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State of the art olfactometers. Different types

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Research Article

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Abstract

The complexity of human olfaction is very high and the importance of being able to measure it directly, objectively and qualitatively has led experts to search for mechanisms that can be applied. Human beings use this sense, which is one of the oldest, to recognize danger and distinguish between pleasant and unpleasant odors. Smells are mixtures of molecules that, at different concentrations in the inhaled air, stimulate the olfactory area and are recognized at the brain level. Therefore, there is a coding and decoding system.

Human olfactometer techniques use equipment designed to be able to measure its intensity and quality of volatile substances. If we are able to measure this sense, we will be able to know its variations and be able to make clinical diagnoses in normal and pathological conditions and diagnose the losses that occur in certain infectious, degenerative diseases, traumatic processes and other variants.

For many years, systems have been developed that can measure subjective olfaction in humans, as well as objective forms, but it is also true that there is no equipment available that is fast, simple handling and that can be applied in daily clinical services.

Aim of the Study

- *Present the recent achievements in olfactometer technology;*
- *Elaborate the scientific articles about olfactometry published mainly in the last 10 years;*
- *To gather the information published in the last years in relation to the usefulness, existence in the market and purposes of equipment that can measure the odors, what we will call the Smell-o-meter or olfactometer for human use.*

Material and Methods: *In the first part of this research we will gather most of the information existing so far in international bibliography, as well as the achievements and utilities obtained to date. Following, we will analyze all the new concepts related to smell-o-meters devices that exist on the market and assess the possibility, based on what has been done so far, to seek new practical systems for application in the medical field.*

Keywords : odor human detection, odor concentration, sensory methods, dynamic olfactometry, electronic nose, sensors, sampling methods, GC-O

Introduction

The mammalian olfactory system is considered undoubtedly the most complex, sensitive, and broad range odor sensor. The process of smelling has been of interest to researchers for decades. First, the volatile compounds reach the nasal cavity through the anterior nostrils. In the nasal cavity, which is covered with the olfactory epithelium, the odor is spread. The endings of olfactory neurons – olfactory cilia, are sunk in a layer of the mucus membrane, where also compounds dissolve. Odors interact with specific receptors on the dendritic cilia, which corresponds to the key and lock model. However, most olfactory neurons can recognize multiple odorants, even from different chemical classes. Moreover, one odorant can activate various receptors, which respond with different power to a particular odor. The properties of odorant receptors were described by Malnic (1). who defined an idea of recognizing an odor by a few various receptors, a combinatorial odor code The signal initiated by the odor and receptor is changed into an electronic signal and transmitted through an axon to the olfactory bulb in the brain. The information is further passed on to the cerebral cortex, where it is processed, which allows discriminating from plenty different odors. (2).

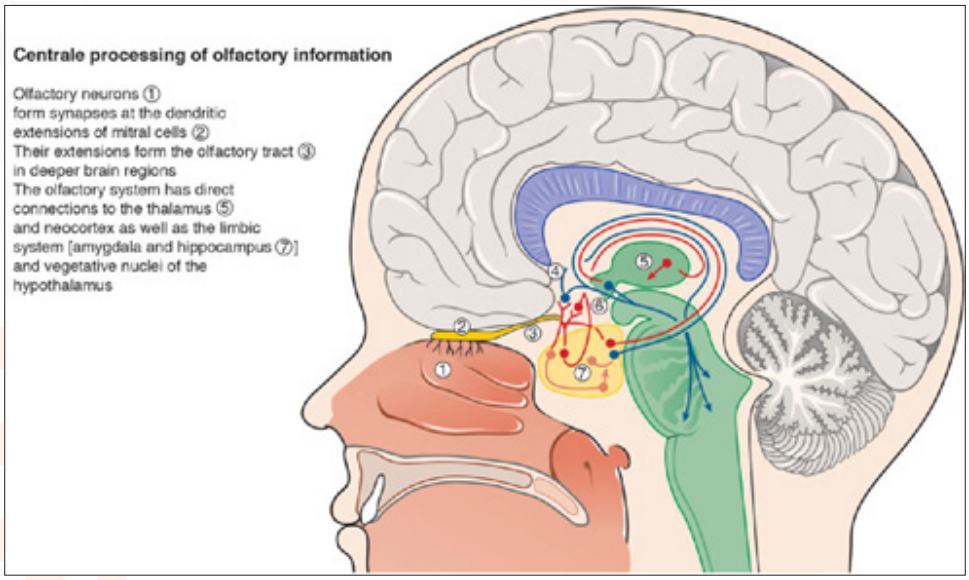

Figure 1: Anatomy of the human olfactory system

https://www.semanticscholar.org/paper/The-Human-Sense-of-Olfaction-Walliczek-Dworschak-Hummel/1770676b1fe8a281e-4ae4b1fca3b7110f6c23f79

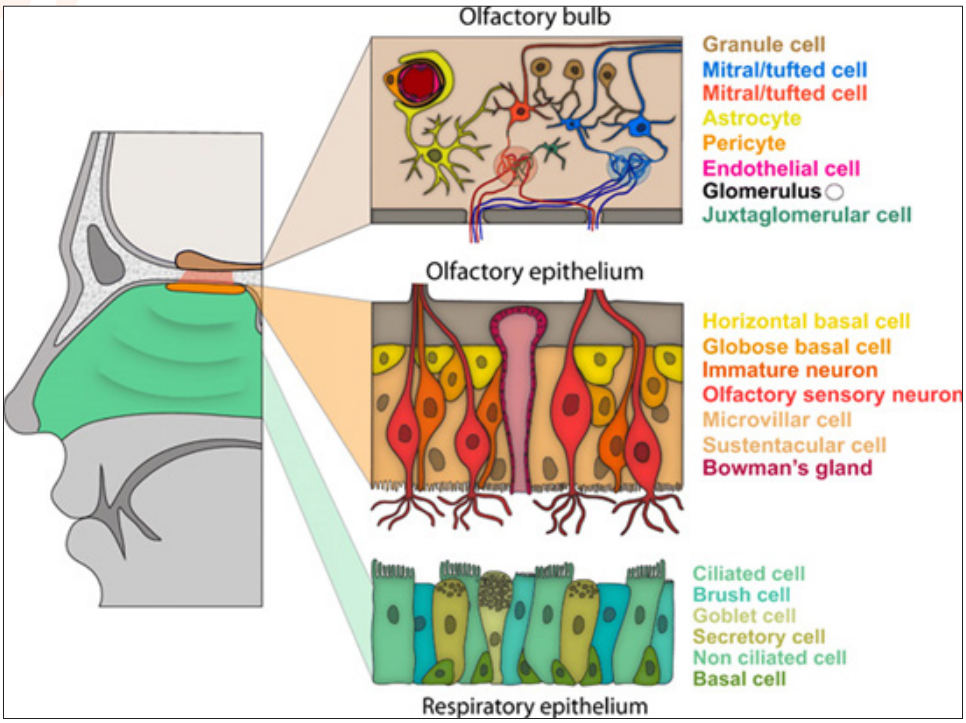

Figure 2: Schematic of the nasal respiratory epithelium, olfactory epithelium, and the olfactory bulb (3).

Buck and Axel received the Nobel Prize in Physiology or Medicine in 2004 for the identifica¬tion odorant receptor genes. Odorant receptors (ORs) belong to the G protein-coupled receptor (GPCR) superfamily and consist of seven-trans¬membrane domains, which create a ligand-binding pocket. There were identified around 350 functional OR genes in humans and 1200 in mice, which makes OR the largest mammalian gene family. ORs are also found in other tissues (2).

