ANALYSIS OF POSITION OF TRIAL SEQUENCE AND TYPE OF DILUENT ON THE DETECTION THRESHOLD FOR PHENYL ETHYL ALCOHOL USING A SINGLE STAIRCASE METHOD

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Summary.—Although detection thresholds for odors are commonly measured in academic and medical settings, the influences of procedural factors on threshold values are poorly understood. The present study evaluated the influences of (i) trial sequence position and (ii) diluent type on the threshold value for the rose-like odorant phenyl ethyl alcohol. In Exp. 1, detection thresholds were measured in 24 subjects on two occasions in which different diluents were used in the concentration series, propylene glycol and light mineral oil. The thresholds were estimated using a 7-reversal initially ascending single-staircase procedure. Threshold values were significantly influenced by the type of diluent (lower for mineral oil) and trial sequence (lower for later threshold reversals). In Exp. 2, 24 subjects were administered a staircase threshold test which continued through 15 staircase reversals. Continued testing resulted in a significant lowering of the threshold measure. These findings demonstrate the importance of both diluent and test length on detection threshold values measured by a single staircase procedure and emphasize the need for standardization of procedures for threshold testing.

Numerous psychophysical techniques have been developed for measuring olfactory thresholds, including detection and recognition paradigms, single-series ascending method of limits procedures, and various staircase methods (for review, see Doty and Kobal, 1995). The reliabilities of such procedures are variable, with single series ascending and nonforced-choice procedures producing less reliable measures than staircase and forced-choice procedures (Doty, McKeown, Lee, & Shaman, 1995). As with a number of psychological tests, reliability is related to test length. For example, within a staircase threshold series incorporating the odorant phenyl ethyl alcohol, reliability is related ($r^2 = .984$) to the number of reversals included in the

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threshold estimate by a function derived from the Spearman-Brown formula, namely, reliability = 0.455 × number of reversals / [1 + 0.455 (number of reversals – 1)]. The location of the reversal per se has little effect on reliability (Doty, et al., 1995).

Few studies have examined the influences of procedural factors on the measure of odor detection threshold. Doty, Gregor, and Settle (1986) demonstrated that detection thresholds were relatively invariant across intertrial intervals of 8 to 64 seconds but were inversely related to the volume of the sniff bottle. More recently, Doty, Diez, Turnacioglu, Kris, McKeown, Armstrong, and Lee (submitted) found, in a study of 128 college students, that perithreshold odor detection is little influenced by the direction of series sampling, i.e., ascending vs descending trials, and that feedback has minimal, if any, influence on such performance.

To date, the influence of diluent type on odor detection thresholds has not been systematically examined. An ideal diluent is one that is odorless, resistant to evaporation, and a good solvent for most organic odorants. Water is a commonly used diluent; however, many odorants are not chemically stable in water, e.g., esters slowly hydrolyze to acids and alcohols (Dravnieks, 1975), many odorants have low water/air partition coefficients, water evaporates relatively rapidly (thereby altering stimulus concentration), and water promotes bacterial development (Amoore, 1994). Propylene glycol and diethyl phthalate are popular diluents (e.g., Berglund, Berglund, Ekman, & Engen, 1971); however, these agents, particularly diethyl phthalate, are known to vary in quality from sample to sample and frequently emit odors which may mask the dissolved stimulus. More recently, United States Pharmaceutical (USP) grade light mineral oil has been used as a diluent, in large part because it is comparatively odorless and appears to be a reasonably good solvent for many organic compounds.

The purpose of the present study was to compare detection thresholds obtained from dilution series using USP grade light mineral oil to those obtained using propylene glycol. An additional goal was to establish the influence of the position of the trial sequence upon the detection threshold measure. We hypothesized, based upon data from both animal and human studies (e.g., Doty & Ferguson-Segall, 1989; Engen, 1960), that detection threshold values decrease as a function of trial sequence.

