Behavioral Determination of Olfactory Thresholds to Amyl Acetate in Dogs

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KRESTEL, D., D. PASSE, J. C. SMITH AND L. JONSSON. Behavioral determination of olfactory thresholds to amylic acetate in dogs. NEUROSCI BIOBEHAV REV 8(2) 169–174, 1984.—By use of a modified conditioned suppression technique, olfactory thresholds to amylic acetate were determined for four beagle dogs. Using the same odorant and olfactometer and a similar breathing chamber, olfactory thresholds were obtained in eight human subjects. It was determined that the olfactory sensitivity of the dogs was about 2.5 log units better than that of the human subjects.

Dog    Olfaction    Amyl acetate    Conditioned suppression

Adequate olfactometry and behavioral control have frequently been lacking in studies of canine olfactory sensitivity. As Moulton pointed out, this has contributed to discrepancies in both measured canine thresholds and estimates of differences in sensitivity between dog and man [14,15]. The range of methods of odor delivery have included handkerchiefs impregnated with odor (Hemning, cited in [13]), funnels strapped to the subject’s nose with odors delivered via tubing (Heizenerroeder, cited in [13]), floor-mounted screen-covered crucibles [15], and the modern-day olfactometers [16]. One of the first laboratory studies in which olfactory measures were carefully controlled was Neuhaus’s [18] use of a capillary olfactometer. He estimated the dog’s olfactory threshold for butyric acid and acetic acid to be 8 logio units below man. Renzvanz [20], using field trials, estimated threshold for butyric acid to lie between 5 x 10^4 and 2 x 10^5 molecules per cc, which was consistent with Neuhaus’s estimate of 9 x 10^6 molecules per cc. Kaise [10], using a classical conditioning technique and clove oil, found absolute threshold for dogs to be approximately 6 logio units below that of man. Others have not been as successful in obtaining canine thresholds this far below that of man. Becker, Markee, and King [3] failed to train dogs to detect clove oil at concentrations below that detectable by man. Moulton et al. [14] estimated canine threshold for butyric acid to be 2 logio units below that of man. This differs from Neuhaus’s [18] canine-human comparison by a million-fold for the same substance. Discrepancies in estimates of differential sensitivity between dog and man are attributable in large part to differences in means of odor delivery, type and purity of odorants, and psychophysical method.

A recent attempt to overcome this problem is a well controlled study by Moulton and Marshall [16] in which seven human subjects were tested with alpha-ionone using essentially the same apparatus as that used for dogs. Results indicated that minimum detectable concentrations for dogs were three to four logio units below that for humans. This study made significant progress in establishing conditions adequate for directly comparing canine and human olfactory psychophysical thresholds, thus shedding some light on a long standing issue.

The present study extends the direct canine-human comparison to amylic-acetate. The same air-dilution olfactometer and sniff port apparatus was used for both species. In addition, the technique of conditioned suppression was used to determine canine olfactory sensitivity. The efficacy of conditioned suppression as an olfactory psychophysical method has been demonstrated for several species and sensory modalities [21]. It has been used for threshold determinations in vision [8], audition [5], x-ray detection [17], and olfaction [9,19]. Henton [9] determined threshold to amylic-acetate in pigeons. Pierson [19] determined amylic-acetate threshold for rats. As yet, conditioned suppression has not been used to determine canine olfactory sensitivity.

Amylic-acetate was chosen as the olfactory stimulus because it has been demonstrated, in the tortoise and rabbit [22,24], that at lower concentrations its effect is primarily on the olfactory system. Trigeminal influence doesn’t occur until much higher concentrations are used. Vomeronasal activation seems to occur at concentration levels slightly higher than that required for olfactory stimulation. Because of these properties, amylic-acetate has been a popular stimulus in both electrophysiological and behavioral studies of olfactory function.

EXPERIMENT 1

METHOD

Subjects

Three purebred male beagles and three female beagles served as subjects. They ranged in age from two to two and one-half years of age. One of the males was purchased from Ridglan Research Farms, Inc, Mount Horeb, WI. All others were whelped at the Florida State University Research Kennel.

Dogs were housed in indoor-outdoor runs with kibbled dry food freely available. Subjects were placed on a 23-hour water deprivation schedule. During the experimental session they were provided with approximately 210 ml of water. Occasionally the water intake was supplemented after a session when deemed necessary.

