

## Research Reports

### Nasal Trigeminal Chemoreception: Responses to *n*-Aliphatic Alcohols

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Odorant molecules can stimulate nasal trigeminal receptors, but the properties of such molecules which make them effective stimuli are largely unknown. In the present study, we obtained integrated multiunit responses from the ethmoid branch of the rat trigeminal nerve to a homologous series of aliphatic alcohols. Our aim was to determine whether lipid solubility might correlate with stimulus efficacy. Response thresholds (ranging from 3000 ppm for methanol to 3 ppm for octanol) decreased with increasing carbon chain length, suggesting that lipid solubility is important for stimulus effectiveness. One plausible explanation for the importance of lipophilicity is that the more lipid soluble a substance, the more easily it can penetrate epithelial layers to reach chemoreceptive trigeminal nerve endings. Since all stimuli at vapor saturation elicited responses within 0.5 s, and because diffusion of stimulus molecules through epithelium is slow, we speculate that trigeminal nerve endings lie closer to the epithelial surface than previously thought.

#### INTRODUCTION

Several receptor systems in the nasal cavity are capable of detecting chemicals in the external environment. These include the olfactory, vomeronasal and trigeminal systems. Olfactory and vomeronasal receptors are primary sensory neurons located in discrete areas of the nasal cavity. In contrast, trigeminal receptors are reported to be free nerve endings distributed throughout the respiratory epithelium<sup>3</sup>.

Pain, touch, temperature and proprioception are mediated by the trigeminal system, but nasal trigeminal receptors also respond to many (often noxious) odorous volatiles. Trigeminal chemoreception is often included in the common chemical sense<sup>27</sup>. Trigeminal stimulation by noxious substances elicits marked respiratory, cardiovascular and hormonal responses<sup>23</sup> that may protect the organism from further exposure. However, non-irritating substances also elicit responses from nasal trigeminal nerves<sup>29,31</sup>.

Although it has been demonstrated that many odorous molecules can stimulate nasal trigeminal receptors, little is known about the properties that make these molecules effective stimuli. The presence of carbon-carbon double bonds, carbonyl and halogen groups, and large dipole moments may enhance efficacy, as may the ability of stimuli to react with SH-groups on proteins<sup>2,11,21</sup>. Still another factor which may be important is stimulus solubility<sup>2,11,18</sup>. Certainly, for both olfactory and gustatory stimuli, solubility is important. Increasing lipid solubility (which occurs as the carbon chain length of an aliphatic alcohol or acetate is increased) leads to an increase in stimulus effectiveness (olfaction<sup>22,25</sup>, gustation<sup>10,15</sup>). In the present study we obtained responses from the ethmoid branch of the rat trigeminal nerve to a homologous series of aliphatic alcohols in order to determine whether lipid solubility may play a role in trigeminal chemoreception.

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## MATERIALS AND METHODS

*Surgical preparation*

Eighteen male Sprague-Dawley rats, weighing 300–400 g, were anesthetized with Urethane (ethyl carbamate 2.5 g/kg) and tracheotomized. A cannula was inserted into the caudal end of the severed trachea to allow the rat to breathe room air. Another cannula was inserted into the rostral end of the trachea up to the nasopharynx and connected through a flowmeter to a vacuum line. This permitted either clean air or test stimuli to be drawn through the nasal cavity. The rat was placed in a head holder which allowed the head to be rotated as well as moved up and down. A portion of the skin overlying the parasagittal ridge of the frontal bone was removed on one side, and the eye and other contents of the orbit were retracted by means of hooks inserted into the tissue, thus forming a cavity. The ethmoid branch of the trigeminal nerve was then exposed for several mm distal to its foramen, cut, freed from the surrounding tissue and gently stripped of its connective sheath.

*Electrophysiological recording*

Electrical activity from the ethmoid nerve was recorded by placing the whole nerve, or portions of it, on a pair of platinum-iridium wire electrodes. The preparation was grounded through the head holder. Mineral oil pipetted into the cavity covered the nerve, prevented it from drying out, and ensured electrical insulation. The two electrodes were connected to the high impedance probe of an AC amplifier, the output of which was monitored by a storage oscilloscope and audio monitor. In addition, the neural activity was stored on audio tape with an instrumentation recorder, and passed through a leaky integrator (cf. ref. 17) with a rise time of 1.0 s and displayed on a chart recorder.

