The construction of a programmable olfactometer

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A new type of multichannel olfactometer is described, and the results are given for gas chromatographical standardization of this equipment. The olfactometer keeps 24 odorous substances available for presentation, consecutively, two per trial, or combinations of the two. It produces concentrations of odorous gas in the air between 1×10^{-5} and 1×10^{-11} molecules/liter. The principle of construction is gradual dilution of an odorous gas in streaming purified air. The

design provides for electronic control of stimulus sequence and response registration via punched tapes.

Starting with the pioneering work of Zwaardemaker (1889), olfactometry has created numerous techniques designed for precise quantitative determination of odorous substances in the respired air. The relevant unit of measurement is molecules/liter of air intake. By now, we know that only a so-called "flow olfactometer" may render stable and precise stimulus concentrations. With this type of equipment, a defined quantity per time of an odorous gas is injected into a constant stream of conditioned air. Earlier instruments using stagnant gas lost an

uncontrollable amount of molecules by adsorption on the surrounding surfaces. Due to this contaminating adhesion, they did not readily allow an alternation of smells within the outfit. Johnston (1967) describes some recently developed olfactometers; other designs are outlined by Bozza et al (1960), Köster (1965), Ekman et al (1967), and Goldenberg (1967).

Flow problems create the main difficulties in the construction of an olfactometer. Being unsolved theoretically, most of these need experimental solutions. At least the following principles should be observed:

(1) The odorous air to be inhaled by the O should constitute only a small portion of the O should constitute only a small portion of

Fig. 1. Diagram of the total olfactometer. The second cabin is not drawn out. Also, the exhaustor (EX) is omitted. The explanation of symbols and abbreviations is given in the text.

the total flow. Normal intake of a sitting person is 5 liters of air per minute.

(2) The total surface in contact with the stream of odorous gas should be as small as possible.

(3) The flow should be turbulent, i.e., of high speed, in order to ensure a homogeneous mixture of gases. In addition, the high speed minimizes the relative loss of odor molecules and maximizes the removal of adsorbed layers between trials.

These principles are not generally respected. This applies especially to smelling devices that may be as large as hoods (e.g., Stone, 1964), offering enormous surfaces for adhesion. Furthermore, the hitherto published designs generally show two limitations of their applicability: (1) For a single experimental session, the number of different odorous substances and concentrations to be presented is usually small; and (2) the designs do not provide for simultaneous presentation of two or more stimuli, a desirable feature for comparative judgments. Both limitations had to be overcome in the construction of the new olfactometer described here. The design was influenced by suggestions cited in Köster (1965).

THE DESIGN OF THE OLFACTOMETER²

Air Purification

Figure 1 shows the scheme of the apparatus. A compressor, K, delivers 120 liters of air per minute at a pressure of 3 pounds per square inch from the laboratory into the system. An oilless eccentric compressor had to be reconstructed to eliminate contamination. The air is filtered by activated carbon at C and flows through Check Valve R. The air is forced through a filter plate of medium porosity into the vessel, W, filled with distilled water to remove soluble

substances. A part of this purified and humidified air is dried in TR by silicon gel; this part is proportioned to keep the relative humidity constant (usually at a 60% level). The conditioned air then reaches the bulb, L, which has a volume of 60 liters and is kept under constant pressure of 0.70 pounds per square inch by an adjustable safety valve, SV1. Temperature and humidity of the air are indicated by the gauges, T and H. The air reaches the two distributing systems through two valves and the flowmeters, M1 and M2. Surplus air escapes through the safety valve and is conducted to the experimental cabins.

The Carrier Gas System

Nitrogen is used as a carrier gas for the odorous molecules. The liquid smelling substances are kept in the gas-washing bottles, F. The nitrogen flows from the compressed-gas cylinder, N, through a pressure-reducing device and a micro-regulating valve to the flowmeters, M3 and M4, before reaching two sets of 12 bottles each. In these bottles, the nitrogen may bubble via filters of medium porosity through about 30 cc of odorous liquid to be saturated with odorous molecules. The nitrogen passes those bottles if the electromagnetic valve, DF, at the outlet to the distributing system is opened. If all of these valves are shut, the overpressure escapes through Valve SV2. The bottles are kept in a thermostatically controlled water bath, wherein the nitrogen is also tempered (usually at a temperature of 26°C). In the flowmeters, the position of the float is controlled by selenium transistors.

