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Odors, Aerosols, Laminar Air Flow, and Electrostatics

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The electro-olfactogram (EOG) has been widely used to study the nature of odor perception and to evaluate the effect of various contaminants. The typical EOG procedure has been to puff or flow odorant laden air at the receptor tissue through a small diameter tube located within centimeters of the tissue (Gesteland et al 1982; Ottoson 1956; Thommesen and Doving 1977). Electrodes in contact with the tissue typically show a negative wave response, often with a very small initial positive wave. This response has been the basic datum in EOG experiments, but there have been conflicting findings and interpretations. This has made it difficult to define the stimulus material's relationship to the receptor cell and mucosa chemistry.

Complex electrical fields exist in all spaces. They interact with particles, water droplets, and adsorbed gasses, which are almost always charged. These interactions, in large part, determine the deposition of substances in and on people, objects, and walls in a space. In an analytical review, the writer defined how these interactions affect the nature of olfactory data obtained with humans and the interpretation of such data from such experiments (Frey, 1968). It was found that electrostatics and air flow characteristics have a critical effect. Frictional charging of the odorant, the charge on the subject, the interactions that occur in the typically complex and uncontrolled electrical environment, and air turbulence influenced the nature of the olfactory data obtained from humans. Thus, experimentation was undertaken to determine if these same factors operate in EOG experiments.

MATERIALS AND METHODS

The air handling apparatus consisted of a mixing box, duct, a perforated cloth ceiling, and a steel exposure chamber with a perforated steel floor. Air from the mixing box passed through a 1.8 meter long, 10 cm diameter herculite cloth duct at a flow rate of 6 meters/min. The air diffused into the exposure chamber through a perforated herculite cloth ceiling. The air passed down through the perforated steel bottom of the exposure chamber to achieve laminar air flow. Programming equipment controlled

the insertion of odorant into the mixing box. The temperature and humidity of the odorized and non-odorized air were approximately the same when they reached the exposure chamber. The odorant used was filtered cigar smoke since it was faintly visible and could be seen through a viewing port in the chamber. With such a visible stimulus, we could precisely manipulate laminar air flow characteristics. At the same time, the filtered smoke odorant produced very reliable stable olfactory receptor responses for at least three hours with no apparent damage to the tissues as determined by microscopic inspection. Other more classic odorants, i.e., heptane and 1-butanol, were also used.

The olfactory receptor tissue was obtained from 37 grass frogs. The frog's head was severed behind the eyes, and the roof of one nasal cavity was removed and the eminentia olfactoria exposed. This preparation was placed on a test platform and held in place with a rubber band. The test platform was constructed from a block of paraffin 11.5 cm long, 6 cm wide, and 2 cm thick. The paraffin block was mounted upon a 10 cc hypodermic syringe body. A stainless steel plate 4.5 cm long and 2.5 cm wide was attached to the top of the paraffin block by dripping melted paraffin around the edges of the plate and allowing it to harden. This plate served as the indifferent electrode.

A hole 6 mm in diameter through the center of the steel plate and the paraffin block allowed air to be sucked from the exposure chamber past the receptor tissue. The air was drawn into what was the top of the oral cavity and passed through the hole in the plate, paraffin block, and into the syringe. From the syringe, it was drawn through rubber tubing to a water bath and then to a vacuum pump. This set-up made possible the maintenance of laminar flow conditions.

The receptor tissue was periodically dampened with frog Ringer solution to maintain it in good condition. This was done remotely with a 1.75-meter length of 1 mm O.D. polyethylene tubing attached to a 1 ml syringe. The free end of the tubing was passed under the frog's skin on top of the head and so positioned at the edge of the nasal cavity that droplets of saline, when ejected by pressure on the syringe plunger, would fall onto and wash the receptor tissue. It was found that .02 ml of saline was the correct volume for washing. Larger quantities occasionally shorted out the system and smaller quantities did not maintain the tissue in good condition.

