out that expert tasters were better than novices at familiar odor discrimination and identification tasks. Some other approaches have highlighted a positive impact of training on discrimination performance. Labbe *et al.* (2004) observed a better discrimination of coffees after a panel was trained, whereas Chollet *et al.* (2005) and Valentin *et al.* (2007) only observed this phenomenon for beers that panelists had already sampled during previous tastings. Cardello *et al.* (1982) noticed a greater textural differentiation on the part of trained experts than of novices. Similarly, Rabin (1988) demonstrated that professional wine tasters performed better than novices in odor discrimination tasks. When it comes to verbalizing perceptions, the experts are more efficient than the novices. This can be explained by a better potential of the experts for generating accurate descriptors for the product tasted, as the vocabulary used is standardized and shared (Chollet and Valentin, 2001; Gawel, 1997; Labbe *et al.*, 2004). The level of expertise is closely linked to learning and memory, especially with regard to verbal learning and a focus on the relevant characteristics, and less on learning perception (Bitnes *et al.*, 2007b; Soufflet *et al.*, 2004).

Influential contextual factors

Numerous studies have shown that the response linked to the sensory image is affected by temporary factors such as context, adaptation, order of sample evaluation, number of samples to evaluate or sensory fatigue (Lawless and Heymann, 2010; Meilgaard *et al.*, 2007; O'Mahony and Rousseau, 2003; Schifferstein, 1996). According to O'Mahony and Rousseau (2003), in the case of discriminative tests such as the Two-Alternative Forced Choice (2-AFC) and Three-Alternative Forced Choice (3-AFC) tests, the variability of the responses, based on an instability of memory, grows with the number of samples and the time between two stimuli. Compliance with good practices (for example, controlling the tasting environment, presenting the samples using random three-digit coding and using random and counterbalanced orders, minimizing the number of samples to taste, etc.) makes it possible to limit these biases (Lawless and Heymann, 2010). The panelist's state of mind may influence the way of scoring the palatability of the solutions tasted, except for the bitter taste, which could provoke a significant biological mechanism (Greimel *et al.*, 2006).

Sensory analysis uses human subjects as measuring instruments (Meilgaard *et al.*, 2007) but is intended to be as objective as possible, by controlling the stimuli, intrinsic and contextual parameters.

Measurement of perception by sensory analysis

Sensory analysis, also called sensory metrology, has been defined as a scientific discipline used to evoke, measure, analyze and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 2004). Sensory analysis

expanded during the second half of the 20th century, especially in the food industry when consuming behaviors increased dramatically. Until then, it was used to check the physical, chemical, nutritional and microbiological safety of products. Manufacturers were trying to qualify the product's performance by comparing it with a reference whose characteristics were well-known, the objective being to standardize the making of the product and to keep it stable throughout the production process (Brocas, 1997).

Currently, the main objective of sensory analysis is to translate the consumer's desires and preferences into the well-defined and tangible properties of a given product. The hypothesis used to link them is that a part of sensation is structured by preference. By comparing and analyzing the product characteristics that consumers like or dislike, sensory analysis contributes to identifying both positive and negative aspects and adapting them in order to match the consumer's tastes.

Sensory tests

It is interesting to study several parameters in a product: its quality, intensity and hedonic dimension. In this paper, quality is defined as "*qualitas*", i.e. a set of inherent characteristics. Intensity represents the degree to which a characteristic is present in the product. The hedonic dimension of a product is its capacity to provide pleasure or displeasure. There are three main categories of sensory tests, each having different objectives and using panelists chosen on the basis of different criteria. Discriminative tests are the simplest ones; these aim to detect the presence or absence of perceptible differences between two products (Amerine *et al.*, 1965; Lawless and Heymann, 2010; Meilgaard *et al.*, 2007; O'Mahony and Rousseau, 2003; Peryam,

1958; Stone and Sidel, 2004). The triangle test (Sauvageot *et al.*, 2012), the paired comparison and the duo-trio test are examples of these. The second kind of method is one which can quantify the intensity of the perceived sensory characteristics of the products. These are descriptive methodologies, the two main ones being the Flavor Profile[®] method and Quantitative Descriptive Analysis[®]. In both these kinds of test, subjectivity (cultural past, personal experiences, etc.) is controlled as much as possible. In the case of water analysis, the Flavor Profile Analysis is the reference, and is described below. On the contrary, and by definition, the hedonic approach is subjective as the consumer is asked to evaluate the degree of pleasure or displeasure given by a certain product (Lawless and Heymann, 2010).

Flavor Profile Analysis

Among the methodologies used to evaluate the intensity of flavors in water, the Flavor Profile Analysis (FPA) is a reference (AWWA, 1993), and was adapted to water evaluation by Krasner *et al.* (1985) and Suffet *et al.* (1988). For each FPA descriptor from the water taste and odor wheel (Suffet *et al.*, 1999), a semi-quantitative 7-point scale is set. The scale ranges from 1, corresponding to the threshold, to 12, “very strong odor”, going by 2, “very weak”; 4, “weak”; 6, “moderate”; 8, “moderate to strong” and 10, “strong”. This method only requires a few panelists but they need to be highly trained. The major criticisms of FPA are that it is not a statistical method and that it is subjective. The profile obtained is generally treated qualitatively and intensities are not very well interpreted (Suffet *et al.*, 2004). Teillet *et al.* (2010) used sensory profiling and comparative methods for the sensory analysis of water and showed that these methods were not suited to the description of tastes and odors in water. They developed another

methodology, called Polarized Sensory Positioning, which enables the comparison of similarities between a sample and three prototypes named “poles” on a continuous scale ranging from “exactly the same taste” to “totally different taste”. This PSP method provides a discrimination of water samples but has some disadvantages. In fact, the PSP scale is subjective, due to the use of the words “totally different taste” which is an individual point of view. Moreover, the choice of the sensory poles (prototypes), with which the samples are compared, defines the results obtained. Finally, to date, this methodology does not include descriptive or hedonic data.

Classic sensory methodologies are difficult to apply and remain subjective in the domain of tastes and odors of water; thus, the challenge is now to go back up the perception chain and access the involuntary and unconscious events which occur when tasting or smelling water. In fact, it has been proven that unnoticed odors in the environment can alter mood and behavior (Zucco *et al.*, 2009). The central and autonomic nervous systems intervene in an involuntary way thus measurement of the physiological response might bring objective information which could supplement expressed perception. In order to understand the central and autonomic response, the functioning of the nervous system is now described.

Nervous system

Nervous system functions

The nervous system performs many complex tasks, particularly those concerning perception, language and memory. It also emits signals that determine the movements of the body and

regulate the functioning of internal organs. These tasks can be grouped into three basic functions: sensory, integrative and motor.

Regarding the sensory function, the sensory receptors detect internal or external stimuli then sensory neurons, also called afferent neurons, transmit sensory information to the brain and to the spinal cord through the cranial and spinal nerves (Guénard, 2001; Martin *et al.*, 2006).

Thus, the nervous system integrates and processes the sensory information. In order to do this, it analyzes the information, stores part of it, then decides how to respond. Neurons that contribute to the integrative function are either interneurons or short axon neurons that communicate with neighboring neurons of the brain or spinal cord. Interneurons constitute the vast majority of neurons in the human body.

Once the sensory information is integrated, the nervous system can respond. It determines the motor response; for example, muscle contraction or glandular secretion. Neurons performing this function are the motor neurons, also called efferent neurons or motoneurons. They transmit information either from the brain to the spinal cord or from the brain and the spinal cord to the effectors (muscles and some glands) via the cranial and spinal nerves. By stimulating the effectors, motoneurons trigger muscle contractions and glandular secretions.

Nervous system organization

The nervous system consists of two subsystems: the central nervous system (CNS), composed of the brain and spinal cord, and the peripheral nervous system (PNS), which includes all the parts of the nervous system located outside the CNS. It integrates all kinds of afferent sensory

messages and is also the processing center of thoughts, emotions and memories. Most of the nerve impulses responsible for muscle contraction and the secretory activity of glands come from the CNS. The PNS includes the cranial and spinal nerves as well as their respective branches, the ganglia and sensory receptors. The PNS is divided into three parts: the somatic nervous system (SNS) (soma: body), the autonomic nervous system (ANS) and the enteric nervous system (ENS).

Somatic nervous system

The somatic nervous system consists of two types of neuron. Sensory neurons transmit information from the head and skin somatic sensory receptors and from proprioceptors located in the joints and muscles to the CNS, and also information from the specialized sensory receptors of sight, hearing, taste and smell. Motor neurons carry impulses from the CNS to the skeletal muscles only. Given that motor responses thus produced can be consciously regulated, the activity of this part of the PNS is called voluntary.

Autonomic nervous system

The autonomic nervous system also consists of two types of neuron. Sensory neurons transmit information from the autonomic sensory receptors, mainly located in blood vessels and viscera, for example the stomach and lungs, to the CNS. Then, the motor neurons transmit the nerve impulses from the CNS to the smooth muscles, heart muscle and glands (Figure 3). Since the motor responses produced by the ANS are not usually regulated consciously, the activity of this part of the PNS is called involuntary. The autonomic nervous system itself is traditionally divided into two systems, sympathetic and parasympathetic (Furness, 2006), although there are

many interactions between them, usually antagonistic but complementary (Serratrice and Verschueren, 2005).