*Stimulus production and delivery*

Homologous aliphatic alcohols were purchased from Fisher Scientific and redistilled. Stimuli were presented via an air dilution olfactometer (e.g. ref. 24). An odorant-saturated airstream, flowing at a known rate, was diluted with various quantities of filtered air. Dilution steps were controlled by rotameters. A stream of air with a flow rate of approximately 1 liter/min was drawn through the rat's nose

TABLE I

*The stimuli, their formulae and the concentration ranges tested*

Compound	Formula	Concentration range tested (ppm)*
Methanol	CH <sub>3</sub> OH	3,020–120,225
Ethanol	CH <sub>3</sub> CH <sub>2</sub> OH	1,380–54,955
Propanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> OH	460–18,620
Butanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub> OH	160–6,610
Pentanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> OH	60–2,345
Hexanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub> OH	25–1,025
Heptanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>6</sub> OH	7–275
Octanol	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>7</sub> OH	3–120

\* The highest concentration tested was at vapor saturation (20 °C).

via the nasopharyngeal cannula attached to the vacuum line. For stimulus presentation, the vacuum was turned on for approximately 30 s, and during the middle of that period while the vacuum was still on, odor was delivered for 10 s at a flowrate of 2 liters/min. Battery powered miniature solenoids were used to switch from the filtered background airstream to the odorant stimulus. Table I lists the alcohols, their formulae, and the concentration ranges tested.

*Data collection and analysis*

The integrated response magnitude was measured in arbitrary units from baseline to the peak of the phasic response. Responses are reported as a percent of the response to approximately 2200 ppm cyclohexanone. Concentrations reported for cyclohexanone as well as for the alcohols were estimates of reproducible but unmeasured concentrations. This standard stimulus was presented periodically throughout each experiment to check the reliability of the preparation, i.e. as long as the responses to cyclohexanone did not differ by more than 10%, the data from that particular experiment were included in the analyses.

Relative latencies were defined as the time from the switching of the battery powered solenoids to the onset of the response. The onset of the response was measured on the chart paper subsequently by 3 independent observers, and arithmetic means of these measurements were calculated and recorded.

## RESULTS

All of the alcohols elicited responses from the eth-

## ETHANOL

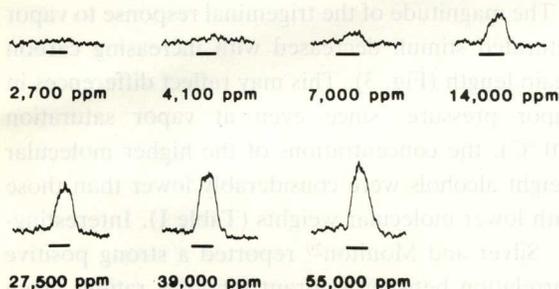


Fig. 1. Integrated multiunit responses to 7 concentrations of ethanol ( $C_2$ ). The bars under the responses represent the 10 s stimulus duration.

moid branch of the trigeminal nerve in all the rats tested. High stimulus concentrations produced responses with an initial phasic component followed by a decline to a steady state tonic level. Lower concentrations led to a gradual increase in nerve activity for the duration of the stimulus presentation. Responses at all concentrations rapidly returned to baseline levels after removal of the stimulus.

Response magnitude increased with increasing stimulus concentration (Fig. 1). Concentration–response curves for methanol ( $C_1$ ), propanol ( $C_3$ ), pentanol ( $C_5$ ) and heptanol ( $C_7$ ) are presented in Fig. 2A, while curves for ethanol ( $C_2$ ), butanol ( $C_4$ ), heptanol ( $C_6$ ) and octanol ( $C_8$ ) are presented in Fig. 2B. Response magnitudes are reported as a percent of the standard response to 2200 ppm cyclohexanone. The largest responses were elicited by the lower molecular weight alcohols. This is seen at vapor saturation (Fig. 3) where the response to methanol was approximately 6 times greater than the response to octanol.

Although the shorter chain alcohols elicited the largest responses, those with the higher molecular weights were more effective stimuli. Concentrations of approximately 4800 ppm methanol and 90 ppm octanol were necessary to elicit a response equal to 20% of the response to 2200 ppm cyclohexanone. Fig. 4 shows the concentrations necessary to elicit 20% of the cyclohexanone response for all 8 alcohols tested.