Some researchers support the vibration theory of smell and call it the "swipe card" model. The volatile compounds vibrate with different frequencies. First, they have to fit to OR biding place and tie-up with a receptor to generate a piece of information about the smell. After that, the receptor undergoes a conformation change from its inactive state to its active state. The volatile compound's vibrational energy, which is compatible with the difference in energies between two energy levels on the receptor, begins the flow of electrons through the molecule

via inelastic electron tunneling, realizing the signal transduction pathway and activates the central nervous system (4).

The History of Measuring the Smell

The methods of measuring odors have been developed through the years. One of the first ones was proposed by Valentin in 1850 (4) an air-dilution method, which consisted of some amount of odorous samples placed in a tiny, thin-walled glass tube inserted inside a larger container, and thereafter an individual opened the container and sniffed it. If he was able to recognize the odor, the same quality of samples was put in a bigger container and the test was repeated. In 1895 Zwaardemaker proposed an olfactometer (6) which was built of two tubes, one inside the other, the inner tube was open at both ends and fitted to an individual's nostril. The smell was transported between both tubes. When the subject detected the smell, he took out the tube, and then the length of the tube appointed one "olfactive" (a unit of odor stimulus). In 1921 he designed "*camera inodorata*", which was a glass-wall box with a top and bottom made of aluminum with a capacity of 400 liters (7) the odor-free atmosphere was maintained by an ultraviolet lamp and an exhaust fan. A subject had to put his head inside through an opening in the bottom and his sensitivity was measured by the Zwaardemaker olfactometer outside of the box (8) another idea was from Woodrow and Karpman (9) they bubbled air through a test sample and changed the temperature; the odor was delivered to the individual's nose. Allison and Katz in 1919 (10) introduced a complex instrument consisting of "Venturi" tubes, which gave the possibility to measure the volume of air flowing with a determined speed through the chemical substance. The concentration of odor was counted by measuring the loss of weight of the substance. Another solution was proposed in 1925 by Hofmann and Kohlrausch (11) they built a positive pressure olfactometer; saturated vapor was mixed with air in mercury columns and transported to the subject's nose. It was possible to count the concentration with known pressure, volume, and specific gravity. Then Gundlach and Kenway in 1939 (12) constructed an instrument based on manometers, which regulated the concentration of odors. Elsberg examined patients with brain tumors and single-sided or bilateral anosmia in order to localize the tumor. He released specific gases into nostrils with different pressure and volume. Wenzel constructed an instrument, which could control temperature, pressure, and volume of the odor and calculate molecular concentration using test materials of known vapor pressure. In 1953 Jones verified Elsberg's theory (13) He proved that concentration did not have an equal effect, and the data could not be transformed to molecular terms. He constructed a motor-driven syringe filled with air and tested gas, in which molar concentrations of odors could be changed. However, Jons' assumptions were not proven in experiments. He concluded that the aerodynamics of the nose might have an impact on thresholds achieved by the blast-injection method. The next solution was proposed by Castello, Fortunato, and Niccolini (14).

The electronic olfactometer was based on the blast-injection method and could control pressure, temperature, and humidity. Odors diluted in nitrogen were passed through an ionization chamber and the amperage was measured. Unfortunately, the results have not quantitative significance. Fortunato and Niccolini (14)) modified the technique in their research on olfactory fatigue. They built a revolving plate with a few boxes with a vertical column in the center, which could serve different gases placed in containers to the syringe with inlet and outlet valves. In 1963 Schneider (15) detected the changes in swelling of the nasal mucosa by "*nasal patency meter*". He constructed the device based on the injection technique; the examined gas was diluted in odorless gas (nitrogen) and measured by the device. He proved that the increased flow rate of the gas causes decreasing of thresholds, faster arrival to olfactory mucosa, and a smaller loss in adsorption during the passage (4). Wenzel proposed an air-dilution olfactometer similar to Zwaardemaker's "*camera inodorata*", which better imitated natural breathing. The individual had to place his head in a Plexiglas container filled with purified air and a specific amount of odor substance was released in a defined time into the box (16).

Cheesman-Kirkby (17) designed an instrument with capillary tubes, which was able to deliver odor at the same concentration to 12 people simultaneously and measure their responses. Unfortunately, it was impossible to measure or compare different substances. In 1961 Ough and Stone (18) introduced an instrument, which saturated volatile samples by the use of vapor pressure at a certain temperature and flowmeters. They were able to count the present concentration of the mixture. The subject's head was placed in a Plexiglas hood. Next modifications were put into by Bozza (19) who placed the individual's nose and mouth in an opening, and then a stream of odor or purified air was released to the closed glass and polyethylene chamber to reduce the impact from the external environment. In 1963 Guadagni (20) used polyethylene and Teflon squeeze-bottles, which contained different odors, for food analysis (5).

Gas chromatography started being developed in 1947 when German physical chemist Erika Cremer together with Austrian graduate student Fritz Prior prepared its theoretical foundations and proposed the first liquid-gas chromatograph. However, her work was recognized as irrelevant and ignored (21). The next steps taken in this field was by Archer John Porter Martin, awarded the Nobel Prize for developing liquid-liquid (1941) and paper (1944) chromatography. The fast development of gas chromatography is observed after the invention of the flame ionization detector (22). The work of gas chromatograph (GC) is based on the chemical separation of volatile compounds in a complex sample. It is made of narrow tubes ("columns"), through which chemical substances flow diluted in a gas stream (carrier gas, "mobile phase"). The column is filled with a specific filling called "*stationary phase*", which is responsible for separating compounds. The process begins from the injection of a certain volume of gas or liquid at the start of the column. While the analyzed substation flows through the mobile phase, its motion is slowed down by adsorption either onto the column walls or filling in the column. The rate of the flow is adjusted to certain chemical and physical properties of the examined mixture and depends on the strength of adsorption, type of molecules, and the stationary

phase substances. At the end of the column, compounds are detected and identified electronically by a detector, which measures the time and quantity of substances. Various substances appear at the end of the column at different times, because they have a different rate of progression, which enables to separate each compound. It is called "retention time". Parameters such as the carrier gas flow rate, column length, and temperature have an impact on retention time and can be modified in the examination process. Compounds are identified by the order they appear at the end of the column and the retention time (23).

The idea of improving gas chromatography (GC) by mass spectrometry (MS) was raised in 1959. The use of both methods allows achieving a much finer degree of differentiation. The function of gas chromatography has been described above. At the end of the chromatography process, mass spectrometry joins in a job. When compounds are selected by different retention times, mass spectrometry breaks each compound into an ionized state and detects the ionized molecules separately by the use of their mass-to-charge ratio. However, both of those methods have some limitations. GC is often not precise enough, which may result in separating a few compounds with the same retention time. On the other hand, MS may similarly ionize various molecules, which makes that their mass spectrum coincide. Combining GC and MS allows avoiding such errors and similarities between two different compounds. To sum up, achieving a certain mass spectrum with a characteristic retention time in a GC-MS indicates a huge probability that the wanted analyze is in the sample (24). Unfortunately, the technique may have difficulties in the differentiation of complex odor in low concentrations; the instrumentation is expensive and must be operated by qualified employees.

The invention of the artificial nose (*e-nose*), which mimics a mammal nose, caused a rapid development in odor detection. E-nose consists of many different chemical sensors with partial specificity to a wide range of volatile compounds, placed on an array, connected with software based on artificial intelligence. The instrument is able to recognize objectively a variety of simple and complex odors by the entire system of connections across the sensory array. Thanks to the storage of data, compounds can be fast identified (25), (26).