The sympathetic system is often heavily involved in stressful situations. It stimulates the organs involved in the defense of the body through the action of norepinephrine which leads to physiological changes. In these situations, heart rate, respiratory rate, blood pressure, sweating, muscle strength and mental activity are increased, which enables the individual to perform a more intense physical activity. The whole of these reactions is called the sympathetic response to stress and is observed in intense emotional states. However, in other circumstances, the sympathetic system has a local action. It controls the blood flow of the skin (thermoregulation) and the muscles (muscle activity) independently. Some sympathetic reflexes involve local circuits that pass through the spinal cord but not through higher centers.

On the contrary, the parasympathetic nervous system usually intervenes in a localized manner, involving only one or a few organs directly associated with large reflex functions that contribute to rest, assimilation and reproduction. The heart rate and the respiratory rate are slowed, the blood pressure decreases, the pupils contract, and the mechanisms of digestion are favored. The balance between these two systems enables the organism to react appropriately to the environment.

Autonomic nervous system activity has been studied and measured in order to obtain objective information about gustatory and olfactory stimuli. Many different methods exist. The main ones allowing ambulatory and easy measurement of autonomic activity are described in this paper.

Depending on the method, some parameters, such as the quality, intensity and hedonic dimension of the stimulus or the emotion induced by the stimulus, can be predicted, as summarized in Figure 4.

Methods for exploring central and autonomic activities

Functional central nervous system investigation tools

Central nervous system activity may be measured using different tools, especially functional Near Infrared Spectroscopy (fNIRS) and ElectroEncephaloGraphy (EEG). fNIRS is an optical method based on the property of hemoglobin to absorb near infrared light. EEG measures the electrical activity of the brain. The advantage of fNIRS and EEG is that the subject wears a set of probes on their head, which avoids their confinement in a magnetic resonance imaging scanner.

fNIRS

fNIRS has been used to study brain activity associated with language (Watanabe *et al.*, 1998), emotions (Herrmann *et al.*, 2003; Plichta *et al.*, 2011), cognitive conflict (Schroeter *et al.*, 2004) and, more recently, taste. A study using functional Magnetic Resonance Imaging (fMRI) showed that the activation of the orbitofrontal cortex seemed to be stronger when the gustatory stimulus had a higher hedonic value (Frank *et al.*, 2003). De Araujo *et al.* (2003) showed that the consonance for smell and taste combinations and their palatability are represented in the medial anterior part of the orbitofrontal cortex. Taster expertise also plays an important role in the neural representation of flavors. Wine tasting, which is a multimodal stimulus combining the effects of

taste and smell, activates brain regions modulated by expertise (Castriota-Scanderbeg *et al.*, 2005).

fNIRS detects signals from the lateral surfaces of the cortex, but this method does not measure the activity of deeper gustatory areas, such as the orbitofrontal and insular cortex, which near-infrared light cannot reach. However, recent studies suggest that the lateral prefrontal cortex is a crucial area involved in higher cognitive processes of taste and other eating behaviors (Kringelbach *et al.*, 2004). Using fNIRS during the tasting of different sucrose solutions, Okamoto *et al.* (2006) reported that the dorsolateral prefrontal cortex is involved in the memory encoding of taste. The same research group showed the activation of the ventrolateral prefrontal cortex when the task was simply to taste stimuli without memory processing (Okamoto *et al.*, 2009).

fMRI has confirmed that undetected odors change brain activity in the absence of awareness: this phenomenon is called "blind smell" (Li *et al.*, 2010; Sobel *et al.*, 1999). Bembich *et al.* (2010) have shown that individuals who do not perceive the bitterness of 6-n-propylthiouracil have no significant cortical activation during tasting.

EEG

The majority of studies focus on suprathreshold odors or tastes which can be self-reported. However, other studies have shown that unnoticed odors may affect behavior and mood (Zucco *et al.*, 2009). Using the electroencephalogram (EEG), similar odors perceived as identical by

subjects produced very different EEG activity. It was also found that EEG activity changed in subjects who did not perceive the presence of odorants compared to odor controls (Lorig, 2012).

Further research using EEG found significant differences in brain activity for different odor valences, even when smells were not consciously detected (Owen and Patterson, 2002). These results demonstrate the potential of this technique to highlight differences between hedonic responses, even when the molecule is at very low concentrations, or close to the threshold level. Martin (1998) showed that the theta frequency (4 to 7 Hz) was related to the palatability and relaxing ability of the olfactory stimulus and that the alpha activity (8 to 12 Hz) was reduced with odor perception. The theta frequency may reflect the ability of the smell to attract or distract, and therefore reflects changes in attention associated with the emotional response induced by the odor. An example of a filtered EEG signal for the theta waveband is given in Figure 5.

Measurements of olfactory event-related potentials (OERP) have shown that the amplitudes and latency of signals are related to stimuli intensity (Tateyama *et al.*, 1998). Studying the time-frequency changes in an EEG signal, Hashida *et al.* (2005) found that the signal was dependent on different taste stimuli and they were able to differentiate sweet and salty taste stimuli.

Autonomic nervous system investigation tools

Facial reactions

The gusto-facial reflex is a singular phenomenon, triggered by subliminal stimuli. The psychologist Paul Ekman has contributed most to the modern analysis of facial reactions. He

evidenced automatic, universal and innate moves related to basic emotions (surprise, disgust, sadness, anger, fear, and happiness) (Ekman, 1999). This was systematically studied by Steiner (1973), who provided details about this phenomenon: from the first moments of life, the newborn reacts to stimuli by a facial expression (this response might already exist *in utero*). These expressions and gestures are, on the one hand, present in every child and, on the other hand, different from one stimulus to another (salty, sweet, sour, bitter) but identical for the same stimulus, from one individual to another. This reflex expression, without any intention, occurs in a heavily emotionally loaded social context (food situation in infants): as soon as family or friends perceive the facial expression, they try to interpret it and give it a meaning. Chiva (1979) also followed the phenomenon of the gusto-facial reflex in subjects from birth up to the age of 24 months. Clearly, this is not only about the study of taste sensation but is also part of a general problem of psychology: the metamorphosis of biological into psychological, the establishment of the processes of nonverbal communication, the study of the innate basis that some facial expressions have as an expression of emotions. In the psychological literature, emotion has been defined as the individual response to relevant stimuli, which include behavioral, physiological and experiential components (Gross and Thompson, 2007).

There are at least three main ways for psychologists to assess the facial expressions of emotions:

- The first is to use novice observers who look at the pictures and judge, in a holistic manner (taking the pictures as a whole, and not specific points of the images), the degree to which they see the emotions on the target faces. Although relatively simple and quick to perform, this technique is limited because programmers can miss subtle

facial movements, and the coding may be biased by idiosyncratic morphological characteristics (specific to each individual) of different faces. Furthermore, this technique does not isolate which features in the face are responsible for particular emotional expressions.

- The second approach is to use basic coding schemes in which observers are trained to use a highly regulated process technology to detect facial movements. For example, the Facial Action Coding System (FACS) developed by Ekman and Friesen (1978) is a complete measurement system that uses assessments of facial movements frame-by-frame, called action units. Each action unit is either the contraction or the relaxation of one or more muscles; one facial expression corresponding to an emotion is thus composed of several action units (Table 2). This technique has several advantages including the wealth of data obtained and the ability to discover new facial movements. Its disadvantage is that coding the points frame-by-frame is extremely laborious. However, Hamm *et al.* (2011) have developed an automated FACS based on the latest computer science technology. The system automatically tracks faces in a video, extracts the geometric and texture features, and produces temporal profiles of each facial muscle movement. These profiles are quantified to calculate the frequencies of single and combined action units in videos.
- The last approach consists in obtaining more direct measurements of facial muscle movement by electromyography (EMG) with electrodes attached to the skin of the face. Although this technique can measure sensitive characteristics, electrode

placement is difficult and also relatively restrictive for the individuals who wear them.

Except for the "social smile" for unpleasant tastes, facial reactions remain more or less the same into adulthood for the basic expressions. However, some supplementary reactions may reflect socialization influences (Greimel *et al.*, 2006; Weiland *et al.*, 2010). The research of Wendin *et al.* (2011) shows that facial expressions enable stimuli quality and concentration to be distinguished. The intensity of most facial reactions increases with increasing stimulus concentration while its pleasantness decreases in parallel. Bailenson *et al.* (2008) demonstrated the possibility of finding good connections using statistical algorithms to predict emotions from the natural facial expressions of subjects. When models are adapted to the individual level, they give better results than other, more general, models. This study also demonstrated the importance of conducting experiments on emotions in as natural an environment as possible. It is very important to supplement the information obtained from the analysis of facial expressions with physiological data in order to increase model accuracy. Negative hedonic sensations seem to be associated with higher electromyographic activity in the levator labii muscle region, while positive hedonic sensations are associated with lower EMG activity in the same muscle region. Facial EMG activity can be used as an indicator of palatability in humans (Hu *et al.*, 1999). Reactions to pure water have been described as neutral (Ganchrow *et al.*, 1983).