Plots of log threshold (ppm) vs carbon chain length, and log of the oil/water partition coefficient<sup>20</sup> vs carbon chain length are shown in Fig. 5. Thresh-

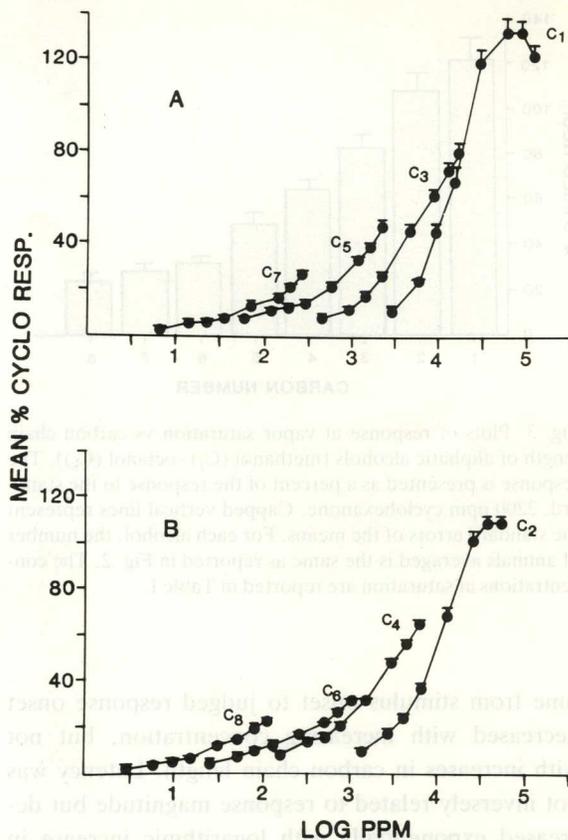


Fig. 2. A: concentration (log ppm)–response curves for methanol ( $C_1$ ,  $n = 11$ ), propanol ( $C_3$ ,  $n = 9$ ), pentanol ( $C_5$ ,  $n = 10$ ) and heptanol ( $C_7$ ,  $n = 10$ ). B: concentration–response curves for ethanol ( $C_2$ ,  $n = 11$ ), butanol ( $C_4$ ,  $n = 11$ ), hexanol ( $C_6$ ,  $n = 11$ ) and octanol ( $C_8$ ,  $n = 11$ ).  $n$ , the number of animals averaged. Mean responses are reported as a percent of the response to the standard, 2200 ppm cyclohexanone. Capped vertical lines represent the standard errors of the means.

olds decreased with increasing carbon chain length while oil/water partition coefficients increased (i.e. as carbon chain length increased, the alcohols became more lipid soluble).

As stated above, relative latency was measured as the time between the switching of the battery powered solenoid and the onset of the response. Fig. 6 shows records obtained from one animal in response to methanol, and demonstrates how latency was determined. A plot of relative latency vs log concentration is shown in Fig. 7. As for Fig. 2, curves for methanol ( $C_1$ ), propanol ( $C_3$ ), pentanol ( $C_5$ ) and heptanol ( $C_7$ ) are presented in Fig. 7A, while those for ethanol ( $C_2$ ), butanol ( $C_4$ ), heptanol ( $C_6$ ) and octanol ( $C_8$ ) are presented in Fig. 7B. For each of these alcohols, the

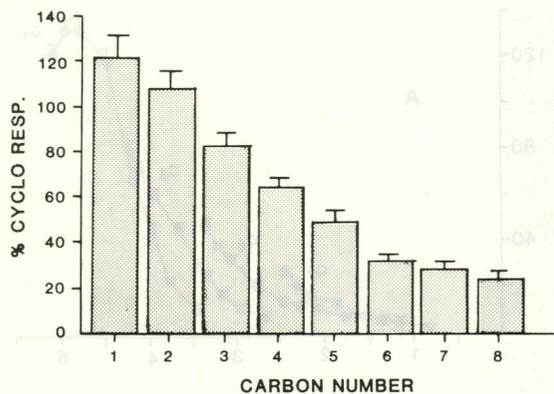


Fig. 3. Plots of response at vapor saturation vs carbon chain length of aliphatic alcohols (methanol ( $C_1$ )–octanol ( $C_8$ )). The response is presented as a percent of the response to the standard, 2200 ppm cyclohexanone. Capped vertical lines represent the standard errors of the means. For each alcohol, the number of animals averaged is the same as reported in Fig. 2. The concentrations at saturation are reported in Table I.

time from stimulus onset to judged response onset decreased with increasing concentration, but not with increases in carbon chain length. Latency was not inversely related to response magnitude but decreased exponentially with logarithmic increase in response magnitude. The longest latency measured was 4.8 s for the lowest concentration of methanol (approximately 3000 ppm). Latencies were less than 0.5 s for all the alcohols, when tested at vapor saturation.