How does an olfactometer work?

An olfactometer is a device designed to detect and measure odor dilution. It is also used to quantify and qualify human olfaction, as well as to reveal a substance's odor detection thresholds (27). The term "olfactometer" refers also to an instrument designed to examine the behavior of insects as a response to different odors (28).

There are two main types of olfactometer: dynamic dilution and flow-olfactometer. A dynamic dilution olfactometer requires the participation of the panelists, who are trained and evaluated automatically. Moreover, various complementary techniques may be adapted, such as odor concentration and odor threshold determination, odor suprathreshold determination with comparison to a reference gas, hedonic scale assessment to define the degree of appreciation, evaluation of the relative intensity of odors (27). A flow-olfactometer provides a constantly heated and humified flow of pure air to an individual's nose to produce specified, reproducible odor or pain stimuli. This objective method gives a possibility to register reflex reactions and changes in the central nervous system such as olfactory evoked potentials (OEPs) (29).

A field olfactometer is a device, which is broadly used in the commercial market. Although they are easier versions of laboratory olfactometers which can be used by one panelist, they have to provide precise dilutions of odor mixtures and present the sample in standardized airflow. They are dedicated to determining odor levels at different places and validating odor complaints (30).

Flow olfactometry

The need for objective measurement of smell stimulation caused the creation of a flow olfactometer. The most commonly used flow olfactometer was created by Kobal in 1985 (31). The main feature of the instrument is the ability to generate precise, constantly heated, and humidified airflow into the nose. It does not use any thermal or tactile stimulation. In 1993 Evans proposed requirements for determining olfactory evoked potential with high accuracy (32): odors should be put in the flow of odorless air as a pulse; the stimulus must be introduced as a rectangular wave-form, which means that 70% of maximum concentration has to be reached in 50 ms; during the whole measurement, odorless air must be present in the nasal cavity. There is a risk of drying the nasal mucosa, which is why the humidity of pure air must be maintained over 50% and the temperature around 35–37 °C (33).

As emphasized by Hellwig (34) the dilution flow olfactometer allows changing the ratio of odor and the clean air stream at various airflow speeds, at the same time, and it does not influence the rate of changing of mixing ratio. Mechanical stimulation of nose mucosa is also limited thanks to the stability of the olfactometer output flow.

Tichy (29) designed an airflow dilution olfactometer in order to examine the ability of olfactory receptor neurons of cockroaches to detect and process small changes in volatile compound concentration with varying rates and amplitude. The instrument consists of electronic valves controlled by a computer for diluting the odor-saturated with pure air at various ratios at any tempo. The valves are supervised by a Proportional–Integral controller (PI controller or two-term controller) and the stream is kept constant by phase-shifting voltages by 180°. The main advantage of the device is a high precision in controlling the flow rate and concentration and slowly oscillating concentration changes. Moreover, the instruments allow using the quick rise and fall stimulation times with pauses of pure air. The authors found that transient concentration changes quickly pass the excitation threshold and the discharge rate considerably exceeds the neural noise, which can be easily measured. Rates of dilution can be quickly changed and delivered, however, those oscillation frequencies over 4 Hz cause

turbulent flow. Unfortunately, the proposed olfactometer can analyze only a single odor. New sets of delivery pieces should be used to avoid contamination during the transportation of various odors.

Burton (35) constructed a new module olfactometer characterized by efficient and flexible odorant transmission and minimal contamination. Despite the turbulent flow, it provides certain amounts of odor samples with precise concentration and allows for flexible realizing volatile mixtures and fast sequences. Instead of tubing, which favored contamination, the device was built of changeable, cheap, and disposable containers, and the open airflow was adapted. The outstanding features of the new olfactometer are the separation of containers with odors from the delivery path by periods of open airflow and the ability to mix odorant vapor with a carrier stream in free space. The olfactometer was used in the experiment, in which the mouse's neural responses to odorants were monitored. Odorant-evoked activity patterns were observed, which gives a potential for investigating the neural encoding and behavioral perception of odorant stimuli. Neither intertrial nor interchannel contamination was found. The expected price of a new device is around 1,500 USD and each testing costs an additional 1 USD in disposables

Plenty of new different solutions were adapted in the new flow dilution olfactometer to avoid the contamination of odorant mixture and to streamline the process of dilution. Olfactometers are built of sets of 12 small disposable boxes, which allows for quick changing of odorants during experiments. It contains two main elements: a delivery arm and flow control housing with airflow controls. A delivery arm is responsible for directing volatile compounds to the experimental preparation. It consists of a single base and modular barrels. All steps are controlled by computer software systems, which supervise valves to direct air to the appropriate container, and then the solution is referred into a diluting carrier stream and to the experimental preparation. Another advantage is that new olfactometer can integrate spans of open airflow upstream and downstream of the containers for odors. To minimize intertrial contamination the flow of air downstream should be opened to the maximum. Thanks to spans of open airflow upstream the possibility of backflow into upstream and interchannel contamination is reduced. A narrow and curved tip of an odorant reservoir was designed to effectively limit diffusion at the same time to allow pressurized air to expel and direct volatile compounds into the airstream. Channels are independent and can deliver combinations of mixtures of 12 odorants. They can also control the concentration of the mixture by a separately regulated pressure source. There are possible short intervals between various odorants or even they can overlap in time, which increases the efficiency and can be used to evaluate temporal relationships of different smells (34).

However, some limitations exist. Researchers found difficulties in the delivery of odors due to the longer than typical distances in this type of olfactometer. The incomplete mixing of odorant with vapor stream can be a problem, which makes the olfactometer not reliable in measurements of odorant molarity. Flow rates are higher than in typical flow dilution Olfactometers, which can cause desiccating and/or irritating the nasal epithelium. Such high carrier stream flow rates can result in fast running out of odors that is why a small air pump with an in-line charcoal filter was used. Some of the odors can escape from containers to carrier stream even through a closed valve. Also, the turbulent flow may affect neural activity. The researchers try to use a Proportional–Integral–Derivative controller (PID controller or three-term controller) to simultaneously monitor odorant delivery and potentials in the brain. The educator can help with variance in the instantaneous concentration of odorant delivery also inhalation may decrease the turbulent flow by Low-Pass (LP) filtering stimuli. However, no difference was observed in neural activity between turbulent and more stable delivery (35).

Schriever (36) presented a study based on an affordable and portable olfactometer and time-frequency analysis (TFA) of olfactory-induced EEG in clinical examination of smell function. TFA was applied in order to increase the signal-to-noise ratio of chemosensory event-related potentials responses. He collected 78 volunteers. The research was divided into three parts. Part I was dedicated to optimizing odor stimuli and recording conditions. The researchers found that the most successful model for odor presentation was cued signal, with stimulus duration of 1000 *ms*, an interstimulus interval of 18-20 s to clean air, and each stimulus introduced 60 times in blocks of 20 stimuli each. The stimulus was indicated to the participants by the change of the screen from black to red, the change of colors appeared between 1000 to 3000 *ms* before the onset of the stimulus. Four various conditions were checked for each stimulus (60 recordings per stimulus condition). EEG fragments lengths of 4000 *ms*, beginning 1000 *ms* before stimulus onset, were recorded with a 16-channel amplifier at a sampling frequency of Fs= 250 *Hz* and with the use of a Band-Pass (BP) filter of 0.2-30 *Hz*. During the measurements to prevent fast eye movements and blinking and to assure attention, the participants had to make a computer exercise, which depended on following slowly moving square by the computer mouse. To mask the clicking sounds of the olfactometer, headphones with white noise (~ 60 dB) were used. In part II researchers compared the EEG power (PSD) changes after stimulation of olfactory or trigeminal nerve between healthy individuals and patients with olfactory disorders. They proved that differences in the central processing of olfactory stimuli between two groups exist. The analysis performed by TFA revealed 75% sensitivity and 89% specificity. The test-retest reliability of the technique among the control group was conducted in part III (36).