Pupillary response measurement

Sympathetic stimulation causes a contraction of the radial fibers of the iris and therefore pupillodilation (mydriasis). On the contrary, parasympathetic stimulation causes a contraction of the sphincter of the iris and thus pupillary constriction (Neuhuber and Schrödl, 2011).

Pupillometry was first used for medical purposes; for example, to adjust the amounts of anesthetic used. Pupillodilation has been associated with cognition effort in face memorization and recognition tasks (Goldinger *et al.*, 2009), the memorization of number, word or phrase sequences (Papesh and Goldinger, 2012; Piquado *et al.*, 2010; Vö *et al.*, 2008) and lexical translation tasks (Hyönä *et al.*, 1995). Pupil size also plays a role in the communication of emotions between people. For example, it has been shown that women's pupil size affects men's judgment; they are more attracted to women with more dilated pupils (Tombs and Silverman, 2004). The relationship between emotional processes and changes in pupil size has been studied and debated. Metalis and Hess (1982) used photographs with different emotional themes and suggested that there is a continuum from extreme dilation for interesting or pleasant stimuli to extreme constriction for unpleasant or painful stimuli. This view was challenged by Janisse (1974) who argued that there is no pupillary constriction in response to a negative stimulus, or that this response is limited to a few individuals and a limited range of stimuli. Earlier, Loewenfeld (1966) had also studied the effects of various sensory and psychological stimuli on changes in pupil diameter and argued that none of them, except an increase in light intensity, caused pupil contraction, (Partala and Surakka, 2003). Janisse (1974) suggested that pupil size is linearly related to the intensity of the stimuli but that the variation in pupil size seems to behave

curvilinearly regarding the stimulus valence. Thus, the pupil is larger for positive and negative emotions and smaller in the center, for a neutral emotion. Recent studies of Partala and Surakka (2003) using auditory stimuli and of Bradley *et al.* (2008) using images with different hedonic valences seem to confirm that pupil dilation is caused by stimuli of both positive and negative valences, and that a smaller expansion is observed for a more neutral stimulus.

Very few studies have been undertaken to evaluate the effect of olfactory or gustatory stimuli on the pupillary response. Schneider *et al.* (2009) studied the effect of olfactory stimuli with different intensities and different hedonic valences on pupillary responses. They showed a reproducible pupillodilation as a sign of sympathetic activity. There seemed to be a negative correlation between the stimulus intensity and the latency of the reaction onset and between the stimulus intensity and the latency of the pupillary reaction maximum. The stronger the stimulus, the shorter the latency time, both for the reaction onset and to reach the maximum. No correlation between the stimuli hedonic valence and the pupillary response was really found.

Heart rate variation

The heart rate (HR) is controlled by the sympathetic and parasympathetic nervous systems. Sympathetic stimulation leads to an increased heart rate, whereas a parasympathetic one causes its reduction. Horio (2000) as well as Rousmans *et al.* (2000) showed that the heart rate increased for all taste stimuli compared to the heart rate before stimulation. Increases in heart rate were negatively correlated with hedonic ratings, except for the sweet taste. Bensafi *et al.* (2002a) showed that pleasantness was the best dimension to predict heart rate variations. The higher the concentration, the higher the heart rate increases. Heart rate variation has been found to be one of

the most relevant parameters for discriminating tastes (Rousmans *et al.*, 2000) or odors (Alaoui-Ismaili *et al.*, 1997b; Robin *et al.*, 1998).

The study of Heart Rate Variability (HRV), by carrying out a spectral or time-frequency analysis, gives information about both autonomic nervous systems. The cardiac rhythm high-frequency component (HF, 0.15 – 0.40 Hz) corresponds to the vagal influence (parasympathetic) of the sinus node while the low frequency (LF, 0.04 – 0.15 Hz) corresponds to mechanisms in both sympathetic and parasympathetic nervous systems (Malik, 1996). The very-low frequency (VLF, 0.003 – 0.04 Hz) corresponds to metabolic and humoral processes. Muroi *et al.* (2011) used time-frequency analysis with pleasant and unpleasant oral flavor stimuli which induced bradycardia and tachycardia, respectively. Figure 6 presents an example of a heart rate signal during five odor stimulations. The four first odors were pleasant and the last one was unpleasant. From the figure, it can be seen that an unpleasant odor induces a longer reaction and higher activity in the HF bandwidth.

Electrodermal response

Electrodermal (EDR) parameters have been studied since the 1880s and have become the bioelectric factors most commonly used in psychophysiology. This success is mainly due to the ease of obtaining a clear electrodermal response, and because of the probable link between response intensity and stimulus intensity as well as its psychological meaning (Boucsein, 1992; Dawson *et al.*, 2000). EDR activity has been defined as the whole of skin electrical phenomena (SR: Skin Resistance, SC: Skin Conductance and SP: Skin Potential), directly related to some extent to sweat gland activity and skin blood flow. An entire set of parameters can be extracted

from the EDR signal (Figure 7). The response may be quantified by its amplitude and its duration, also called the Ohmic Perturbation Duration (Vernet-Maury *et al.*, 1995). Generally, the amplitude and the Ohmic Perturbation Duration increase with the stimulus impact perceived by the subject.

Møller and Dijksterhuis (2003) showed that the use of the amplitude and latency parameters on skin conductance responses were not sufficient to discriminate unpleasant odors from pleasant ones. Rousmans *et al.* (2000) showed a significant effect of the basic tastes on skin resistance amplitude and duration; both were higher for bitter, acid and salty tastes than for the sweet taste and water. The duration of the response was found to be more relevant than its amplitude. Bensafi *et al.* (2002a) found a positive correlation between the arousal and intensity of an olfactory stimulus and skin conductance variations. The more arousing and intense a stimulus, the more the skin conductance level increased.

Potential measurement is simple; the difference in the spontaneous potential is recorded and amplified between two electrodes placed on cutaneous surfaces. Conductance measurement requires a small current with a constant voltage between these two electrodes (Grapperon *et al.*, 2012). An example of the measurement apparatus and the signal obtained is shown in Figure 8. Recommendations for electrodermal measurements are given by Fowles *et al.* (1981).

Skin blood flow

There are different ways to measure skin blood flow variations. Many studies have used a Hematron sensor, developed by Dittmar *et al.* (1992), although the laser Doppler is a more sensitive instrument.

When rats were exposed to odors, the laser Doppler highlighted an increased cortical blood flow (Major and Silver, 1999). It was also used on human subjects during the cephalic phase of digestion, before food had been taken into the mouth. Results showed that blood flow increased under the influence of a stimulus during this phase (Buss *et al.*, 2012). Likewise, an increased blood flow was observed when subjects had to chew or eat solid food (Someya and Hayashi, 2008). The laser Doppler was sensitive enough to detect changes in skin blood flow when small amounts of water samples were administered orally (Wipke-Tevis and Williams, 2007). Using water samples, Rousmans *et al.* (2000) showed that skin blood flow enables each of the basic tastes to be discriminated. The amplitude of the vasoconstriction was significantly higher for bitter, acid and salty tastes than for water and significantly lower for sweet than for bitter taste. Skin blood flow variations can be linked to hedonic responses: the pleasanter the solution, the lower the amplitude and duration. This link was confirmed by Kashima and Hayashi (2011) who demonstrated that sweet, umami, and bitter taste stimuli elicited characteristic facial skin blood flow responses. They showed that many facial skin blood flow aspects reflect the hedonic valence of the stimuli; the skin blood flow responses observed in the eyelid and nose represented signals of gustatory information. An example of a skin blood flow signal in response to basic tastes is shown in Figure 9.

Multiparametric approaches

Rousmans *et al.* (2000) showed that there is a significant effect of basic tastes on skin resistance amplitude and duration, on skin temperature amplitude, and on skin blood flow amplitude (vasoconstriction) and that instantaneous heart rate increases. Skin resistance and cardiac responses were the most relevant parameters. The different tastes have been associated with significantly different autonomic nervous system responses: sweet taste, which has a positive hedonic valence (pleasant) and is innate-accepted, caused the weakest ANS response, while salty, sour and bitter tastes, which are rather unpleasant, elicited stronger ANS responses, the innate-rejected bitter taste inducing the strongest ones. Similarly, a pleasant smell induces weaker autonomic responses than an unpleasant one. Moreover, the same smell can induce a different response depending on the subject's experience (Robin *et al.*, 1998). Changes in heart rate seem to be correlated with whether the sensation is pleasant or not, and changes in skin conductance seem to be correlated with arousal. However, the distinction between the concepts of acceptance and rejection on one hand, and the notions of pleasure and displeasure based on autonomic responses on the other hand is not so clear. Autonomic responses seem rather to reflect an innate acceptance of the taste than a sensory pleasure (Leterme *et al.*, 2008). In addition, there is a strong correlation between the dimensions of arousal and intensity (Bensafi *et al.*, 2002a). Subjects might involuntarily categorize odors by their pleasantness (Bensafi *et al.*, 2002b).