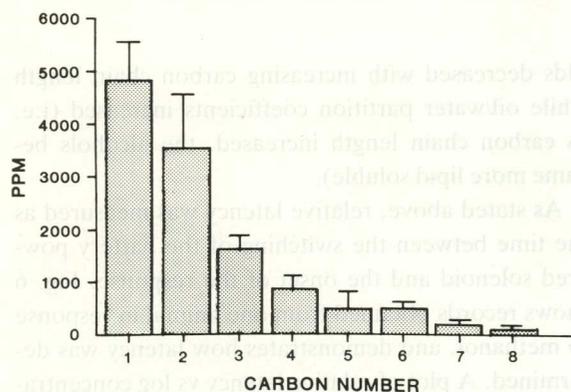


Fig. 4. Plot of the concentration (ppm) needed to elicit a response equal to 20% of the response to the standard, 2200 ppm cyclohexanone, vs carbon chain length of aliphatic alcohols (methanol ( $C_1$ )–octanol ( $C_8$ )). Capped vertical lines represent the standard errors of the means. For each alcohol, (n) equals the number reported in Fig. 2.

## DISCUSSION

### Stimulus effectiveness

The magnitude of the trigeminal response to vapor saturated stimuli decreased with increasing carbon chain length (Fig. 3). This may reflect differences in vapor pressure, since even at vapor saturation (20 °C), the concentrations of the higher molecular weight alcohols were considerably lower than those with lower molecular weights (Table I). Interestingly, Silver and Moulton<sup>29</sup> reported a strong positive correlation between odorant intensity ratings given by human anosmics<sup>11</sup> and rat electrophysiological trigeminal response magnitudes at vapor saturation. We speculate that the higher molecular weight alcohols may be less irritating than the lower molecular weight alcohols, even though they are more effective stimuli in terms of the concentration needed to elicit 20% of the response to 2200 ppm cyclohexanone (Fig. 4) and threshold (Fig. 5).

Thresholds obtained in the present study for butanol (355 ± 91 ppm) and heptanol (41 ± 14 ppm) are consistent with at least one previous investigation. Silver and Moulton<sup>29</sup> reported threshold values for butanol that ranged from 164 to 500 ppm and those for heptanol that ranged from 21 to 137 ppm. However, the threshold for pentanol reported here (182 ± 63 ppm) is considerably lower than the value (approximately 2500) reported by Kulle and Cooper<sup>18</sup>, who recorded the nasopalatine nerve of the rat. This

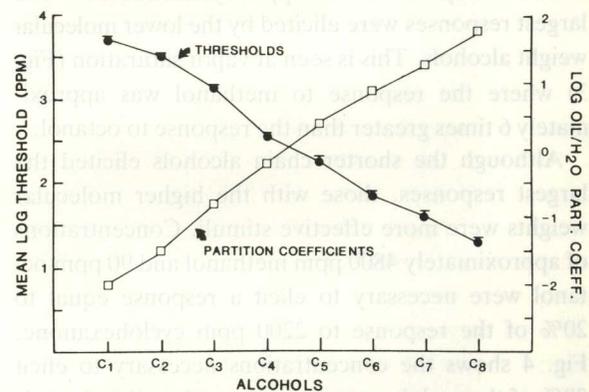


Fig. 5. Plots of log threshold (ppm) and log oil/water partition coefficient<sup>10</sup> vs carbon chain length of aliphatic alcohols (methanol ( $C_1$ )–octanol ( $C_8$ )). Capped vertical lines on the threshold curve represent the standard errors of the means. For each alcohol, (n) equals the number reported in Fig. 2.