Lowen (37) proposed a low-cost functional magnetic resonance imaging (fMRI)-compatible constructed olfactometer with the possibility to estimate the changes in the BOLD signal. The solenoid valves were localized at a big-distance from MRI scanner to ensure more space. Odors were directed to the nose via nasal cannula with a subtle, low-speed flow, without large assemblies on the face of examiners. The con-

stant flow rate is kept by a single flow regulator and meter. A multiport connector with six ports was added in aim to examine more odorants. The olfactometer was positively validated and is controlled both manually and by a computer interface. He proved that higher the flow rate is, fewer errors and shorter response latency times.

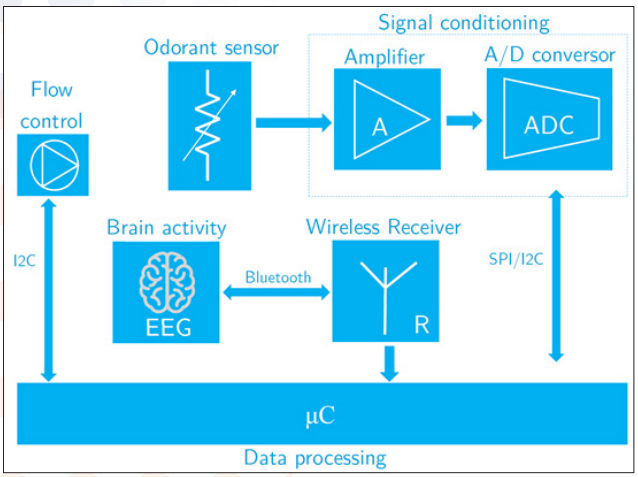

Figure 3: The diagram presents the schematic function of the EEG-based smell-O-Meter. Brain activity is recorded by EEG and sent via Bluetooth to the microcontroller (µC) (according to Pérez R, Marimon X, Portela A. Artificial nose. UIC Barcelona).

Dynamic dilution olfactometry

Dynamic olfactometry is a method of determining the concentration of smell in a gas sample. It is based on the standardized methodology and panels of sensory-trained evaluators. An olfactometer is responsible for presenting to the human nose a diluted odor sample in pure air at specific ratios and constant temperature and humidity. A sensory perception threshold test is performed by a panel to specify odor thresholds. The volatile sample is gradually diluted by odorless gas in a specific ratio and presented to the panel of experts. Members of the panel sniff the mixture through a port with a nose mask. In Europe, olfactometers are designed according to the European Standard (EN)<13725:2003 standard (38) which specifies construction materials, gas flow velocity, dilution precision, measurement range, recommended ways of presentation of the gas mixture to the panel, etc. The result of the measurement is the degree of dilution of an analyzed gas when the panel reaches the odor threshold and is determined as one European odor unit per cubic meter (*ouE/m*^{3}). New dynamic olfactometers are broadly used in the trade industry to value food, beverages, perfumes, etc (39).

Panelists are chosen from the group of examiners according to a standardized procedure by the criteria of predetermined repeatability and accuracy in the determination of reference gases, the most commonly used is n-butanol. Panelists, during their work, have to be continuously screened and trained. Those who suffer from cold or other indispositions cannot take part in measurements (40).

Researchers have tried to improve the reliability, accuracy, repeatability, and robustness of a dynamic olfactometer. The proposed optimization of the panel selection was to make it less repetitive and to avoid guessing by panel members (41). At least ten individual threshold estimates (ITEs) of the reference gas, which are individual odor detection thresholds, should be collected. The qualification is performed by dynamic olfactometry during at least three sessions on consecutive days. There are two inclusion criteria: the geometric mean of the ITEs should be between 20 *ppb* and 80 *ppb*; and the antilog of the standard deviation counted from the logarithms of the individual ITEs should be less than 2.3. The ITE is specified every twelve measurements (41), (42). The other aspect was time exposure to odorants. A standard or the same environmental odor sample was presented to a panel several times during one test. The experiment revealed that the optimal time of analysis in one session is two hours (43), (44).

The most often used unit to express odor concentration is *ou/m*^{3}. One *ou/m*^{3} which is equal to the perception threshold is a point at which 50% of the panelists cannot detect the odor, at the same time 50% of them can perceive it. To specify how many odor units were in the sample at the beginning, all the dilutions before reaching the perception threshold should be added (e.g. if the sample was diluted 89 times to reach 1 odor unit, then the sample odor concentration was 89 *ou/m*^{3}). This method is sometimes called dilution-to-threshold, or D/T. The perception of odor is a logarithmic phenomenon (40).

Odors are presented in a standardized way. Samples may be delivered manually presenting odorant reservoirs to the experimental preparation. However, this method is rather ancient, provides long, changeable, and relatively uncontrolled odorant delivery (45) Nowadays, automated flow dilution olfactometers have become more common. In such devices, airflow, odor concentration, duration, onset, and offset are precisely controlled, using a combination of tubing, computer-controlled valves, and mass flow sensors. Some instruments can supervise temperature, humidity, and other features (46) However, surfaces of each element of the instrument, where odor mixture flows, as well as pressure fluctuations during valve actuation that may generate backflow of the sample, may have an impact on adsorption or desorption and cause intertrial and interchannel contamination and alteration of examined substances. To avoid that the olfactometer should be constructed from low-absorbency materials, e.g. stainless steel, polytetrafluoroethylene (PTFE), tetrafluoroethylene hexafluoropropylene copolymer (Teflon™), polyvinylfluoride (Tedlar™), polyterephtalic ester copolymer (Nalophan NA™), or glass. Also, pure air should be provided between the following examinations, and components of the olfactometer should be carefully washed or replaced, as it might reduce delivery efficiency and flexibility (47) Some olfactometers may present odor at a certain time, e.g. during inhalation. Factors such as olfactometer design, test procedure, differing sensitivity of panelists, data quality, measurement uncertainty may affect measurements (48).

Two standardized methods are usually used in the presentation of odor samples to the panel. The first is a forced-choice method at least two ports are necessary, the volatile compounds are set free at one port, pure air is placed to the others. The task of panelists is to compare samples and choose the port with odor. The second is the yes/no method, which is that each panelist smells each port and has to communicate if an odor is detected or not. In each sample is presented pure air or solution of pure air and odor (38), (49), (50).

A sampling of volatile mixtures relies on presenting to a group of panelists samples with different odor concentrations. The first presentation has a very low concentration, non-detectable by examiners. The next samples are diluted in decreasing order by a predetermined and constant factor. The panelists' answers are recorded. On the basis of measurements, it is possible to establish a geometric progression of dilutions and the logarithmic relation between odor intensity and concentration. The research is continued till the moment when all individuals positively reveal an odor. This concentration of odor marks the detection threshold, which is calculated as a geometric mean between the dilution of the last negative answer and the first positive. Different cycles are repeated and the final score is calculated for series altogether (40), (33), (50) Gotow (33) designed an olfactometer with an attachment, which inserts a pulse of examined odor into a stream of air. The instrument was designed in such a way as to determine reaction time in detecting the onset of examined odor during the presentation in a mixture of smells. She noticed that the reaction of participants was subtly significantly shorter under odorless conditions than with a background.