**Experiment 1**

**Subjects**

Twelve men (M age = 21.6 yr., SD = 4.1 yr., range = 16 to 30 years) and 12 women (M age = 22.6 yr., SD = 1.5 yr., range = 20 to 25 years) served as subjects. All had normal olfactory function as determined from scores on the University of Pennsylvania Smell Identification Test (Doty, Shaman, & Dann, 1984). None of the subjects were cigarette smokers.
OLFACTORY THRESHOLDS

**Odorant**

Gold label grade phenyl ethyl alcohol with a purity of over 99% served as the odorant (Aldrich Chemical Co.). This pleasant rose-like smelling compound has comparatively low trigeminal stimulative properties (Doty, Brugger, Jurs, Orndorff, Snyder, & Lowry, 1978). The diluents used were propylene glycol and USP grade mineral oil (Fisher Scientific, King of Prussia, PA). Half-log dilution steps ranging from \(-10.5\) to \(-1.00\) (log vol/vol) were used for propylene glycol and from \(-10.5\) to \(-2.00\) (log vol/vol) for light mineral oil.

**Procedure**

Each subject received two detection threshold tests, one with the phenyl ethyl alcohol diluted in propylene glycol and the other with the phenyl ethyl alcohol diluted in mineral oil. These tests were presented successively and in counterbalanced order. The subjects received a 25-min. break between the two threshold tests, during which time they completed the University of Pennsylvania Smell Identification Test (Doty, et al., 1984) and had a brief rest.

Detection thresholds were assessed using an initially ascending single-staircase method with forced choices. A trial consisted of the presentation of two 120-ml volume glass sniff bottles in rapid succession. The order of the presentation was random. One bottle contained 20 ml of a given phenyl ethyl alcohol concentration dissolved in a diluent, and the other contained 20 ml of diluent alone. On a given trial, each bottle was opened and immediately placed over the subject’s nose in a standardized manner (Doty, et al., 1978; Doty, et al., 1986). Following presentation of both bottles, the subject reported which bottle appeared to have the stronger smell. If not sure, the subject was required to guess one or the other bottle, i.e., make a forced choice. The intertrial interval was about 30 seconds.

For each test, the staircase was begun at the \(-6.50\) log concentration step and moved upward in full log steps following incorrect responses until detection occurred on five consecutive trials at a given concentration. When the five correct trials occurred, the staircase was reversed and subsequently moved in 0.5-log steps, with a maximum of two trials at each concentration. When the subject correctly identified the odor on both of these trials, the next trials were presented 0.5-log unit lower. If a miss occurred, the next highest 0.5-log unit concentration was presented. Subsequent reversal points were determined by two correct responses at a given concentration when moving up the staircase and a single incorrect response when moving down the staircase. The geometric mean of the last four of seven total reversals was used as the estimate of the threshold.
Results

Analysis of variance was used to determine the effects of gender, diluent type, and position in the trial sequence on the measure of the threshold. The latter factor was comprised of successive pairs of reversals subsequent to the first reversal point, i.e., means of the second and third, fourth and fifth, and sixth, and seventh reversals. In this analysis, thresholds from the concentration series based upon mineral oil were significantly lower than

![Graph showing detection performance (mean ± SEM) to phenyl ethyl alcohol as a function of diluent type and reversal number within the staircase series. See text for details.](image-url)
those from the concentration series based upon propylene glycol ($F_{1,23} = 10.58, p < .004$). No effect of gender was present ($F_{1,23} = 0.12, p > .50$). Thresholds decreased as trial sequence increased (Fig. 1), with the trend being composed of both linear ($F_{1,23} = 14.04, p < .001$) and quadratic ($F_{1,23} = 8.39, p < .008$) components. Pairwise comparisons, using Bonferroni corrections, indicated that the mean of the second and third reversals was significantly higher than the mean of the fourth and fifth reversals ($F_{1,23} = 17.77, p < .001$) and the sixth and seventh reversals ($F_{1,23} = 14.04, p < .001$). No other comparisons were statistically significant ($p > .20$).

**Experiment 2**

The purpose of Exp. 2 was to determine whether the decrease in detection threshold values observed across trial sequences continues beyond seven staircase reversals. Thus, subjects were tested on an analogous staircase series, using mineral oil diluent, which was extended through 15 staircase reversals.

**Subjects**

Twelve men ($M$ age $= 21.8$ yr., $SD = 4.9$ yr., range $= 18–33$ years) and twelve women ($M$ age $= 24.0$ yr., $SD = 6.3$ yr., range $= 18–34$ years) served as subjects. None reported any problems smelling and tasting, and all scored within the normal range on the University of Pennsylvania Smell Identification Test. At the time of testing all subjects were nonsmokers.