## METHANOL

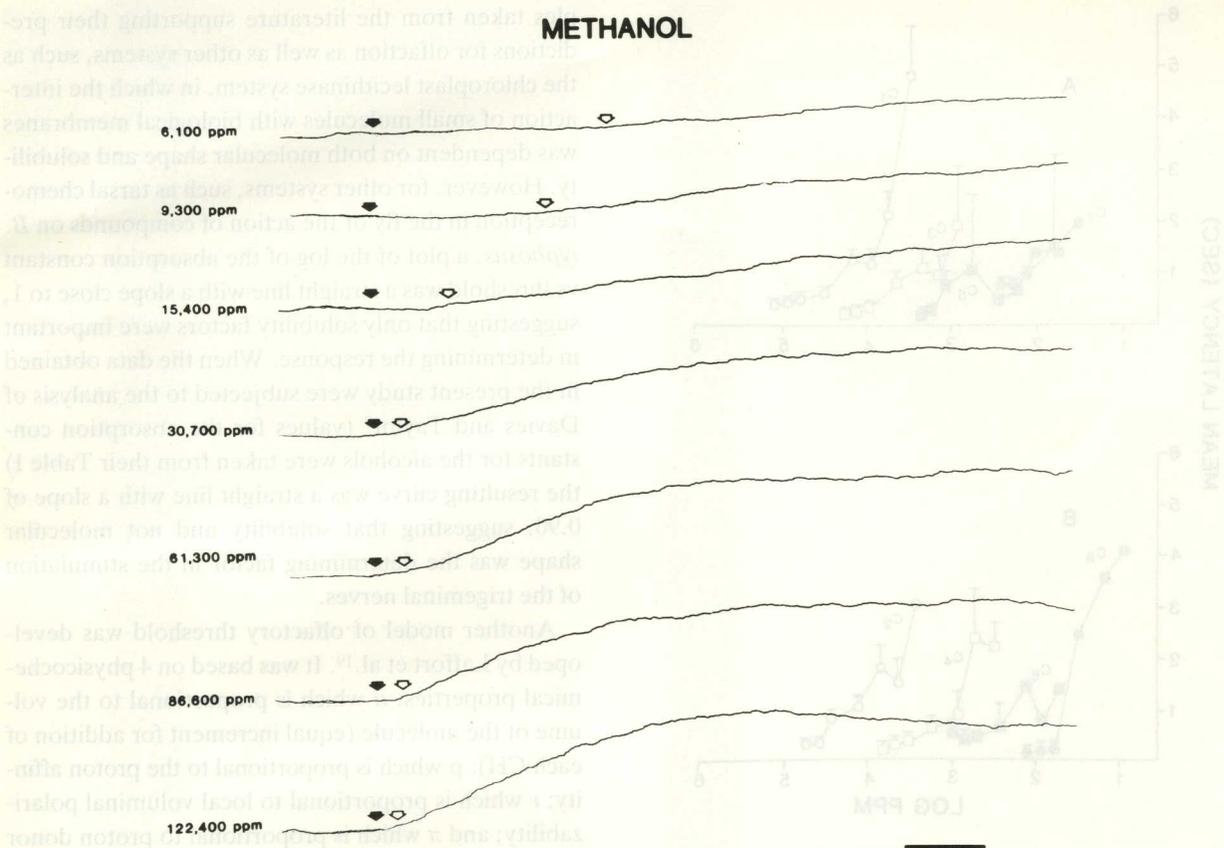


Fig. 6. Determination of latency for 7 concentrations of methanol ( $C_1$ ). The records are from one animal. The solid arrow denotes switching of the stream flowing through the solenoid valve from clean, background air to odorant. The open arrow denotes the judged onset of response.

discrepancy for pentanol thresholds may reflect differences in sensitivity between the ethmoid and nasopalatine branches of the trigeminal nerve, differences in stimulus flowrate through the nasal cavity<sup>30</sup>, or perhaps differences in the duration of stimulus presentation<sup>29</sup>. Regarding the latter two possibilities, Kulle and Cooper<sup>18</sup> used a flowrate of 350 ml/min through the nasal cavity and a stimulus duration of 25 s. The flowrate through the nasal cavity used in the present study was 1000 ml/min and stimuli were presented for 10 s.