The Distributing Systems

The two distributing systems (Fig. 2) are designed to proportion the influx of saturated nitrogen into the stream of conditioned air. The existence of two systems allows for comparative judgments

and exact mixtures of odors. The main elements of these systems are glass bifurcations with precision capillaries, C. Air coming from the bulb, L, will be divided by the first bifurcation, leading two-thirds of the flow to the exhauster and transmitting one-third to the next bifurcation. Here, the air is merged in a stream of fresh air, which is then divided according to the same principle, i.e., two-thirds go to the exhauster and one-third goes on to the third bifurcation, and so on, up to the 10th bifurcation.

An electromagnetic valve is positioned 3 cm ahead of each bifurcation. Here, carrier gas is injected if Valve D opens, and it will be partitioned also in the ratio of 1:2 at the next bifurcation. In case the gas is injected at the last valve, D10, it flows to the smelling device without loss. Only one-third of the gas reaches this device if it is injected at D9, i.e., the proportion of this gas is reduced continuously to a third at each bifurcation, thus constituting a geometric progression of possible concentrations ranging from 16.67% to .00075% of carrier gas in the total volume.

The layout of these distributing systems demanded extensive experimental determination of pressures and flow conditions. These measurements led to a choice of capillaries that allow constant partition at all bifurcations, independent of the injected amount of carrier gas. The diameters of the capillaries vary from 1.15 to 2.0 mm. The carrier gas is injected through a tube of 1-mm width. The dimensions of the capillaries at each bifurcation had to be determined individually since the pressure decreases from D1 to D10.

A very compact design of the system was desired in order to minimize the total surface of the tubes leading from the liquid substances to the place of presentation. This surface covers a maximal 300 cm² and consists of glass. In the valves and at the fittings, it consists exclusively of PTFE (Teflon). These materials have low adhesion constants and do not interact chemically with the odorous substances. Each of the two distributing systems conducts 40 liters/min at speeds of 217 to 2,567 m/sec. This speed keeps up

Fig. 2. Diagram of the distributing system. The incoming air goes through the capillaries, C, being conducted partially directly to the exhauster tube (EX), and partially via the zig-zag way to the smelling device. The odorous carrier gas is injected via Valves D1 to D10. This gas is pressed at N through Bottles F and Valves DF into the zig-zag way of the distributing system. For further explanation, see text.

Fig. 3. Smelling device. Two alternative versions are pictured: on the left, the version for comparative judgments, and on the right, the version for absolute judgments. The odorous air is inhaled through the nose pieces.

turbulence, according to the calculated Reynold numbers (Fluegge, 1959). During intertrial intervals, Valves DP and all Valves D of the distributing system are opened automatically, allowing pressurized air to rinse the whole system.

The Smelling Device

At the outlets of the distributing systems, 13 liters of gas per minute are available for presentation. This odorous air is conducted to two experimental cabins simultaneously. In each cabin are two smelling devices. By means of the distribution valves, VK, it is possible to direct the stimulus to the right or to the left device or to both. Thus, position learning may be avoided in comparative studies. The tubes that are not carrying odorous gas conduct purified air. It is also possible to direct two different stimuli to each of the devices or a mixture of both to one or both of them.

The smelling devices (Fig. 3) consist of two semicircular glass tubes in the wall of each cabin. Each tube carries a nozzle on which bulbous nose pieces may be fixed. The O introduces these pieces into his nostrils so that he may inhale through two tubes, each leading to one of the systems. An alternative is provided for comparing two different stimuli. In this case, on each nozzle a bifurcation is fixed, ending in two nose pieces, allowing the O to sniff by turns samples from the right and the left system.