It should be underlined that the descending order of contamination of a sample can increase adsorption/desorption effects, but also can cause olfactory adaptation in panelists. If the next samples are presented in a specific order (in increasing or decreasing concentrations), they can have an impact on panelists' response, as they predict the dilutions of solutions. That is why an ascending order presentation is preferred (48).

Another important factor in olfactometry is data quality. It can be estimated by the use of one of two sources of uncertainty: the panel referability to a standard and the coherence of panel responses. To achieve referability, the results are evaluated by accuracy and precision measures. The coherence of panel responses depends on the validation process (e.g. retrospective screening), which allows excluding panelists who gave incorrect answers (38) One of the largest limitations of dynamic olfactometry is that it only gives a piece of information on odor. It is not possible to perform continuous and field measurements especially important for monitoring the industrial processes and environmental pollution. It analyses the whole mixture without discriminating each chemical compound of the solution. In laboratories, a problem is the need to storage collected odors and their transportation. During that process, samples may be absorbed or chemical reactions may take place between compounds. Moreover, concentrations of odorants in atmosphere air are often too little and too fluctuating in time to use a technique recommended by EN 13725: 2003 standards.

Field Olfactometric Measurements

The idea of performing measurements of odors *in situ* without the need for storage and isolating the panel from the influence of the environment to avoid olfactory adaptation or fatigue caused the development of an instrument and a procedure for field olfactometry. It is a form of dynamic olfactometry but works in real-time. The scentometer, designed as a hand-held analyzing odors on-site device, was the first field olfactometer. It provides different dilutions of odor in odorless carbon-filtered air (51). However, the instrument is economically attractive and measurements are made rapid. On the other hand, there appears a phenomenon of smell fatigue. As a panel is exposed to the environment before the analysis, it is unable to rate the substance in comparison to a known reference dilution, therefore it is impossible to change the concentration of the mixture. It should be taken under consideration, if a panel preserves objectivity when is exposed to the source of the odor. Different weather conditions, such as temperature, humidity, and wind speed, during the measurements, are also important (52), (53).

Another field olfactometer available commercially and the most common in the USA, is the Nasal Ranger™, which has a filtration system that can create almost odorless air. It is a light, portable device with built-in two replaceable carbon filters to purify the air and a channel system to mix and divide gas samples. A built-in airflow meter limits the stream up to 16-20 l/min. A panel breathes for at least a minute of pure air to clean the olfactory sense, and then gradually, concentration of odor is increased, which is always followed by a purifying of the nose. The dilution degree of a mixture may be manually changed by regulating the valve. The dilution-to-threshold ratio (D/T) is the step on the dial when the sniffer first smells the odor (38). The Nasal Ranger was designed to assess the nuisance of odor sources and determine the validity of complaints of bad smells from animal farms, landfills, compost piles, sewage plants, factories, etc. (54). Local governments used it to regulate recreational marijuana's use and production (55).

The IDES Scentroid SM 100 is a new model of portable field olfactometer dedicated to determining the concentration of odors from ambient air, sample bags, or directly from stacks. Concentrations of samples are presented in increasing order and are regulated by sniffers by a sliding valve called dilution regulator till the moment they detect the smell. Samples are diluted in a fresh air stream from an air container filtered by a carbon filter (56). Damuchali (57) compared its possibilities to a standard laboratory olfactometer in determining volatile compound concentrations of samples with n-butanol gas and poultry barn exhaust air. Both olfactometers met the criteria of the European EN 13725 Standard (2003) (32) Two olfactometers obtained similar results for full-strength and diluted samples with odor concentrations. SM 100 gave 28%–41% higher values for poultry barn smell. This relationship was not observed for n-butanol gas samples. The author concluded that the difference of chemicals may influence the concentrations of odors measured by various devices (30).

Figure 4: Trained panelists using field ofactometers in detecting odors *in situ*

https://www.flickr.com/photos/acwa/27967249765(PEO ACWA).

Static olfactometry (Triangle Odor Bag Method)

Measurements of odor concentration, according to PN-EN 13725, demand to the use of a complex device for precise tested gas in clean air. Similar measurements can be made without the use of olfactometers - the method is called static olfactometry or Triangle Odor Bag Method. Static concentration is mixing the exact amount of tested gas and clean air. The Ministry of the Environment in Japan recommended evaluating the environmental pollution. The sensory (organoleptic) measurement is controlled by specific procedures, which ensure the accuracy and repeatability of the obtained results. The procedures defines teams of panelists, sampling and transport to the laboratory, dilution of samples with clean air and presentation them to the team of experts, the analysis of the results (58). The olfactory sensitivity tests of each of the evaluators are performed using five chemical compounds in paraffinic solutions with various smells - from floral to fecal. The concentrations of these solutions were selected based on research on a large amount of population. The mean values of the detection threshold and standard deviation were determined for solutions. A "two out of five" test is used to determine sensitivity. An examined person receives five numbered papers with odors, two of which contain the standard smells, and three are immersed in pure paraffin. The task is to smell all the stripes and identify the ones that smell. To join a panelist team a person cannot make any mistake. Samples are taken into PET bags with the use of pocket Teflon pumps or evacuated glass cylinders (59). Measured with glass syringes odors are introducing into airbags with a capacity of 3 dm^3 containing air purified in an activated carbon filter and then they are presented to odor evaluation teams. Triangle tests are performed to state the concentration of odor in a sample that leads to odor detection threshold ("Which of the three samples is contaminated?"). Six selected people participate in a test. They receive three numbered bags (two contain pure air and the other one has a solution of air and odor). A panelist has to determine which bag contains odor as well as its intensity. The amounts of the sample keep getting smaller in the following step, depending on the result of the evaluation of the preceding sample. The measurement ends when the correct answers reach approximately 33%. The result of the measurement is expressed as an odor index (60). Fig.4. ofactometers in detecting odors *in situ* during the odor test.

In Table1, a comparison between broadly used dynamic olfactometry and static olfactometry is presented.

Table 1: Comparison of static and dynamic olfactometry (58), (49).

Stage in the measurement procedure	Features	Triangle Odour Bag Method	Dynamic olfactometry
Panelist selection	Model odours	five different smelling compounds	n-butanol
	Presentation of odours	paraffinic solutions on perfume paper	air streams with different n-butanol amount
	The type of psychophysical test	two out of five	type of test depends on the olfactometer
	Selection criteria	all answers correct	detection threshold range for the mean and standard deviation
	The minimum number of people in a team	6	4
Sampling		flexible foil containers, lung method, direct pumping method	flexible foil containers, lung method, direct pumping method
Measurement	The method of diluting the sample with clean air	static dilution in bags	dynamic dilutions in the olfactometer
	The lower limit of the measuring range	< 2 ou/m³	several dozen ou/m³
	The type of psychophysical test	triangle test	type of test depends on the olfactometer
Standardization		Odour Index Regulation and Triangular Odour Bag Method	PN-EN 13725

Gas Chromatography-Olfactometry (GC-O)

Hybrid solutions such as chromatography-olfactometry (GC-O) are also very popular. The technique was created to analyze complex mixtures of odorous compounds by a joined instrument consisting of gas chromatography and sensory measurement. It allows to overcome the subjectivity of the human olfaction organ and delivering more objective measurements (61). GC-O consists of GC-MS system combined with an olfactory detection port – a sniffer mask. The trained panelist smells the gas and gives information about his feelings. In the GC column, the gas mixture is separated into single chemical compounds and an equal stream of odor hits MS detector and the panelist's nose. When a panelist smells an odor, he pushes a button and describes the type of smell. The instrument allows for deeper analysis of a gas mixture based on chemical properties and the noticeable smell, reliable and repeatable identification of each compound, and quantification (61). A weak point of GC-O is that panelist's subjec-

tivity and inattention may have an impact on the results, especially when stimuli are short and not intensive. However, in many situations, a human nose is much more sensitive than GC-MS. Unfortunately; the technique does not determine the concentration of a gas mixture. Another disadvantage is that samples are not analyzed as a whole, because of the separation of compounds. This is why it is not a good method for dispersion modeling and does not provide knowledge about an odor impact (62).