Moulton and Eayrs<sup>22</sup> obtained behavioral thresholds in rats to the same alcohol stimuli used in the present study. The behavioral threshold for each alcohol was lower than the trigeminal electrophysiological thresholds determined in the present paper. For methanol and ethanol, behavioral thresholds were less than 1 log unit lower than trigeminal elec-

trophysiological thresholds. The propanol threshold was 1.7 log units lower. Behavioral thresholds for butanol through octanol were between 2.5 and 4 log units lower than electrophysiological trigeminal thresholds.

Moulton and Eayrs<sup>22</sup> obtained behavioral thresholds from rats with both their olfactory and trigeminal systems intact (as well as their vomeronasal systems). Therefore responses to the different alcohols could have had a trigeminal component. The observation that the trigeminal thresholds were closer to the behavioral thresholds for the lower molecular weight alcohols suggests that the trigeminal component plays a greater role in eliciting behavioral responses to these compounds.

For the alcohols used as stimuli in the present study, threshold concentrations were inversely related to carbon chain length (Fig. 5). This suggests

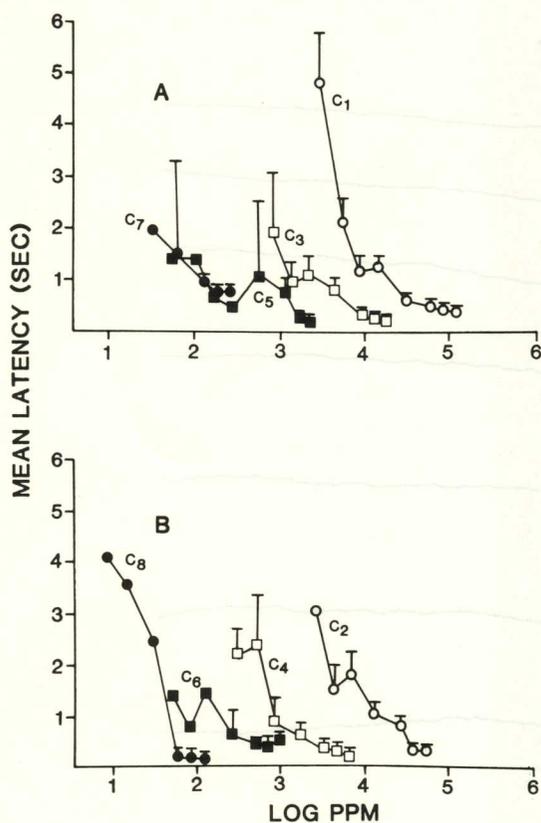


Fig. 7. A: concentration (log ppm)-latency (s) curves for methanol ( $C_1$ ), propanol ( $C_3$ ), pentanol ( $C_5$ ) and heptanol ( $C_7$ ). B: concentration-latency curves for ethanol ( $C_2$ ), butanol ( $C_4$ ), hexanol ( $C_6$ ) and octanol ( $C_8$ ). Where present, capped vertical lines represent the standard errors of the means,  $n = 7$  ( $C_1$ ),  $n = 6$  ( $C_2$ ),  $n = 5$  ( $C_4$ ),  $n = 3$  ( $C_5$ ),  $n = 4$  ( $C_3, C_6, C_7, C_8$ ). Absence of a standard error bar indicates that the data point was obtained from only one animal.

that lipid solubility is one determinant of stimulus effectiveness for trigeminal chemoreception, as it is for olfaction<sup>22,25</sup> and gustation<sup>10,15</sup>. We speculate that the more lipophilic a substance, the more easily it can penetrate epithelial layers to reach the chemosensitive trigeminal nerve endings.

Davies and Taylor<sup>7</sup> presented a model of olfactory thresholds based on both solubility and molecular shape. They concluded that olfactory thresholds were dependent on both of these parameters. From their predictions, a plot of the log of the adsorption constant vs log olfactory threshold should deviate from a straight line if both molecular shape and adsorption factors (i.e. solubility) were significant. Indeed, Davies and Taylor<sup>7</sup> presented several exam-

ples taken from the literature supporting their predictions for olfaction as well as other systems, such as the chloroplast lecithinase system, in which the interaction of small molecules with biological membranes was dependent on both molecular shape and solubility. However, for other systems, such as tarsal chemoreception in the fly or the action of compounds on *B. typhosus*, a plot of the log of the absorption constant vs threshold was a straight line with a slope close to 1, suggesting that only solubility factors were important in determining the response. When the data obtained in the present study were subjected to the analysis of Davies and Taylor<sup>7</sup> (values for the absorption constants for the alcohols were taken from their Table I) the resulting curve was a straight line with a slope of 0.90, suggesting that solubility and not molecular shape was the determining factor in the stimulation of the trigeminal nerves.