To avoid the escape of odorous air into the cabins, a tiny glass cone is placed over a contraction of the tube. This cone is lifted if the O inhales, otherwise it shuts the outlet. This type of nonreturn valve was first used by Köster (1965), who also found that the mechanism keeps the air intake fairly constant since too vivid sniffing leads to a block in the passage of air. Gas that is not inhaled is extracted by the exhaustor. Lamps over both devices signal when and where the observation is

wanted. The Os use a keyboard to indicate their judgments, which may be answered by optical feedbacks. Electronic control of the whole system is in preparation. It will provide a programming of the sequence of stimuli and concentrations by means of a punch tape. Also, the reactions of the Os will be recorded directly on punched tape. The cabins are air-conditioned, using the surplus of purified air that does not go into the distributing systems.

STANDARDIZATION OF STIMULUS CONCENTRATION

The definition of odor concentrations given by this system depends on (1) a knowledge of the flow characteristics of the system; (2) the homogeneity of the mixture of air and carrier gas; and (3) the negligibility of adsorbed quantities of the odorous substance in the total flow of gas.

The fluxional characteristics of the distributing systems were determined experimentally. These depend mainly on the dimensions of the capillaries. In order to test the homogeneity of mixtures and the importance of adsorption on the surfaces of the system, we used gas chromatography.³ In this standardization procedure, purified nitrogen was substituted for the conditioned air and ethylene for the carrier gas. These gases were chosen since extremely small proportions of ethylene in nitrogen are detected by the gas chromatograph.

A sample of nitrogen-ethylene mixture was taken from the smelling device for each stimulus concentration. These samples were analyzed by gas chromatography. The results confirm nearly perfectly the concentrations that were calculated on the basis of the flow characteristics of the distributing systems alone. This indicates homogeneous mixing of air and carrier gas and low adsorption in the system. Figure 4 shows the ratios of ethylene to nitrogen for the 10 possible concentrations that are equivalent to the ratios of carrier gas to purified air. The solid line indicates the theoretically expected function, assuming constant partition of the flux at all bifurcations. The small deviations of some gas chromatographical data from this expected line are readily explained by imperfect choice of capillaries, the diameters of which are available only in steps of .05 mm. The expected distribution characteristics were thus extremely well confirmed by gas chromatography.

A definition of concentrations in terms of molecules/liter presupposes not only the relative but the absolute amount of odorous gas in the inhaled air. The quantity of a substance transported by a given volume of carrier gas may be calculated if its constants in Van der Waal's

Fig. 4. Concentrations of carrier gas in the total flow of gas according to gas chromatography. The abscissa gives the number of the valve opened, the coordinate, the ratio of carrier gas to air (C) on a logarithmic scale. For further explanation, see text.

equation are known. For many odorous substances, this is not the case. Furthermore, we did not know if saturation of the carrier gas with odorous molecules would be reached under the given speed of the streaming nitrogen. According to experimental determination by weighing and by determination of evaporation under different temperatures, the saturation is reached. The amount of molecules that goes into the system has to be determined, in most cases, by weighing the chemical to be used as an odor stimulus before and after nitrogen is sent through it for about 1 h.

The concentrations produced by this olfactometer vary for different substances, depending on their saturation level in nitrogen. However, the ratios between the concentrations remain constant. The example of tetrahydrolinalool may indicate the range of these concentrations: Using a flow rate of 40 liters/min of air in one system and a flow rate of .921 liters/min of nitrogen, we get 10 concentrations in a geometric progression with 3.10^{-6} molecules/liter as the highest and 2.10^{-10} molecules/liter as the lowest concentration.

This range is very suitable for determining absolute thresholds. For other purposes, the distribution of the 10 concentrations may be changed by altering at least one of the following variables: the flow rate of air, the flow rate of nitrogen, and the temperature of the odorous substances as well as of nitrogen.

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NOTES

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2. Specifications of technical equipment (flowmeters, valves, etc.) and of capillaries are given in a technical report that is available on request.