In Table 2, different types of olfactometry were compared. In Table 3, the advantages and the disadvantages of mentioned techniques were compared.

Table 2: Characteristics of odour measurement techniques

	Flow olfactometry	Dynamic olfactometry	Field olfactometry	Static olfactometry	GC-O
Objective methods	yes	no	no	no	yes
Quantitative measurement of odour concentration	no	no	no	no	yes
Measurement standardization	yes	yes	no	yes	yes
Continuous measurement	no	no	no	no	no
Single substances determination	no	no	no	no	yes (but limited)
Participants selection	no	yes	yes	yes	no
Time of measurement	short	medium	short	medium	long
The need for complex equipment	yes	yes	no	no	yes
Active participation of people	no	yes	yes	yes	yes
Costs of measurement	high	high	low	very low	high
Mobile	no	no	yes	no	no

Table 3: Summary of advantages and disadvantages of the analyzed methods

	Advantages	Disadvantages
Flow olfactometry	objective method, quality criteria, results as a threshold of detection of odours	high cost of analysis, complex procedures, conducting measurements in specialized laboratories, no standards for sampling, not intensive odours can give false-negative results
Dynamic olfactometry	quality criteria for the performance of full measurement, result of the test in the form of the odour concentration	high cost of analysis, no standards for sampling, conducting measurements in specialized laboratories
Field olfactometry	determination of odours *in situ*, low measurement cost, sufficient measuring range to determine emissions of odours, results of measurements in real-time, results are obtained in the form of odour concentration	limited amount of dilution caused by the number of orifices on the D / T disc, difficult to determine the moment of a breakthrough of filters, no specific quality requirements for sensory measurement, limitations in the scope of organized emission testing
Static olfactometry	very low measurement cost, quality criteria for measurement, results in real-time, ability to determine the concentration, standards for sampling	limited amount of dilution, conducting measurements in specialized laboratories, evaluating only strong odours
GC-O	qualitative analysis of several compounds at the same time, determining the concentration values for averaging times of a few minutes or several seconds, continuous monitoring, quality criteria for the performance of a full measurement	high cost of analysis, a single detector enables the analysis of a selected group of compounds, insufficient sensitivity of detectors for odourants, often occurring in very low concentrations, errors in quantitative analysis, resulting from the polarity and chemical activity of odourants, an option of odourants on the surface of sample containers, the need for pre-concentration and desorption for odourants occurring in low concentrations, Inability to determine odour concentrations on the basis of qualitative and quantitative analyzes of the mixture of odourants

Other Methods of Measurement of the Smell Experience

Despite the presence of many kinds of olfactometers, which depend on the subjective feelings of examined subjects, scientists were interested in searching objective signs for odor detection. Many pieces of research have been performed, which have proved the influence of odorants on the sympathetic and parasympathetic nervous system, and neurophysiological brain activity. Odorants can also stimulate the neuroendocrine system, neurotransmitters, and neuromodulators, changing not only body function but also psychological behavior (63). Emotional and behavioral effects of odor stimulation can be evaluated by a few techniques detecting brain activity, such as electroencephalogram (EEG), near-infrared spectroscopy (NIRS), and functional magnetic resonance imaging (fMRI), which is a more expensive method (64),(65),(66),(67). Some researchers proposed alternative ways to test the impact of smell on human body reactions. The literature review on the detection of olfactory stimuli in humans can be found below.

Sokolow (68) noticed that regular breathing changes under the influence of sudden stimulation of smell. Gudziol (70) proved that pleasant odors stimulate breathing whereas unpleasant ones shorten it and the changes can be identified with computer measurements. He conducted the experiment on Kobal's olfactometer (69). Also in another study performed with the application of respiration-olfactometry, he found that inhaled pleasant odor is deeply and longer inhaled. Bad smells caused curtailment time of exhalation. The individuals exhaled rapidly to inhale pure air. He did not observe either any changes in inhalation time or the impact of strong stimuli on the olfactory evoked respiratory response Guziol (70) also proved that Kobal's flow-olfactometer was very useful for subjective and respiration-olfactometry. He performed an examination, in which individuals were stimulated by hydrogen sulfide and phenyl ethyl alcohol (PEA) solutions at different concentrations and had to signalize when they smell the odor. A differential pressure transducer monitored continuously the respiratory nasal pressure. He showed that the detection rate depends on odor quality and its concentration, while these two values do not influence the frequency of detection with simultaneous alteration of breathing pattern. Bad smells were more frequently detected.

According to Ehrlichman's study (72) the relationship between the eye blink and unpleasant odor exists. He measured base tension *in orbicularis oculi* muscles by electromyography during unpleasant, pleasant, or non-odor conditions and providing acoustic probes. He delivered white noise to individuals via headphones after 400 ms of the second or third inhalation of an odor, from one of the 18 bottles, in various computer-generated random orders. During the test, the subjects breathing by a Grass ONT2 thermocouple was measured. They were visually monitored to ensure that procedures are followed properly. EMG reactions were controlled by two Beckman *Ag/AgCl* miniature disk electrodes placed on the orbicularis oculi muscles beneath the left eye and a ground electrode on the center of the forehead. Ehrlichman found that participants achieved higher values in EMG experiencing unpleasant smell. The results for pleasant and non-odor were similar. The author also suggests that pleasant smells demand accurate background to generate a positive state. The same author conducted a similar study, in which the participants smelled pleasant and unpleasant odors before presenting to them an acoustic startle probe. Grass ONT2 thermocouple measured an inhale or an exhale of individuals. The reactions were measured by the same EMG. Heart rate (HR) was recorded by two Beckman *Ag/AgCl* electrodes placed in the middle of the left clavicle and the left ankle. Four odor blocks and four odorless blocks were delivered. The researcher found that the unpleasant odor samples invoked greater blink magnitude during the odor blocks in contrast to pleasant odor conditions (73) Hermann examined Pavlovian aversive and appetitive odor conditioning in humans based on subjective, peripheral, and electrocortical changes. Slides of two different neutral faces that were easy to discriminate served as conditioned stimuli (CS). The reactions were measured by a 9-electrodes EMG from the musculus corrugator supercilii and musculus zygomaticus. The *musculus orbicularis oculi* provided the startle response. Furthermore, the electro-oculographic (EOG) activity was controlled by electrodes placed above and under the eye - vertical EOG activity, and at the outer canthi - lateral EOG activity. Heart rate and skin conductance response were monitored. Odors were delivered by a 1-channel olfactometer. Aversive conditioning was confirmed by the subjective data and the skin conductance response while pleasant odor did not influence appetitive odor conditioning. The study revealed that the musculus corrugator is strongly connected to the expression of negative affect, which is a non-voluntary response, unlike the startle reflex, which does not depend on conditioning (74).