Another model of olfactory threshold was developed by Laffort et al.<sup>19</sup>. It was based on 4 physicochemical properties:  $\alpha$  which is proportional to the volume of the molecule (equal increment for addition of each CH);  $\rho$  which is proportional to the proton affinity;  $\epsilon$  which is proportional to local voluminal polarizability; and  $\pi$  which is proportional to proton donor ability. When the values of these 4 parameters (taken from Laffort et al.<sup>19</sup>, Table I) were compared with the trigeminal threshold values determined in the present study for the aliphatic alcohols, only  $\alpha$  and  $\epsilon$  were significantly correlated ( $P \leq 0.05$ ).  $\alpha$  relates to the volume of the molecule (which obviously increases proportionally as an aliphatic series is ascended) and  $\epsilon$  relates to its solubility.

In addition to lipid solubility, other physical properties, including surface activity and vapor pressure, vary logarithmically as carbon chain length increases within a homologous series<sup>12</sup>. Thus when threshold concentrations for the alcohols are plotted against the log of their oil-water partition coefficients (Fig. 5) a linear relationship is obtained. A linear relationship also is seen when thresholds are expressed as pressures (mm Hg) and plotted against their saturated vapor pressures (Fig. 8). The pressure of the alcohol vapor at threshold (pt) was calculated from Equation 1 (ref. 8):

$$\text{pt} = \text{concentration in mol/liter} \times 22.41 \times (T+273/273) \times 760 \quad (1)$$

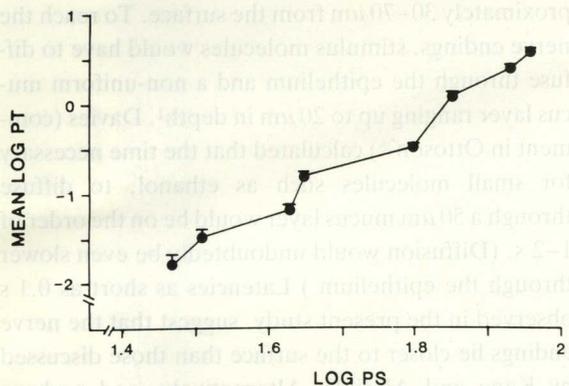


Fig. 8. Relationship of mean threshold, expressed as partial vapor pressure (PT), to saturated vapor pressure (PS) for a series of aliphatic alcohols (methanol ( $C_1$ )–octanol ( $C_8$ )). Capped vertical lines represent the standard errors of the means. Partial vapor pressures were calculated for the thresholds presented in Fig. 5. For each alcohol, (n) equals the number reported in Fig. 2.

For the present study, saturated vapor pressures were calculated from the Clausius-Claperton equation<sup>6</sup>:

$$\log(P_2/P_1) = (\Delta H_{vap}(T_2 - T_1)) / (2.303 R T_2 T_1) \quad (2)$$

where  $P$  = vapor pressure at temperature  $T_2$  (°K),  $P_1$  = vapor pressure at  $T_1$  (°K),  $\Delta H_{vap}$  = heat of vaporization, and  $R$  = the gas constant. Values for  $P_2$ ,  $T_2$  and  $H_{vap}$  were obtained from ref. 32.  $P$  was calculated for a temperature of 20 °C. The linear relationship between log pressure at threshold and log pressure at saturated vapor is characteristic of narcotic and toxic phenomena in which an apparent equilibrium exists between the external phase and the internal biophase<sup>12</sup>. Ferguson<sup>12</sup> argued that when such an equilibrium exists, the thermodynamic activity of the test compounds will be equal in all phases. Thermodynamic activities at threshold were calculated from equation (3):

$$A = pt/ps \quad (3)$$

where  $A$  = activity,  $pt$  = the pressure of the alcohol at threshold and  $ps$  = the saturated vapor pressure at 20 °C<sup>13</sup>. (For a discussion of the rationale for the application of analysis in terms of thermodynamic activ-