Electrodermal, cardiac and respiratory responses can discriminate between each basic emotion but no single index or single group of parameters can distinguish each emotion; in order to do

this, responses must be taken as a whole (Collet *et al.*, 1997). There is a specificity of the response depending on the subject, who generally shows a preferential channel of specific responses to stimuli. This is why the study of multiple autonomous parameters is necessary (Lacey *et al.*, 1953). A high correlation has been found between hedonic evaluation and the autonomic estimation of the basic emotions (Alaoui-Ismaili *et al.*, 1997b; Ekman *et al.*, 1983; Robin *et al.*, 1998). A multiparametric analysis of the autonomic nervous system can identify the response quality, that is to say the type of basic emotion, in addition to its intensity (Alaoui-Ismaili *et al.*, 1997b; Vernet-Maury *et al.*, 1999). There is a good correlation between verbal and ANS-estimated basic emotions (Alaoui-Ismaili *et al.*, 1997a).

Sensors have been developed to measure these parameters of autonomic nervous system activity (Dittmar *et al.*, 1992; Dittmar *et al.*, 1995). They enable the real-time measurement of six parameters simultaneously with no interference. Many indexes have been defined to analyze the results of these measurements (Dittmar *et al.*, 1991; Vernet-Maury *et al.*, 1995).

Table 3 summarizes the studies which have used physiological measurements in response to gustatory and olfactory stimuli.

Factors influencing autonomic responses

Age

Studies on the effects of age on EEG responses have shown that there is an age-related decline in the amplitude of the response, as well as in the processing speed (Evans *et al.*, 1995; Morgan *et al.*, 1997; Murphy *et al.*, 2000).

Gender

Robin *et al.* (2003) examined the influence of gender on emotional responses during tasting, by comparing autonomic responses and basic emotions associated with primary tastes between men and women. The results showed that mean changes in each autonomic parameter were not significantly different between genders, although skin resistance and cardiac responses were stronger in men than in women. However, the study by Bailenson *et al.* (2008) suggested that women are more expressive than men in their facial reactions to emotions. With regard to brain activity during an olfaction activity, the left orbitofrontal cortex was more active in women than in men in the study by Royet *et al.* (2003), possibly related to the advantage of women in odor identification. Pupillodilation in response to an auditory stimulus did not seem to be influenced by the sex of the individual (Partala and Surakka, 2003) while response amplitudes of olfactory event-related potentials were larger in female subjects (Evans *et al.*, 1995).

Lateralization

Like other sensory systems, higher olfactory functions are lateralized (Brand, 1999); left-handers discriminate odors presented to the left nostril better, and this pattern is reversed in right-handers (Hummel *et al.*, 1998). The emotional processing of odors might be lateralized (Royet *et al.*, 2003). Right-handed subjects exhibited larger skin conductance amplitudes when measured on the left hand while comparing pleasant and unpleasant odors (Brand and Jacquot, 2001).

Discussion and future challenges

Perceptions are the result of complex phenomena processed by the nervous system. Instrumental measurements cannot predict them accurately because of their lack of sensitivity to reflect this complexity compared to the human sensory system. Perception measurement using humans as instruments can be approached from two points of view: psychological or neurophysiological. The first one measures expressed judgment using sensory analysis, but the perception is subjective due to multiple factors of influence. These influencing factors have been reviewed and are divided in three categories: stimuli characteristics, human intrinsic factors and contextual factors. The sensory methodologies described in this paper aim at controlling these factors but they remain subjective.

In order to avoid this subjectivity problem, it is proposed to go back up the perception chain and to measure the involuntary and unconscious responses which occur when tasting or smelling a water sample. These events have been shown to take place at the nervous system level, particularly in the central and autonomic nervous systems. Thus, the physiological approach measures an objective response. The choice of methodology will vary depending on the objectives. For example, physiological methodologies provide an objective approach to the quality discrimination of low-intensity tastes and odors, intensity and hedonic perceptions, and the associated emotions.

Electrodermal and cardiorespiratory responses, as well as skin conductance, facial reactions and EEG measurements, can discriminate between different tastes and odors. Heart rate,

electrodermal and cardiorespiratory responses, pupillary responses, EEG signals and facial reactions provide information about the intensity of the olfactory or gustatory stimulus. The hedonic tone of the stimulus might be predictable from heart rate responses, electrodermal measurement, skin temperature and skin blood flow, facial reactions, electromyographic and EEG measurements. On the contrary, it seems that skin conductance variations are not correlated with the hedonic tone of the stimulus. Electrodermal and cardiorespiratory responses as well as facial reactions can be associated with each basic emotion. The only methodology used with infraliminal concentrations is EEG, which has shown its ability to provide significant results even when the odor or taste was not consciously perceived. Pupillary responses to taste and odors are quite unknown, thus this could be a future research area. However, cardiorespiratory and electrodermal measurements have been widely used to assess taste and odor in water and are less restrictive for the panelists, so these remain a reference in this field.

Most studies have used suprathreshold odors or tastes. In the specific case of odors and tastes in water samples, the stimuli concentrations are very low, and the physiological methods have to be adapted to this constraint. The simultaneous use of sensory and physiological methodologies might provide the whole perception, both expressed and non-expressed, of subjects. Moreover, combining sensory and multiple physiological methodologies could enable the prediction of the quality, intensity and hedonic dimensions of the stimuli at the same time, in an objective manner, even in complex mixtures.

The development of objective methodologies and tools to link emotions and real-time consumer behavior in food and beverage marketing studies is currently a growing field of investigation,

because of its potential to provide information which cannot be measured by classic sensory techniques. Different methods have been developed in order to quantify emotional responses, such as questionnaires, which are the most common method, and physiological measurements, following the rule “the body cannot lie”. This paper has reviewed the physiological approach to measuring perceptions and emotions, which has been found to provide very interesting information. Nevertheless, these methodologies need to be carried out carefully, because of their high sensitivity to the non-oriented stimulation of another sense, parasite tasks, habituation effects or the mental state of the subject.

Acronyms

ANS: Autonomic Nervous System

CNS: Central Nervous System

ECG: ElectroCardioGram

EDR: ElectroDermal Response

EEG: ElectroEncephaloGram

EMG: ElectroMyoGraphy

ENS: Enteric Nervous System

FACS: Facial Action Coding System

FFT: Fast Fourier Transform

fMRI: functional Magnetic Resonance Imaging

fNIRS: functional Near InfraRed Spectroscopy

HR: Heart Rate

HRV: Heart Rate Variability

IHR: Instantaneous Heart Rate

MIB: 2-MethylIsoBorneol

MSG: MonoSodium Glutamate

OPD: Ohmic Perturbation Duration

PNS: Peripheral Nervous System

SBF: Skin Blood Flow

SC: Skin Conductance

SNS: Somatic Nervous System

SP: Skin Potential

SR: Skin Resistance

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Table 2: Example of action units and their combinations to express emotions

Table 3: Summary of published studies of different physiological measurement methods in response to gustatory and olfactory stimulations

Table 1: Odor and taste descriptors and concentration thresholds in water and in air of water contaminants (adapted from Van Gemert, 2011)

Compound	CAS number	Odor descriptors	Odor threshold		Taste descriptors	Taste threshold in water (µg.L ⁻¹)
			in water (µg.L ⁻¹)	in air (mg.m ⁻³) ^f		
a) Pesticides						
Atrazine	1912-24-9	Plastic, polythene, weak bleach ^a	9,200 ^a	-	Bitter, astringent ^a	20 ^a
Bromoxynil	1689-84-5	Disinfectant, glue, hospital, plasters ^a	>11,000 ^a	-	-	-
Carbaryl	63-25-2		37-280 ^a	-	Antiseptic, plasters, earthy ^a	44-140 ^a
Chlorfenvinphos	470-90-6	Insecticide, fertilizer, antiseptic ^a	240-340 ^a	-	Insecticide, fertilizer, antiseptic ^a	3.6 ^a
Chlormequat chloride	999-81-5	-	>8,700 ^a	-	-	>1,400 ^a
Chlortoluron	15545-48-9	-	>9,000 ^a	-	-	>740 ^a
Dalapon	75-99-0	-	>11,000 ^a	-	-	-
Diazinon	333-41-5	Earthy, musty, potato, cabbage water ^a	40-170 ^a	-	-	>55 ^a
Dichlobenil	1194-65-6	Plastic, cardboard ^a	40-200 ^a	-	-	-
Dichlorprop	120-36-5	-	>9,100 ^a	-	-	-
Diquat dibromide	85-00-7	-	>8,900 ^a	-	-	>56 ^a
Isoproturon	34123-59-6	-	>8,000 ^a	-	Bitter, acid ^a	37 ^a
Linuron	330-55-2	-	>9,700 ^a	-	-	-
Maleic hydrazide	123-33-1	-	>9,900 ^a	-	Metallic, menthol ^a	9,900 ^a
MCPA	94-74-6	Antiseptic ^a	460 ^a	-	Bitter ^a	4.1 ^a
MCPB	94-81-5	-	>10,000 ^a	-	-	>950 ^a
Mecoprop	7085-19-0	-	>8,100 ^a	-	-	>650 ^a