Pupillary and ocular responses to olfactory and visual stimuli were the subject of interest of Aguillon-Hernandez (75) 39 healthy participants between 19 and 77 years old were stimulated with ten familiar odors during 10 s, while a board bearing with four-color pictures was presented to them. Three stages of validation needed to be used in the database of visual stimuli. The ocular behavior and pupil size variation was measured by the corneal reflection of infrared light. The author designed three stages: One without olfactory stimulation and two with smell stimuli: objective, while a spontaneous response to the olfactory stimulation was measured, and subjective, while individuals had to identify odors. The pupillary diameter was measured every 100 ms during a presentation of an image during the two first stages (non-olfactory and objective). A baseline pupil diameter was verified 500 ms before the start of stimulation. The pupil diameter variation was measured by subtracting the baseline and the mean change in pupil size between 1 s and 4 s while presenting an image. Measurements of the pupil were done only when a subject correctly recognized odors during "a subjective stage". Event-related gaze focus (ERGF) was determined by measuring the position of the eye while individuals were watching the board with images. The time of exploring and fixating the vision on regions of interest was detected by a dispersion based-algorithm. The author observed that odor presentation caused a pupil dilation and focus of visual on the picture corresponding to the odor, which leads to the conclusion that odors stimulate the sympathetic system and increase attention to the visual clue.

The Galvanic Skin Response (GSR, or electrodermal activity, EDA) can trigger an emotional reaction and attracts attention, which causes an increased vigilance. The response can be detected around 2 s after the trigger, across the palms of the hands or soles of the feet, as an increase in the electrical conductance of the skin and a decrease in resistance. Parts of the brain's premotor cerebral cortex appear to take part in producing GSR by the activation of postganglionic sympathetic fibers of the sweat glands (76). Van Toller (77) described two experiments. One, while exposing individuals to 5-α-androstan-3-one (androstanone) and, a control odor, aurantiol, he measured skin conductance. Subjects also rated odors by analog scales. They were asked to sniff a perfumer's smelling strip with a solution of androstanone. According to their reactions, they were subordinated to three groups: smells "pleasant" or "unpleasant", anosmic. In the second part, subjects were visually and audi-

torily masked. While presenting them odors on the perfumer's smelling strips, The GSR was measured on the medial phalanges of the first and second fingers of the right hand by Grass 7D polygraph and a Birkbeck Skin Conductance Unit. Individuals' answers were controlled by a polygraph. In the first experiment, reactions between subjects who found the androstanone as pleasant or unpleasant and between controls were compared. The author found an influence of cognitive factors between male, female, "pleasant" and "unpleasant" groups. In the second experiment patients with specific anosmia to androstanone were examined.

Omam (78) measured the responses of human skin and brain to different pleasant odor stimuli by the use of galvanic skin response (GSR) and electroencephalography (EEG) accordingly. He observed that the complexity of GSR and EEG signal changes along with the increasing molecular complexity of olfactory stimuli. The fractal dimension was considered as the indicator of complexity. Odors were presented on a small piece of paper in the front of subjects' noses for one minute. First EEG and GSR signals were measured for a minute while subjects had a rest, they did not receive any external stimuli, afterward, and signals were analyzed during sniffing substances again for one minute. One-minute rests were made between each stimulus. The more complex were odor stimuli, the more complex were reactions from the brain and human skin. The author concluded that the reaction of human skin changes according to the activity of the human brain. The differences in the complexity of GSR results are strictly connected with changes in EGG signals). Similar studies were performed by Møller and Dijksterhuis (79) who detected various skin conductance responses to pleasant and unpleasant odors. While subjects were exposed to iso-intense and non-trigeminal pleasant and unpleasant odors, they measured skin conductance on both. Four different odors were presented twice to subjects; they were interspersed three times with a blank stimulus, in a random order of 12 stimuli. The study revealed that various odors induce different skin conductance responses, at the same time unpleasant odors do not produce larger skin conduction responses than pleasant odors. The authors also suggest that skin conduction and emotional responses are controlled by an ipsilateral system.

Sutani (80) studied differences in the brain responses between pleasant and unpleasant odors in order to evaluate the impact of certain smells on each individual. Psychophysical experiments were performed by the use of an olfactometer before magnetoencephalography (MEG) tests to arrange olfactory sensory thresholds of individuals and the concentration of olfactory stimuli. Next subjects were stimulated by different odors during 300 ms. Brain reactions were monitored by MEG scanner and breath was detected by the fiber optic sensor. MEG is similar to EEG, however, instead of an electric field, it measures the magnetic field generated by the brain, which makes it possible to accurately locate the focused activity of the brain. The main disadvantage is the high cost of the device and this is why it is mainly used in scientific researches.

Problems with the standardization of a method and accurate control of stimuli also exist. The whole-cortex-type MEG system was used to detect neuromagnetic responses. Six different MEG sensor groups covered frontal and temporal cortical areas. Local signal power from the MEG waveforms was calculated by Root-Mean-Square value (RMS). Sutani (80) reported significant differences in the MEG signals recorded from frontal/prefrontal regions bilaterally between different smell conditions. Increased MEG signals were observed in the frontal/prefrontal regions both in pleasant and unpleasant conditions.

It was possible to detect potentials of olfactory receptor cells by electroolfactograms (EOG), which collected signals from the surface of the olfactory mucosa. EOG represents a summated potential generated by olfactory sensory cells (81). It should be underlined that to perform such examination an olfactometer, which does not change mechanical or thermal conditions of the nasal mucosa, must be utilized. Otherwise, it can influence the final results. Very often it is difficult to distinguish between the brain responses from the olfactory and trigeminal nerve (82). According to Kobal and Hummel (83) the main technical problem in detecting olfactory evoked potentials from cortical neurons was that onsets of stimuli had to be steep to invoke synchronous activation of enough amount of cortical neurons to start detection of the Event-Related Potentials (ERP) (. They achieved monomodal chemical stimulation by mixing pulses of odors with constant airstream at constant temperature and humidity. The concentration of the gas solution and interstimulus intervals, when pure air was delivered to a nose, was controlled by a switched system based on two separate sources of vacuum. It gave also an opportunity to manage the total flow rate and stimulus duration. Moreover, a very important aspect is breathing control to avoid artifacts or the impact of respiratory air on odorant transportation. In order to observe the time of the odor responses should be independent of breathing. That is why the technique of velopharyngeal closure was adapted to avoid the flow of respiratory air in the nose. General anesthesia is contraindicated because it might cause temporary anosmia (83). These observations were used by them in another research. They detected peripheral electrophysiological responses to olfactory stimulation. Authors delivered a constantly flowing air stream with pulses of odors by olfactometer without altering mechanical or thermal conditions of the nasal mucosa. White noise was used to mask the sounds of a simulator. During the experiment eye blinks and individuals' movements were measured. The electrode was inserted in the nasal cavity. Subjects were instructed about the breathing method and the velopharyngeal closure technique was used. Mucosal potentials were measured. The results proved that comparing electro-olfactograms and subjective intensity estimates, the first ones show a smaller degree of desensitization (84) .Scientists observed responses after application stimuli such as coffee (85) amyl-acetate, hydrogen sulfide, and eugenol (86).