**Procedure**

The detection thresholds were assessed using methods identical to those described in Exp. 1, except that 15, rather than seven, reversals were obtained from the subjects and only mineral oil was used as the diluent.

**Results**

An analysis of variance was conducted on the means of successive pairs of reversal points subsequent to the first reversal point. Threshold estimates linearly decreased across trials (Fig. 2) ($F_{1,23} = 5.19, p < .05$). The mean of the eighth through eleventh reversal points differed significantly from that of the fourth to seventh reversal points ($F_{1,23} = 5.98, p < .05$).

**General Discussion**

Measures of detection threshold, which reflect the lower limits of olfactory sensitivity, are influenced by a multitude of factors. Whereas changes in olfactory sensitivity are commonly noted for different types of odorants, the results of Exp. 1 clearly demonstrate that the type of diluent likewise affects the measured olfactory threshold. These data show that, on a volume/volume basis, values of detection threshold based on phenyl ethyl alcohol dis-
Fig. 2. Detection performance (mean ± SEM) to phenyl ethyl alcohol diluted in light USP grade mineral oil as a function of reversal number within the staircase series. See text for details.

Solved in USP grade mineral oil are approximately a half log unit lower than those based on phenyl ethyl alcohol dissolved in propylene glycol.

The lower thresholds obtained from the light mineral oil dilution series probably reflect a physicochemical phenomenon. Above an "ideal" solution, the concentration of an odorant in the air phase can be estimated from the vapor pressure of the pure odorant and its mole fraction in the liquid by applying Raoult's Law, i.e., in equilibrium at a constant temperature and pressure, each volatile component in a liquid mixture develops a vapor pressure in the air phase that reflects its own vapor pressure as a pure liquid at the same temperature multiplied by its mole fraction in the liquid mixture. Pro-
gressive dilution of a volatile odorant with the solvent should produce reductions in the vapor pressure of the odorant in the air phase proportional to its mole fraction. However, certain liquid mixtures diverge severely from this theoretical ideal. In unpublished work Amoore, O’Neill, Steinle, and Melvin found that l-butanol, “dissolved” in light mineral oil, maintains its vapor pressure undiminished until it is diluted below 0.5% in the liquid, that is, a more than 200-fold deviation from ideality. Stone (1963) noted a similar phenomenon with mixtures of propionic acid and mineral oil. In each case, the volatile hydrophilic solute behaves as a colloidal solution of hydrogen-bonded ultramicro droplets interspersed among the hydrophobic diluent molecules. These droplets still maintain their maximum liquid vapor pressure. On reaching a sufficient dilution, the droplets break down to form a true molecular solution in the mineral oil, and the vapor pressure becomes proportional to the concentration in the liquid (Henry’s Law).

These deviations from ideality are particularly marked when the diluent is hydrophobic and the odorant is hydrophilic or at least is able to participate as both hydrogen donor and hydrogen acceptor in the formation of hydrogen bonds. According to these concepts, phenyl ethyl alcohol, which is capable of reciprocal hydrogen bonding, would be expected to be abnormally volatile from a solution in light mineral oil. On the other hand, it should behave more normally in propylene glycol solution in which it can hydrogen bond to the hydroxyl groups of the diluent molecules. This likely explains why we found that the olfactory threshold, expressed as the concentration of phenyl ethyl alcohol in the liquid, was lower in the light mineral oil solution than in the propylene glycol solution.

The present study further confirms the observations of earlier studies that, at least under some circumstances, repeated testing within the perithreshold range of odorant concentration results in increased detection. This phenomenon has been observed not only for humans (e.g., Doty, Snyder, Huggins, & Lowry, 1981), but also for rats (Doty & Ferguson-Segall, 1989). In some cases, mere exposure to an odorant seems to alter threshold sensitivity to the agent (Rabin & Cain, 1986). Indeed, exposure of some persons ostensibly anosmic to androstenone results in their subsequent ability to perceive the substance (Wysocki, Dorries, & Beauchamp, 1989). Thus, while the present study yielded an increase in threshold-level sensitivity as a result of increased testing, it is not clear whether it is exposure to the odorant or practice within the threshold series (or both) which contributes to the increased sensitivity.

REFERENCES


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