Paraquat dichloride	1910-42-5	-	>8,600 ^a	-	-	>28 ^a
Pirimicarb	23103-98-2	-	>1,100 ^a	-	-	>580 ^a
Propyzamide	23950-58-5	Dusty, sooty, cardboard ^a	700-3,000 ^a	-	-	-
b) Phenolic and anisole compounds						
4-Chloroanisole	623-12-1	Musty, medicinal, perfume, musky, wet paper ^a	<2.0-20 ^a	0.0029	Musty, stale, perfume, earthy, aniseed, sweet ^a	6.2-10 ^a
4-Chloro-2-methylphenol	1570-64-5	Chemical, TCP, medicinal, acetone ^a	62-1,800 ^{a,f}	-	Chlorine, TCP, medicinal, stale, salty ^a	2.5-10 ^a
4-Chloro-3-methylphenol	59-50-7	Musty, stale, wet paper, woody, damp ^a	2.5-3,000 ^{a,f}	-	Stale, musty, damp, chlorine ^a	2.5-9 ^a
2-Chloro-4-methylphenol	6640-27-3	-	0.15-0.30 ^a	-	-	<0.05-0.3 ^a
2-Chlorophenol	95-57-8	-	0.088-10 ^{a,f}	0.0005-0.019	-	0.14-0.97 ^a
4-Chlorophenol	106-48-9	-	0.5-1,240 ^{a,f}	0.001	-	39-62 ^a
2,4-Dichloroanisole	553-82-2	Musty, stale, chemical, roses ^a	0.21-1.9 ^{a,f}	0.0066-0.01853	Stale, musty, stagnant, roses ^a	0.08-0.4 ^a
2,4-Dichlorophenol	120-83-2	Musty, antiseptic, medicinal, TCP ^a	0.65-210 ^{a,f}	0.00027	Antiseptic, disinfectant ^a	0.98-2.5 ^a
2,6-Dichlorophenol	87-65-0	Musty, antiseptic, medicinal, TCP, metallic ^a	2-200 ^{a,f}	-	Disinfectant, antiseptic, chemical, TCP, musty, earthy, metallic ^a	0.0062-0.02 ^a
Pentachlorophenol	87-86-5	-	9.3-1,600 ^{a,f}	-	-	8 ^a
Phenol	108-95-2	Wet paper, wet newspaper, cardboard ^a	9.5-58,585 ^{a,f}	0.0172-20	Metallic, bitter ^a	<2 ^a
2,4,6-Trichloroanisole	87-40-1	Dusty, musty, earthy, rotten vegetable ^{a,e}	0.00003-0.03 ^{a,e,f}	0.0000037-0.01	Musty, stale, antiseptic, earthy ^a	0.025-0.05 ^a
2,4,5-Trichlorophenol	95-95-4	-	11-350 ^{a,f}	-	-	100 ^a
2,4,6-Trichlorophenol	88-06-2	-	100 ;->1,000 ^{a,f}	0.00016-0.021	-	>12 ^a
c) Naturally occurring organic compounds						

Benzaldehyde	100-52-7	Sweet ^e	0.010-4,600 ^{e,f}	<0.01-3,400	-	-
Cineole (Eucalyptol)	470-82-6	Eucalyptol ^e	1,1-1,000 ^f	0.000069-7	-	-
β -Cyclocitral	432-25-7	Fresh grass, hay/woody, tobacco-like ^c	3-19.3 ^{c,f}	-	-	-
Dimethyl trisulfide	3658-80-8	Decaying vegetation, septic ^e	0.00007-0.1 ^{e,f}	0.00006- 0.014	-	-
Dimethyl disulfide	624-92-0	Decaying vegetation ^e	0.16-90 ^{e,f}	0.0011-5.6	-	-
Geosmin (1,10-Dimethyl-9-decalol)	19700-21-1	Musty, earthy, stagnant, grassy, beetroot mold ^a	0.0008- 0.36 ^{a,b,e,f}	0.000049- 0.0005	Musty, earthy, stale, beetroot, mold ^a	0.0075-0.016 ^a
Hexanal (Hexaldehyde)	66-25-1	Lettuce heart ^e	0.32-750 ^{e,f}	0.00082-84.5	-	-
<i>cis</i> -3-hexen-1-ol	928-96-1	Sharp, grassy, decaying vegetation ^{d,e}	3.9-7,000 ^{d,e,f}	0.004-0.078	-	-
<i>cis</i> -3-hexenyl acetate	3681-71-8	Freshly cut grass, sweet, fruity ^d	1-320 ^{d,f}	0.009-0.036	-	-
Indole (2,3-Benzopyrrole)	120-72-9	Septic ^e	11-25,000 ^{e,f}	0.000033- 0.0081	-	-
2-Isobutyl-3-methoxypyrazine	24683-00-9	Woody, stale, musty, coal- dust, ash ^a	<0.00005- 10 ^{a,e,f}	0.000002- 0.000005	Creosote, stale, dusty, coal-dust ^a	0.0004-0.003 ^a
2-Isopropyl-3-methoxypyrazine	25773-40-4	Earthy, musty, bell-pepper ^e Sooty, dusty, cabbage, wet paper ^a	<0.00003-10 ^{a,f}	0.0000005- 0.000005	Musty, vegetable water, stale, peas, asparagus ^a	0.0099-0.02 ^a
2-Methyl-isoborneol	2371-42-8	Earthy, musty, potato bin ^e Musty, earthy, brazil nuts, peaty ^a	0.0012-0.1 ^{a,b,e,f}	0.000003- 0.0005	Earthy, musty, moldy, peaty ^a	0.0025-0.018 ^a
trans-2,cis-6-Nonadienal	557-48-2	Cucumber ^e	0.0018-0.8 ^{e,f}	0.00006- 0.022	-	-
d) Other organic compounds						
Benzene	71-43-2	TCP, musty, phenolic ^a	72-111,000 ^{a,f}	1.5-1,000	-	-
Chlorobenzene	108-90-7	Medicinal, chemical, musty ^a	80-190 ^{a,f}	0.4-59.3	Bitter, cardboard, musty, paint ^a	190 ^a

Chloroform	67-66-3	Chemical, antiseptic, acetone, estery ^a	90-30,000 ^{a,f}	0.5-6,900	Metallic, sweet, cardboard, stale ^a	1,200-2,000 ^a
2-Chlorotoluene	95-49-8	Nail varnish ^a	10-980 ^{a,f}	0.95-1.4	Musty, stale, nail varnish ^a	980 ^a
3-Chlorotoluene	108-41-8	Disinfectant, TCP, antiseptic ^a	150-500 ^{a,f}	-	Disinfectant, TCP, bitter ^a	390-770 ^a
4-Chlorotoluene	106-43-4	Marzipan, almond ^a	40-150 ^{a,f}	-	Marzipan, aniseed ^a	24-44 ^a
1,2-Dichlorobenzene	95-50-1	Grassy, vegetable water ^a	10-450 ^{a,f}	0.12- <300	Cardboard ^a	200 ^a
1,3-Dichlorobenzene	541-73-1	Medicinal, disinfectant, musty ^a	20-170 ^{a,f}	-	TCP, cardboard, antiseptic ^a	190 ^a
1,4-Dichlorobenzene	106-46-7	Almond, sweet, marzipan, antiseptic ^a	0.3-30 ^{a,f}	0.73- <90	Almond, cherry sweets, aniseed ^a	11-32 ^a
Ethylbenzene	100-41-4	Musty, plastic, resin, oily, chemical, styrene, stale ^{a,e}	2.4-2,205 ^{a,e,f}	<0.01-78.3	Plastic, resin, oily, chemical, styrene, glass fiber ^a	390-780 ^a
HHCB	1222-05-5	Perfume, floral, soapy, musk, sweet ^a	1.4-5 ^{a,f}	0.000489-0.042	Perfume, floral, metallic, stale, plastic ^a	22-50 ^a
Isopropylbenzene	98-82-8	Windowlens, polish, paint, pear drops ^a	10-100 ^{a,f}	0.017-6.4	Stale, bicarbonate ^a	60 ^a
4-Isopropyltoluene	99-87-6	Rubber gloves, paraffin, sweet, TCP ^a	5.01-400 ^{a,f}	-	-	>1,000 ^a
Methyl <i>tert</i> -butyl ether	1634-04-4	Estery, vanilla, sweet ^a	0.36-212 ^{a,f}	0.11-0.63	Estery, bitter ^a	40-48 ^a
Naphthalene	91-20-3	Mothballs ^a	1-500 ^{a,f}	0.007-5.34	Mothballs ^a	25-50 ^a
Styrene	100-42-5	Rubber, paint, sulfurous ^a	3.6-730 ^{a,f}	0.012-258	Paint, rubbery, cardboard, shoe polish ^a	94 ^a
Toluene	108-88-3	Paint, chemical, weak petrol ^a	24-20,000 ^{a,f}	0.08-1,000	Bitter ^a	960 ^a
1,1,1,-Trichloroethane	71-55-6	Dusty, sooty, polish ^a	470-20,000 ^{a,f}	5.3-3,900	Stale, drying, musty, Chinese food ^a	1,500 ^a
e) Inorganic compounds						
Aluminum sulfate	10043-01-3	-	-	-	Musty, moldy, stale	4,000-7,400 ^a
Copper sulfate	7758-98-7	-	-	-	-	>990 ^a

^aYoung *et al.*, 1996, ^bPersson, 1980, ^cYoung *et al.*, 1999, ^dKhiari *et al.*, 1995, ^eYoung and Suffet, 1999, ^fVan Gemert, 2011

Table 2: Example of action units and their combinations to express emotions (adapted from Lepron, 2009)