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The production and fading of entoptic images

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A new apparatus, suitable for obtaining reliable reports of entoptic images, was developed. Basically, this apparatus moved a small light across the S's eyelid (transcleral illumination). While the light was moving, Ss reported the appearance of the three entoptic phenomena of interest—retinal blood vessels, foveal images, and background illumination. After initial training with this apparatus, 12 Ss were tested for the differential disappearance (fading) of the entoptic images after the light source stopped moving but continued its transcleral illumination of the retina. Results indicated that although the background image did fade to a dark field, this background fade time ($\bar{X} = 1.3$ sec) is faster than that reported for externally stabilized images, which may take 2 to 4 sec to fade, and was significantly slower than the very rapid fade times for blood vessels and foveal images. Furthermore, there was no reappearance of these entoptic images.

Entoptic images, or images within the eye, include a variety of phenomena including floaters, afterimages, phosphenes, Haidinger's "Brushes," retinal blood vessels, and foveal images. Some of these (floaters, afterimages, and phosphenes) are quite common everyday occurrences; the others require specific manipulation in order to be experienced. Looking through a polarized filter at a field of blue light produces Haidinger's "Brushes" (see Ratliff, 1958), and illuminating the retinae through the sclera (eyelid) with a moving

light source allows the perception of the retinal blood vessels and foveal images.

Helmholtz (1866) gives an extensive discussion of Purkinje's methods for observing the shadows of retinal blood vessels; in a sense, Purkinje anticipated the results of contemporary work on stabilized images by noting that movement was required to make the retinal blood vessels visible. Normally, retinal blood vessels are true stabilized images; they occur entoptically and are not subject to the types of artifacts, such as slippage of contact lenses (Barlow, 1963), associated with external, optically stabilized images.

The fading of these external images, when the image is cast by a mechanism mounted on a contact lens, has been reported by many investigators (Riggs et al, 1953; Pritchard et al, 1960; Barlow, 1963; and Heckenmueller, 1965). A typical image would consist of a patch of light (about 5 deg of visual angle), with an image (about 2 deg of visual angle) focused at apparent infinity. After a few seconds the image fades and shortly thereafter the field is completely lost. The image may reappear after a short period and then fade again (Pritchard et al, 1960). The complexity of the image (i.e., single line vs human face) determines, in part, its persistence. The more complex images tend to last longer.

A few brief reports (Campbell & Robson, 1961; Cornsweet & Dwelley, 1962) have suggested the use of entoptic images to study image stabilization but no data were presented.

The present study explores the use of such entoptic phenomena to study the effects of image stabilization. With the development of the techniques to be described, it has been feasible to overcome the objections that entoptic phenomena may not be compared to the fading of externally stabilized images on the retinae.

METHOD

The apparatus consisted of three basic parts: a vise-mounted head stabilization

mechanism (a dental "bite bar" made of mouth plates covered with dental wax), a scaffolding for placing the transcleral illuminant (bulb, GE No. 22.5) against the S's upper eyelid, and a spring-operated motor system for moving the bulb horizontally between two rails across the S's eyelid.

The 12 Ss were given preliminary training on the apparatus. All Ss were able to report the orientation and color of the retinal blood vessels, the foveal image, and the general background illumination when the transcleral illuminant was moving.

In this experiment, Ss were provided with a button, which, when depressed, stopped the motion of the transcleral illuminant across the eyelid. Ss continued to depress the button until the entoptic phenomenon being studied disappeared. A timer recorded the fade time of the entoptic phenomena in milliseconds. The Ss were tested with both eyes; the selection of right or left was randomized. Once an eye was selected, however, observations on all three entoptic images (retinal blood vessels, foveal images, background) were completed for that eye. The order in which a particular entoptic phenomenon was observed was then randomized and each phenomenon was observed for a series of 10 consecutive trials. This procedure was adopted to minimize the difficulties of realignment and adjusting the apparatus.

The Ss were preadapted to normal ambient lighting (approximately 45 mL) and, after they were seated and aligned with the apparatus, the unused eye was covered with an eyepatch. All Ss were required to bite the dental impression attached to the scaffolding to ensure head immobilization. The transcleral light was then turned on, and motion across the eyelid began. Ss stopped the motion by depressing a hand-held button and released this button when the entoptic phenomenon had completely faded. At the end of each trial, the transcleral light was turned off. Following each block of 10