The human olfactory evoked potentials (OEPs) were first reported by Allison and Goff (87) and Finkenzeller (88). OEPs are an objective diagnosis of olfaction obtained by electrical stimulation of the olfactory mucosa. They are recorded from the surface of the skull. Kobal (89) showed that patients with

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Heart rate and blood pressure as well, can indicate psychophysiological effects of fragrances. Hongratanaworakit and Buchbauer (65) summarized different types of odor psychophysical effects. For example, sweet orange inhalation causes increased heart rate and subjective alertness While Haze (94) investigated the influence of fragrance inhalation on sympathetic activity in healthy adults, he measured blood pressure fluctuations and plasma catecholamine levels. The results showed that odor inhalation might change sympathetic activity. It can be used as a mild regulator of dysfunctions of the sympathetic nervous system.

Table 4a: Summary of psychophysical test of smell detection

Stimulant	Manner of presentation odours	Main measurement	Other measurements	Results	Ref.
H_2S, clean air	Olfactometer	Spirometry		unpleasant odours shorten breathing	(69)
H_2S, phenylethyl alcohol	Flow-olfactometer OM2S	EEG: olfactory evoked respiratory response (OERR)	Respiratory pressure (manometer)	-	(70)
H_2S, phenylethyl alcohol	Flow-olfactometer OM2S	Spirometry	EEG: chemosensory evoked potentials button press when the odour is detected	the detection-rate depends on the quality of odour and concentration, bad smell was detected more frequently	(71)
Limburger cheese, smoked cigar butt, crushed vitamin B complex pills, butyric acid, isovaleric acid, thiophene, orange oil, coconut, vanilla bean, Muguet, Baghdad water lily, Douglas fir, pure air	Bottles with odour and cotton	EMG: startle reflex	-	Unpleasant odours cause greater muscle tension	(72)
Coconut, Limburger cheese	Bottles with odour and cotton, a burst of white noise	Spirometry	EMG: startle reflex heart rate (HR)	the unpleasant odour samples invoked greater blink magnitude during the odour blocks and higher heart rate	(73)

Table 4b: Summary of psychophysical test of smell detection

Stimulant	Manner of presentation odours	Main measurements	Other measurements	Results	Ref.
Mint, orange, mushroom, bread, vanilla, coconut, strawberry, raw potato, oil paint, wet earth	an olfactory cup on a small rigid device	ERP: pupil dilation, ERP: gaze focus	subjective identification: hedonic and intensity of the odour	pupil dilation is an objective indicator of physiological response to olfactory stimulation, odour stimulation directs visual attention towards the target	(75)
Not known	not known	GSR	-	activation of the sweat glands by the postganglionic sympathetic fibers caused by odours	(76)
-α-androstan-3-, aurantiol	a perfumer's smelling strip	GSR	rating odours by analog scales	an influence of cognitive factors between male, female, "pleasant" and "unpleasant" groups, the concept of specific anosmia requires modification	(77)
Pineapple, vanilla, banana, lemon	a small piece of paper with odours	GSR	EEG	the reaction of human skin changes according to the activity of the human brain and the complexity of odours	(78)
Blank, butyric acid, citral, peach, skatole	under the nose	GSR	-	various odours induce different skin conductance responses, the ipsilateral system controls skin conduction and emotional responses	(79)
Citronellol, trimethylamine, odourless air	olfactometer	MEG	Spirometry	increased MEG signals in the frontal/prefrontal regions as the response to different odours	(80)

Table 4c: Summary of psychophysical test of smell detection

Stimulant	Manner of presentation odours	Main measurement	Other measurements	Results	Ref.
Vanillin	airstream led into the nasal cavity by olfactometer through a Teflon tube	EEG	visual analog scales to evaluate odours intensity	peripheral encoding in the olfactory system is less subject to desensitization compared to the decrease of intensity estimates	(84)
Vanillin, phenylethyl alcohol, limonene, menthol, anethol, benzaldehyde, carbon dioxide, mixture of vanilin and carbon dioxide	constantly flowing air stream with pulses of odours through Teflon tube in the nasal cavity	EEG: Olfactory Evoked Potentials (OEP)	-	lack of evoked potentials in anosmic patients during 2-phenethyl alcohol, vanillin presentation	(89)
Vanillin, phenylethyl alcohol, limonene, menthol, anethol, benzaldehyde, carbon dioxide, a mixture of vanilin and carbon dioxide	constantly flowing air stream with pulses of odours through Teflon tube in the nasal cavity	EEG: chemosensory evoked potentials	Electrooculogram (EOG)	potentials to carbon dioxide, menthol, ammonia, sulfur dioxide evoke chemosensory evoked potentials (from the trigeminal nerve, in the frontal cortex), olfactory and chemo-somatosensory system can be monitored by chemosensory evoked potentials, also in medical practice	(90)
Carbon dioxide, menthol, hydrogen sulphide, vanillin	constantly flowing air stream with pulses of odours through Teflon tube in the nasal cavity by olfactometer	EEG: chemosensory evoked potentials	-	odours stimulate different topographical patterns, hydrogen sulfide, vanillin, acetaldehyde in low concentrations are very specific for the olfactory nerve (the mid-line of the cortex)	(91)
Not known	not known	EEG: evoked potentials	Computer-Tomography (CT)	Diagnosis of different brain diseases	(92)

Table 4d: Summary of psychophysical test of smell detection

Stimulant	Manner of presentation odours	Main measurements	Other measurements	Results	Ref.
Not known	not known	event-related potential (ERP)	Sniffin' Sticks test before treatment and after 1 year of follow-up	OEPs can be a predictor of olfactory recovery in post-infectious olfactory dysfunction	(93)
-	-	heart rate (HR), blood pressure, GSR, EEG	-	various aromas can cause different physiological effects	(65)
Pepper, estragon, fennel, grapefruit, triethyl citrate, rose, patchouli oils, odourless solvent	absorbent cotton	blood pressure, plasma catecholamine levels	GC/MS analysis	odours can be used as a mild regulator of dysfunctions of the sympathetic nervous system	(94)

Conclusion

In this paper we have tried to expose much of the literature we have found on systems that measure olfaction in humans, analyzing their applications and, of course, also their limitations. Advances in the field of olfactometry provided many modern methods of odors detection and measurement. However, most of them are very complex, demand standardized procedures, and are based on subjective feelings, although they are still not fully satisfactory and demand further research. Most of the devices can only evaluate whole mixtures, not single compounds. There is a lack of simple and inexpensive instruments for objective, quick, precise, repeatability measurements of odor concentrations as well as to determine the composition of solutions.

Dynamic olfactometry systems are a standardized method to measure the concentrations of volatile substances to assess odor in emissions, varying their concentrations that can be used to verify the state of human olfaction and provide information that can be used in medical concepts. Other sensory techniques, such as field inspection or GC-O, have, as dynamic olfactometry, the advantage of offering a higher sensitivity of the human nose with regards to analytical methods. All this is very advantageous, but they are not always applicable, as they can be affected by subjectivity, and are more time-consuming and costly.

Analytical tests based on chemical characterizations avoid human errors but are more difficult to measure odors especially in complex compositions and mixtures. If we compare the advantages of the Smell-o-meter with the E-nose we see that the latter have the advantage of offering faster results at a lower cost which offers a continuous monitoring of the receptors' odors. However they cannot measure odor intensity and hedonic tone. Neither can they replace dynamic olfactometry, because the odor concentration of the samples must be known to properly train the instrument.

We can conclude that in many cases it is very difficult to answer which series is the best screening method because we must evaluate each case individually. Perhaps the combination of both methods would allow us to go deeper into their study and better manage the results.

Author Contributions

Clarós P , Dabkowska A and Clarós A., wrote the paper. All authors listed have also made substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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