1	Inner Brow Raiser	Anger	4+5+17+23+24
2	Outer Brow Raiser	Disgust	9+10+17+25
4	Brow Lowerer	Joy	6+10+11+12+13+25+37
5	Upper Lid Raiser	Fear	1+2+4+5+20+25
6	Check Raiser	Surprise	1+2+5+25
9	Nose Wrinkler	Sadness	1+4+11+15+17
10	Upper Lip Raiser		
11	Nasolabial Deepener		
12	Lip Corner Puller		
13	Sharp Lip Puller		
15	Lip Corner Depressor		
17	Chin Raiser		
20	Lip Stretcher		
23	Lip Tightener		
24	Lip Pressor		
25	Lips Part		
37	Lip Wipe		

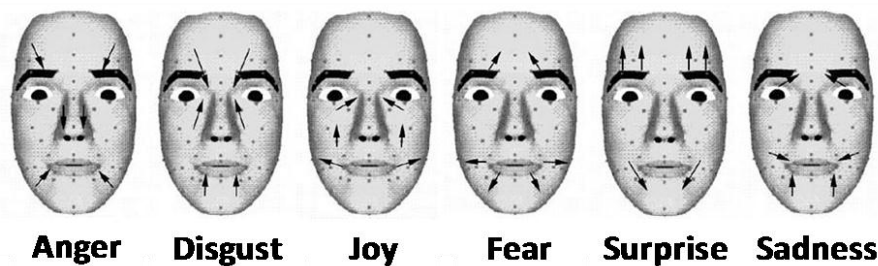
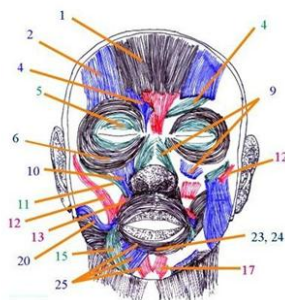


Table 3: Summary of published studies of different physiological measurement methods in response to gustatory and olfactory stimulations

Authors	Stimulus	Apparatus	Parameters	Advantages / Disadvantages
a) fNIRS				
Okamoto <i>et al.</i> , 2009 Okamoto <i>et al.</i> , 2006	Sweet-based taste stimuli	fNIRS topography system OMM-2000 Optical, Multi-channel Monitor (780, 805, and 830 nm), 17 channels, F7 (F8), C5 (C6), C3 (C4) and T3 (T4)	Oxygenated hemoglobin, deoxygenated hemoglobin concentration changes and total hemoglobin change	Non-invasive, supraliminal stimuli, shows brain zone activation, expensive
b) EEG				
Hashida <i>et al.</i> , 2005	Sucrose, NaCl	Ag/AgCl scalp electrodes in position C3-C4, reference electrode in position A1	Fast Fourier Transform (FFT) (adaptive Gabor transform)	Non-invasive but generally used with wet electrodes or closed eyes, infraliminal and
Martin, 1998	Chocolate essence, spearmint, almond, strawberry, vegetable, garlic and onion, cumin	19 electrodes: F3, F4, F7, F8, Fz, T3, T4, T5, T6, P3, P4, Pz, O1, O2, C3, C4, Cz	Power (μV^2). in each frequency band: delta (0-3 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta1 (13-22 Hz) and beta2 (23-30 Hz)	supraliminal stimuli, quality, intensity and hedonic dimensions, cheap
Owen and Patterson, 2002	Beta-damascenone	64-channel, referenced at Cz channel	FFT analysis followed by each subject's air averages and odor average normalization	

			such that the total power in each set totaled 100%. Relative % power for the frequency bins of interest (1-4, 4-8, 8-12 Hz)
Kline <i>et al.</i> , 2000	Isoamyl acetate (1, 0.01, 0.001, 0.0001 ppm by volume in water), Water	19 electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2)	FFT, spectral magnitude bands: theta (4-7 Hz), alpha (8-12 Hz), beta 1 (13-19 Hz), beta 2 (20-29 Hz), and gamma (30-50 Hz)
Murphy <i>et al.</i> , 2000	Amyl acetate	Fz, Cz, and Pz, reference electrodes at the A1 and A2 sites	Evoked Potentials: N1rP2 amplitude, P2and P3 latencies, P3 amplitude
Evans <i>et al.</i> , 1995	Amyl acetate	Electrodes Fz, Cz, Pz, C3, C4, and referenced to A1	P1, P2, N1, N2 latencies , amplitudes and interpeak latencies and amplitudes (P1-N1, P1-P2, P1-N2, N1-P2, N1-N2 and P2-N2)
Morgan <i>et al.</i> , 1997	Amyl acetate	Fz, Cz, and Pz electrode sites	N1, P2, and N2 peak amplitudes and latencies
c) Facial reactions			
Wendin <i>et al.</i> , 2011	Sucrose, sodium chloride, caffeine monohydrate, citric acid, MSG, MilliQ water	Video recording	Modified FACS: 9-point intensity scale for each chosen facial reaction
Weiland <i>et al.</i> , 2010	Sucrose, NaCl, PROP, citric acid, MSG, Evian mineral water	Video recording	FACS
Steiner <i>et al.</i> , 2001	Sucrose, citric acid, quinine HCl, distilled water	Video recording	FACS
			Non-invasive without contact for video recordings, Non-invasive with contact for EMG Supraliminal stimuli Quality, intensity and hedonic

Greimel <i>et al.</i> , 2006	Chocolate drink, quinine, bitter-sweet soft drink	Video recording	FACS	dimensions, emotions Time
Ganchrow <i>et al.</i> , 1983	Sucrose, quinine HCl, urea, distilled water	Video recording	Three decoders on scoring sheets	consuming: FACS and trained observers
Hu <i>et al.</i> , 1999	Apple juice, gatorade, mineral water, soybean milk, pickle juice	EMG: two 6-mm silver–silver chloride cutaneous disk electrodes on the subjects' left levator labii (superioris/alae que nasi muscle) region, third ground electrode on the mastoid behind the left ear	FFT analysis, spectral density (μV^2) for each frequency unit of the frequency epoch. Ratio of EMG frequency band of 100 to 250 Hz between tasting and baseline periods	Affordable

d) Pupillary measurements

Schneider <i>et al.</i> , 2009	Phenyl ethyl alcohol, carbon dioxide, hydrogen sulfide, lime	Infrared pupillograph	Latency and amplitude of responses	Quite invasive (some instruments do not enable head movements), supraliminal stimuli, related to stimuli intensity, not to hedonic dimension, expensive
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e) HRV

Horio, 2000	Sucrose, NaCl, citric acid, quinine-HCl, MSG	Heart rate monitor	Heart rate amplitude variations	Non-invasive with contact, supraliminal stimuli, quality,
Muroni <i>et al.</i> , 2011	Nut chocolate cream, lemon homogenate,	ECG (Ag-AgCl electrodes)	Time domain parameters: (a) the	

	gelatin		standard deviation of all normal durations of the interval between adjacent R wave (RR) intervals (SDNN), (b) the square root of the mean of the squares of successive RR interval differences (rMSSD) Frequency domain parameters: LF, 0.04-0.15 Hz, HF, 0.15-0.4 Hz, integral of power spectrum within each frequency range and percentage changes in LF and HF power areas	intensity and hedonic dimensions, affordable
f) Skin blood flow				
Kashima and Hayashi, 2011	Sucrose, NaCl, citric acid, quinine, MSG	Laser speckle flowgraphy	Percentage of skin blood flow change in specific zones (nose, eyelid)	Non-invasive with contact, supraliminal stimuli, quality, intensity and hedonic dimensions, expensive
Someya and Hayashi, 2008	Solid food with a chocolate taste, paraffin wax	Laser-Doppler flowmetry	Amplitude of the response	
g) Multi-parametric approaches				
Robin <i>et al.</i> , 2003	Sucrose, NaCl, citric acid, quinine sulfate	Skin potential (Beckman 78 mm ² electrodes)	Six indices: (a) the sign (+ or -) of the SP response, (b) amplitude of the SR response (K Ω), (c) duration of the SR response with the OPD index (s), (d) amplitude of the SBF	Non-invasive with contact
Rousmans <i>et al.</i> , 2000	Sucrose, NaCl, citric acid, quinine sulfate	and resistance (15-mA DC current using 25 mm ² Ag/AgCl round electrodes), skin		Supraliminal stimuli
de Wijk <i>et al.</i> , 2012	Bread-type products, sandwich fillings with cheese, sweets, meats and salads, fresh and cooked			Quality, intensity and hedonic dimensions, emotions

				Affordable
Leterme <i>et al.</i> , 2008	vegetables sucrose, orange juice, coke, lemonade, NaCl	blood flow (Hematron patented sensor) and temperature	response (mW/cm °C), (e) the sign (+ or -) of the ST response (°C) and (f)	
Møller and Dijksterhuis, 2003	Butyric acid, citral, peach, skatole	(slow inertia thermistor), instantaneous	magnitude of the instantaneous heart rate (IHR) response	
Bensafi <i>et al.</i> , 2002a	Isovaleric acid, thiophenol, pyridine,	heart rate (three silver electrodes	(bpm)	
Bensafi <i>et al.</i> , 2002b	L-menthol, isoamyl acetate, 1-8 cineole, diluted in mineral oil	in a precordial position)		
Robin <i>et al.</i> , 1998	eugenol, menthol, methyl methacrylate, vanillin, propionic acid, lavender, diluted in alcohol 95			
Alaoui- Ismaili <i>et al.</i> , 1997a	vanillin, menthol, eugenol, methyl methacrylate, propionic acid, diluted in alcohol 95			
Vernet-Maury <i>et al.</i> , 1999	Acetic acid, butyric acid, camphor, ethyl acetoacetate,			
Alaoui- Ismaili <i>et al.</i> , 1997b	lavender, diluted in mineral oil			