The glass electrode placed in contact with the receptor tissue was supported by a 0.5 mm diameter steel rod that was placed in a corner of the paraffin block and rose to a height of 5 cm above the block. A ball and socket joint was fixed to the top of this rod. A sleeve inside the ball held a solid rod which could slide back and forth. On the lower end of the rod, a clamp held the electrode. All metal parts of the platform assembly were electrically tied to the indifferent electrode.

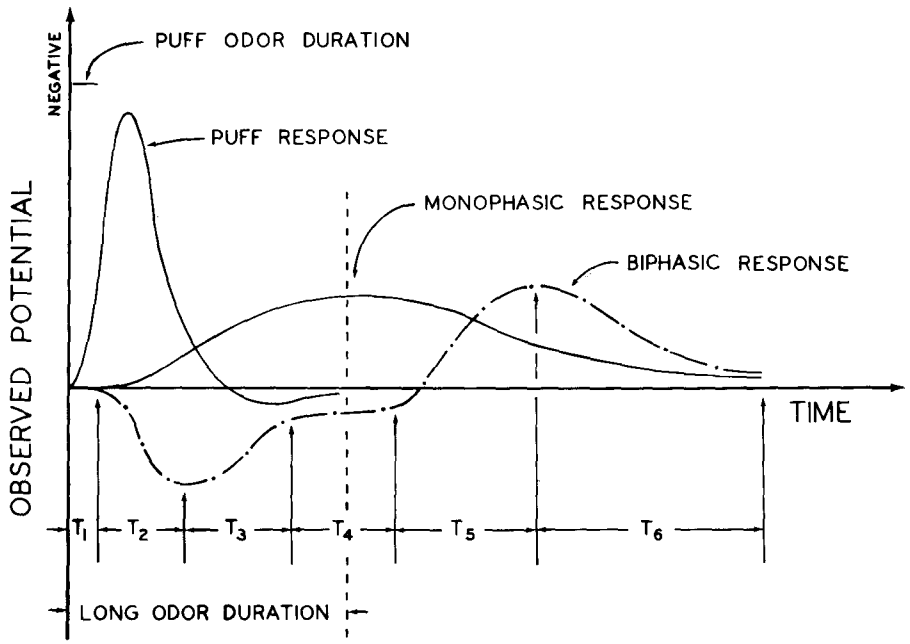

Figure 1. Tracings of characteristic waveforms obtained. Each of the responses could be obtained at will from the same preparation as a function of the electrical environment air flow characteristics, and odorant exposure duration.

The electrode was a 1.2 mm diameter glass pipette, drawn out to an open tip 0.2 mm in diameter. It was filled with a conductive jell. The tip was placed in contact with the eminentia olfactoria. The electrode was connected via RG 196 coaxial cable to a Tektronix 2A61 preamplifier, Tektronix 565 oscilloscope, and Beckman 542 chart recorder. This test platform assembly was used within the exposure chamber, which acted as a Faraday cage.

RESULTS AND DISCUSSION

The odor of filtered smoke yields very reliable stable responses for at least three hours with no apparent damage to the tissue as determined by microscopic inspection. The figures shown are tracings of characteristic responses to the filtered smoke. They are derived from measurements of all waveforms using the parametric points indicated in the figures. The data from the heptane and 1-butanol tests are similar.

Figure 1 shows the three characteristic waveforms obtained. The "puff" response was obtained with a common stimulation procedure. In this, odorant was puffed from a syringe at the receptor tissue. The uncontrolled electrostatic environment and air

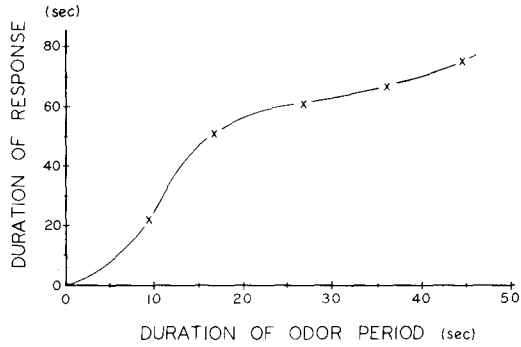

Figure 2. Duration of receptor response of the monophasic wave (T2 to T6), as a function of duration of exposure to the odorant.