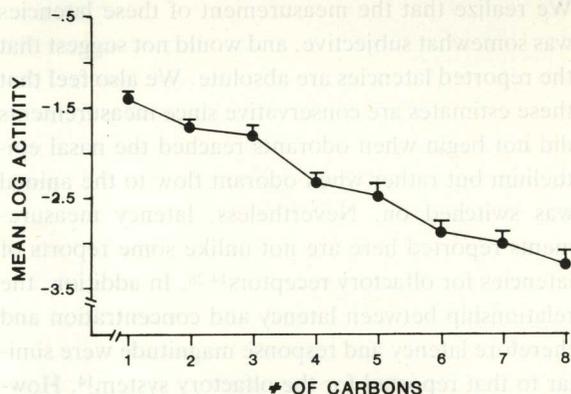


Fig. 9. Relationship of mean thresholds, expressed as thermodynamic activities, to the chain length in a series of aliphatic alcohols (methanol ( $C_1$ )–octanol ( $C_8$ )). See text for details. Capped vertical lines represent the standard errors of the means. For each alcohol, (n) equals the number reported in Fig. 2.

ity, see Ferguson<sup>12</sup>, Ferguson and Pirie<sup>13</sup>, Brink and Posternak<sup>4</sup> and Dethier<sup>8</sup>). This analysis has been applied to behavioral olfactory responses to aliphatic alcohols in blowflies<sup>9</sup> and electrophysiological olfactory responses (EOGs) in frogs<sup>25</sup>. These studies demonstrated experimentally that the thermodynamic activities of the alcohols at threshold were equal (i.e. alcohols stimulated equally at equal thermodynamic activities) and concluded that olfactory responses to alcohols involved an equilibrium process. Moulton and Eayrs<sup>22</sup> used the same method of analysis for behavioral olfactory responses obtained from rats and concluded that medium and long chain alcohols may involve an equilibrium process, while shorter chain alcohols (i.e. methanol ( $C_1$ )–butanol ( $C_4$ )), may not, since the log activities at threshold for the shorter chain alcohols differed from those with a higher number of carbon atoms. A plot of log activity vs carbon chain length for the current trigeminal data is shown in Fig. 9. Because the log activities at threshold are not equal, i.e. they decrease with increasing carbon chain length, it appears that, for alcohols, trigeminal chemoreception does not involve an equilibrium process.

#### Response latencies

Response latencies calculated in the present study ranged from 0.1 s (for the highest concentrations) to 4.8 s (for the lowest concentrations) (Figs. 6 and 7).

We realize that the measurement of these latencies was somewhat subjective, and would not suggest that the reported latencies are absolute. We also feel that these estimates are conservative since measurements did not begin when odorants reached the nasal epithelium but rather when odorant flow to the animal was switched on. Nevertheless, latency measurements reported here are not unlike some reports of latencies for olfactory receptors<sup>14,28</sup>. In addition, the relationship between latency and concentration and therefore latency and response magnitude were similar to that reported for the olfactory system<sup>14</sup>. However, Tucker<sup>31</sup> reported that, in general, trigeminal nerves exhibited longer response latencies than olfactory nerves and Cain<sup>5</sup> demonstrated that human latencies (based on reaction times) for odor (olfaction) were shorter than those for irritation (trigeminal chemoreception). It is conceivable that olfactory latencies to alcohols could be less than the trigeminal latencies reported in the present paper.

It is unclear how chemical stimuli reach trigeminal receptors. Kane and Alarie<sup>16</sup> described the nerve endings in the nasal respiratory mucosa as lying ap-

proximately 30–70  $\mu\text{m}$  from the surface. To reach the nerve endings, stimulus molecules would have to diffuse through the epithelium and a non-uniform mucus layer ranging up to 20  $\mu\text{m}$  in depth<sup>1</sup>. Davies (comment in Ottoson<sup>26</sup>) calculated that the time necessary for small molecules such as ethanol, to diffuse through a 50  $\mu\text{m}$  mucus layer would be on the order of 1–2 s. (Diffusion would undoubtedly be even slower through the epithelium.) Latencies as short as 0.1 s observed in the present study, suggest that the nerve endings lie closer to the surface than those discussed by Kane and Alarie<sup>16</sup>. Alternatively, and perhaps less likely, the nerve endings may not be stimulated directly; stimulation may involve an interaction of odorant molecules with the surrounding epithelial cells.

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