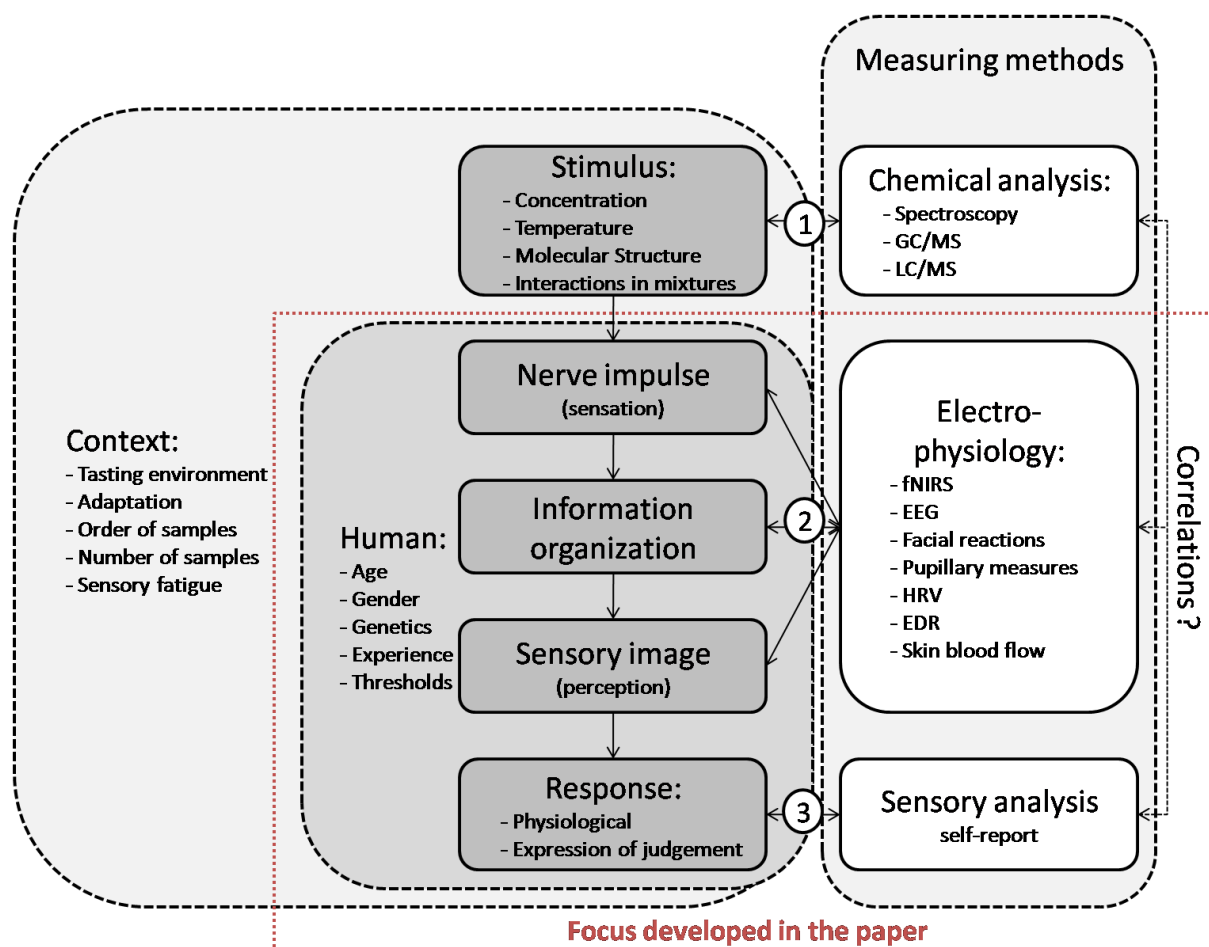


Figure 1: Diagram of the stimulus, human intrinsic and contextual parameters affecting the perception chain and the methodologies used to characterize the stimulus properties at different levels (adapted from Perrin *et al.*, 2008)

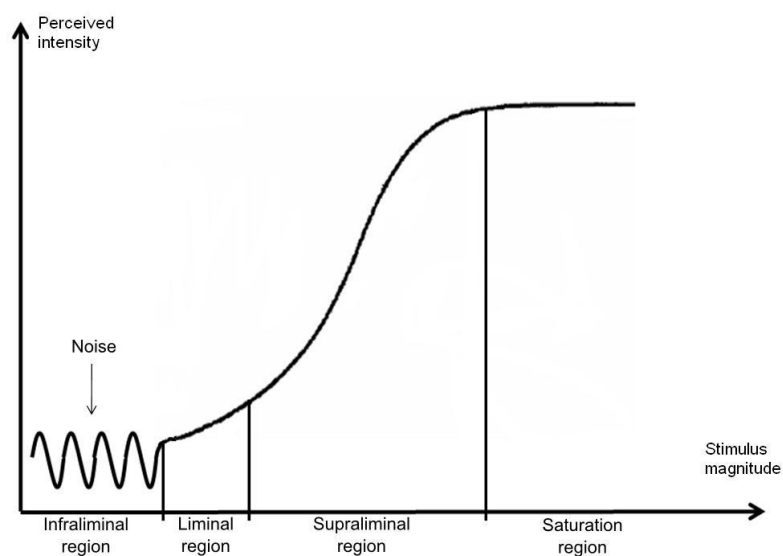


Figure 2: Typical line of the perceived intensity evolution given the stimulus magnitude
(Ramalho, 1999)

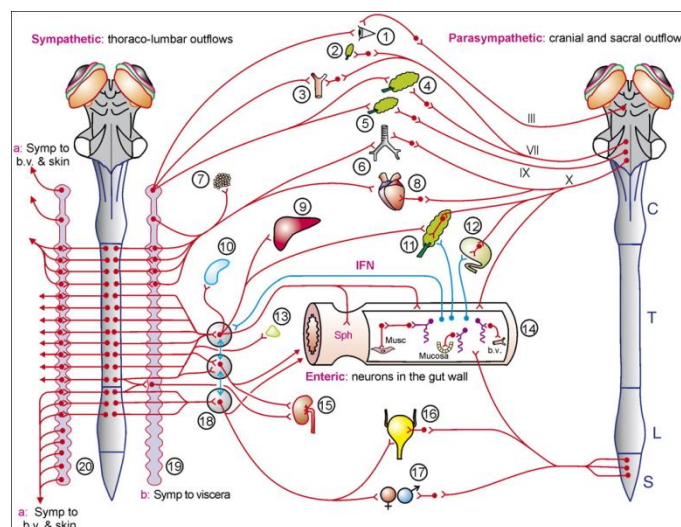


Figure 3: Anatomical representation of the autonomic nervous system (1) Eye, (2) Lacrimal glands, (3) Intracranial arteries, (4, 5) Salivary glands, (6) Airways, (7) Brown fat, (8) Heart, (9) Liver, (10) Spleen, (11) Pancreas, (12) Gallbladder, (13) Adrenal gland, (14) Tubular gastrointestinal tract, (15) Kidney, (16) Urinary bladder, (17) Genital organs, (18) Prevertebral ganglia and plexuses, (19, 20) Sympathetic chains (paravertebral ganglia and their interconnections). Spinal cord levels: C, cervical; T, thoracic; L, lumbar; S, sacral (adapted from Furness, 2006)

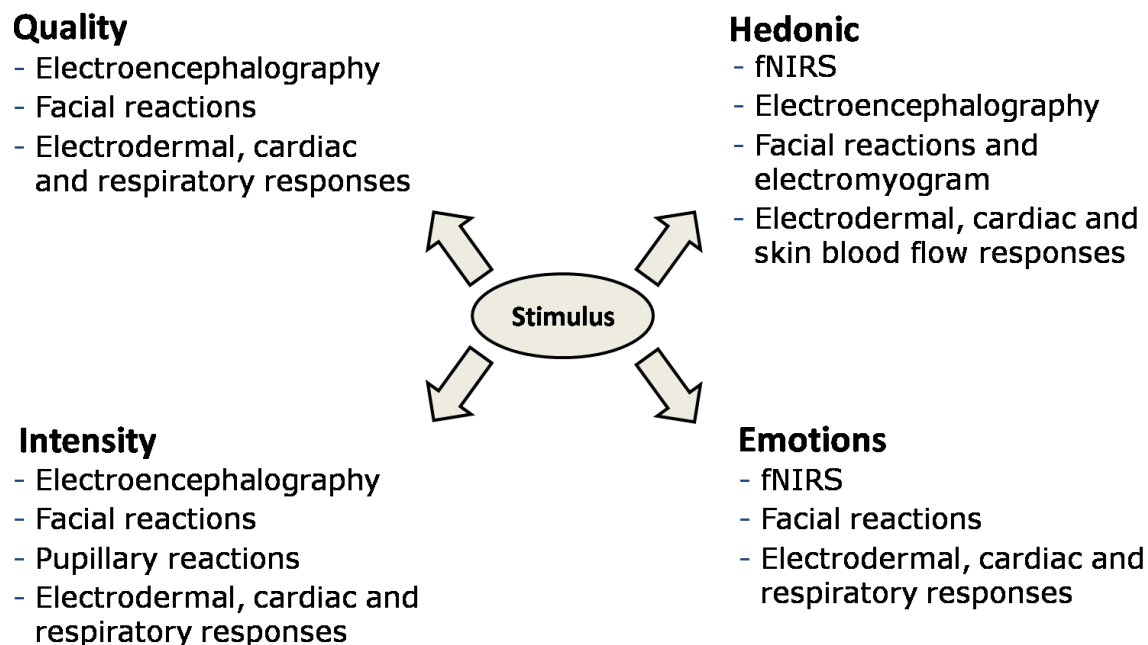


Figure 4: Autonomic parameters related to the quality, intensity and hedonic tone and emotions induced by gustatory or olfactory stimuli