turbulance typically found in EOG experiments were allowed to exist. The typical EOG waveform was obtained with the negative fast rising wave and exponential decay. A somewhat similar picture was seen with the monophasic response. In this case, however, the receptor tissue was exposed to odorant that was diffused in the air entering through the perforated ceiling of the exposure chamber. With the odorant impinging in this manner, the negative wave rises more slowly and the decay is more linear. This is as one might expect. It is also comparable to the response reported by Ottoson (1956) to long duration (15 seconds) odor stimulation.

The other response, however, was quite different. We see here a biphasic wave. Besides controlling the electrostatic environment, a suction pump was used to draw air across the receptor tissue to minimize air turbulence effects. The positive wave occurred with the onset of the odorant exposure, with a negative wave following it when the odor exposure ended. Control tests involving placement of the electrode in other tissue show that this is an olfactory tissue response, not an artifact involving electrochemical effects. Each of the three responses can be obtained from the same preparation. Which response is obtained is a function of the electrical environment, air flow characteristics, and odorant exposure duration.

In Figure 2, the duration of odor exposure is related to the receptor response time for the monophasic response. The rise time of the curve indicates that for odor durations at least up to about 20 seconds, there is an exposure time dependent effect.

In Figure 3, the effect of duration of the odor exposure period on the biphasic wave receptor response is shown. There are

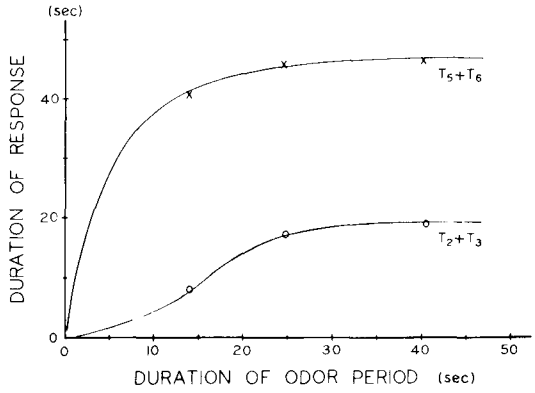

Figure 3. Duration of receptor response showing the positive (T2 + T3) and negative (T5 + T6) components of the biphasic wave, as a function of duration of exposure to the odorant.

substantial differences between the positive and negative wave responses to the duration of the odor exposure. The negative response is quite large even with short exposures such as from puffs. The positive response, on the other hand, is quite small until the exposure time extends for at least 15 or 20 seconds.

In sum, it was found that three different EOG patterns could be obtained from the same preparation as a function of the electrostatic conditions and laminar air flow conditions. The typical negative voltage response was obtained when the olfactory receptor tissue was exposed to the commonly used puff of odorized air without controls for laminar flow and electrostatics. A slowly developing negative voltage response to odorized air was obtained when the tissue was exposed to low velocity but not laminar flowing odorized air. In contrast, a biphasic wave with a large initial positive component was obtained when laminar flow was used and the electrostatic environment was controlled. Placement of the electrode in non-olfactory tissue as a control, indicated that the effects are not electrochemical.

Thus, it is likely that, to a large extent, the conflicting findings and interpretations from EOG and similar experiments are due to the interacting factors identified here. Other relevant reports of experiments are the finding that the perception of animal room odor can be modified by electrostatic fields (Frey 1983), as well as the finding that such fields shift the size distribution of airborne particulates (Frey 1985). Other data on the electrical factors influence on the air environment are discussed in Frey (1986). The broad implications of the interactions are also discussed in that paper. The data reported

here, as well as that reviewed in the above cited papers, indicate that consideration of the electrical environment in a space is relevant and of general consequence for studies in toxicology and contamination.

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