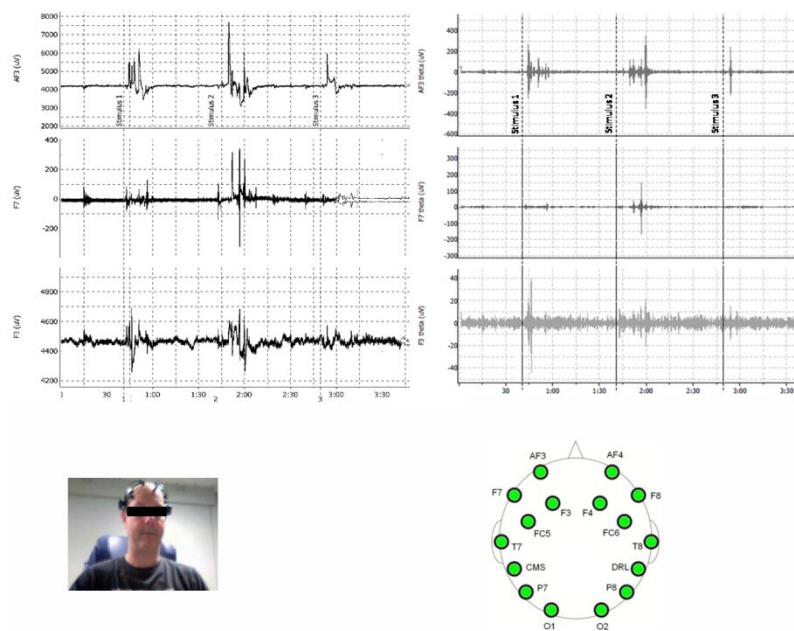


Figure 5: a) Example of an EEG signal obtained with an EEG EPOC neuroheadset for the AF3, F7 and F3 electrodes (stimulus 1 corresponds to the blank sample, stimulus 2 to acid taste and stimulus 3 to sweet taste), b) EEG signal filtered at the theta frequency (4-7 Hz), c) Picture of the EEG EPOC neuroheadset, d) Position of the electrodes on the neuroheadset

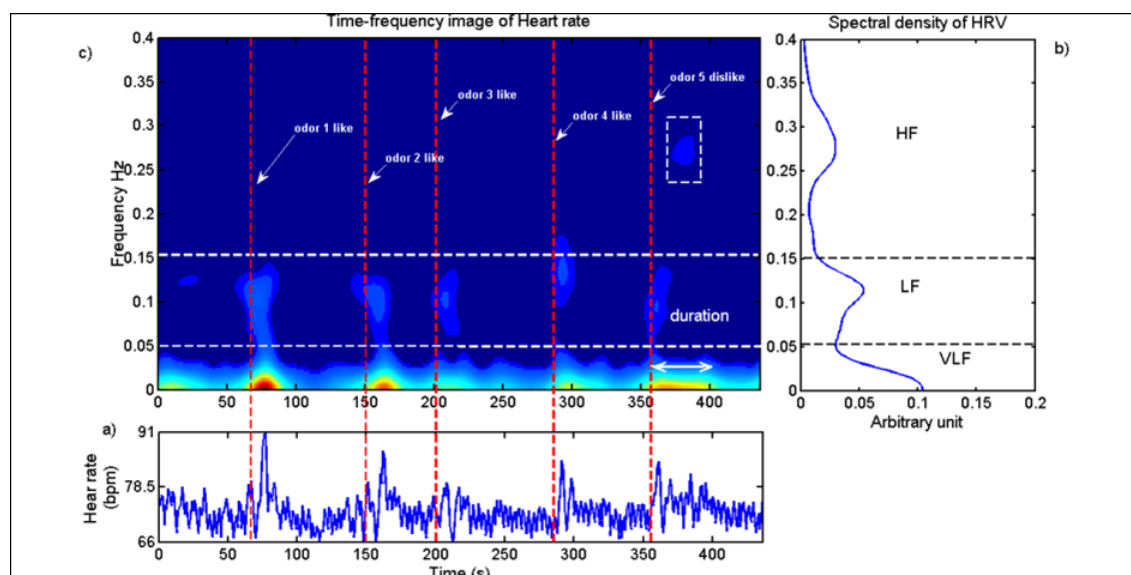


Figure 6: Example of a): HR signal during five olfactory stimulations, b) Spectral density of HRV, c) Time-frequency image of the HR signal where the disliked odor presents the longest duration on the VLF bandwidth and an activation of the parasympathetic nervous system (HF) in order to counterbalance the quick activation of the sympathetic nervous system.

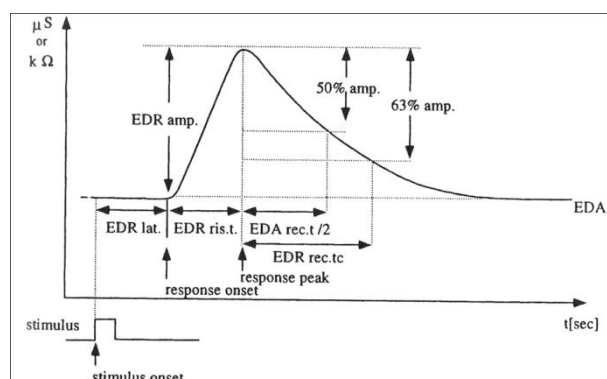


Figure 7: Classic electrodermal response and the parameters to be extracted (Boucsein, 1992)

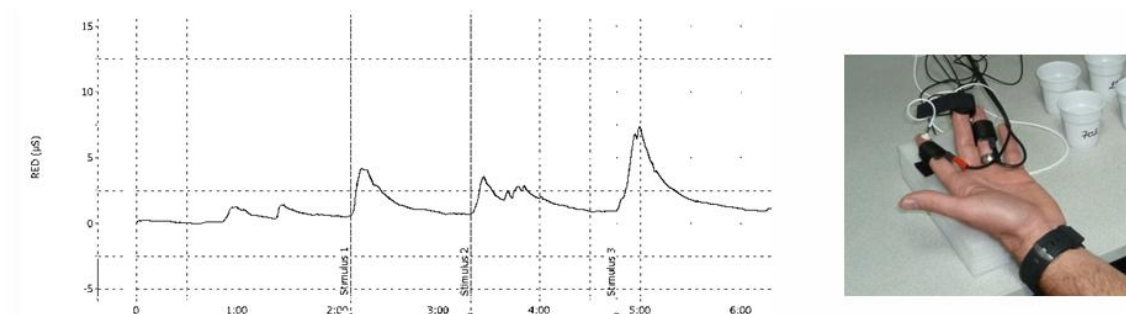


Figure 8: a) Example of an EDR line obtained with two electrodes (stimulus 1 corresponds to the blank sample, stimulus 2 to bitter taste and stimulus 3 to salty taste), b) Position of the electrodes on the second phalange of the forefinger and the middle finger

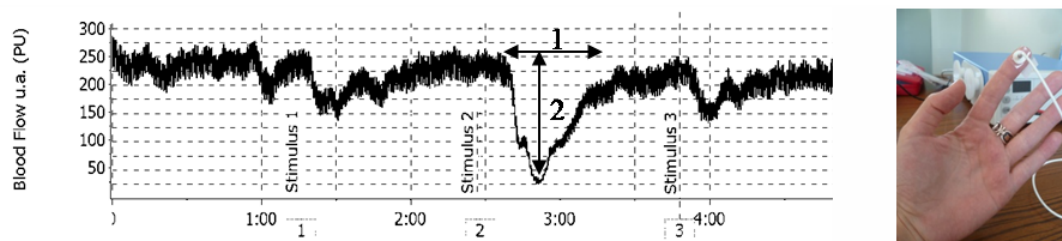


Figure 9: a) Example of a blood flow line obtained with a Doppler Laser from Perimed AB (stimulus 1 corresponds to the blank sample, stimulus 2 to bitter taste and stimulus 3 to sweet taste, 1: duration of the perturbation, 2: amplitude), b) Position of the laser on the pulp of the forefinger