Test Chamber

The testing chamber was constructed of plywood and
stood 116.8 cm in height, 105.5 cm in length, and 73 cm in width. The wood was sealed with epoxy resin to reduce odor absorption. The grid floor was constructed of aluminum tubes (2.5 cm in diameter) and was supported in Plexiglas for electrical insulation. Electric shock was generated by a Lehigh Valley Constant Current Shocker Model 112-04. A one-way mirror was mounted on the side chamber door. Constant masking noise was provided throughout each session via a white noise generator and an exhaust fan. The fan was mounted at the rear of the chamber's roof and vented extraneous odors.

Water reinforcers were delivered into a stainless steel bowl which projected into the chamber from the front wall and was positioned 2.5 cm above the grid floor. Water delivery was controlled by a solenoid valve, timed to allow approximately 6 ml per reinforcement to pass through a stainless steel tube into the bowl. A Teflon breathing chamber, 5.8 cm in width, 7.6 cm in height, and 7 cm in depth, was centered on the chamber's front wall approximately 20 cm above the floor. It was situated such that the face of the breathing chamber was flush with the front wall and the remainder extended outside the experimental chamber (see Fig. 1). Air entered the breathing chamber, passed through a perforated side wall, and was exhausted through perforations in the opposite wall. A positive displacement pump exhausted the air outside the building at a slightly higher flow rate than that of the input. This minimized odor escaping from the breathing chamber into the experimental chamber. A hinged Teflon lever attached to a microswitch was mounted to the ceiling of the breathing chamber. The dog was required to press the lever upwards with its nose actuating a microswitch to obtain the water reinforcers. Responding on the lever provided an operant baseline against which conditioned suppression was measured.

The electromechanical and programming equipment was housed in a room adjacent to the testing room to minimize extraneous noises. The experimenter manually initiated the trials and shock presentations via a control/intelligence panel. This panel was located in the testing room as was the olfactometer allowing constant monitoring of the subject's behavior and the odor delivery.

The Olfactometer

The prototype for this olfactometer was designed by Tucker [23]. Figure 2 depicts the olfactometer used in this study. Compressed air passed through two moisture traps (Parker Hannifin) and a refrigerated air drying unit (Deltich) into a glass feeding manifold at a regulated pressure of 0.35 kg/cm². Branching from this manifold were four air lines and one odor line. Each of the five lines was composed of five gas washing bottles. The first of these bottles housed molecular sieve (Union Carbide) for further moisture removal. The second bottle was filled with activated coconut shell charcoal (Fisher-Scientific) for odor removal. The following two gas washing bottles contained ultrapure water for saturation of the clean air lines. These saturation bottles in the odor line contained amyl-acetate (Malinckrodt). The final gas washing bottle housed Raschig rings which functioned as an aerosol trap. All gas washing bottles were maintained in a 20°C water bath.

All connections between these bottles and elsewhere in the system were Teflon or glass. The use of these materials aided in reducing odor retention within the lines of the system.

Volume flow rate into the breathing chamber was measured by Fisher-Porter Tri-Flat and Gilmont flowmeters. These flowmeters were mounted downstream from the aerosol traps and were controlled by needle-valve stopcocks. Additional flowmeters led into a buffer jar which ensured equalized pressure within the entire system. Air from this buffer jar was exhausted outside the building.

A double dilution feature of the system extended the lowest concentration to 10⁻⁸ vapor saturation at 20°C. This diluting process occurred within the odor mixing manifold after passing through flowmeters. The output of this manifold and the wash line led into opposite ports on a four-port flow valve. This flow valve was constructed of Teflon and anodized aluminum. One of the two remaining ports was an exhaust line leading outside. The other port was the input into the breathing chamber. A final dilution of one log, unit took place within the input line immediately prior to the entrance of the breathing chamber. This additional dilution of 54 l/min of air from the large diluent line extended the lowest concentration to 10⁻⁰ log, saturation. Total volume flow rate entering the breathing chamber was 60 liters per minute, 54 l/min from the large diluent line and 6 l/min from either the odor line or the wash line.

PROCEDURE

Training Phase

All subjects were trained to lever press for water reinforcers prior to this experiment by the method of successive approximations. Training progressed from a continuous reinforcement (CRF) schedule to a random variable interval (VI) 60 second random ratio schedule.

Testing Phase

Two types of trials were presented: (1) odor followed by shock (odor trials) and (2) clean air not followed by shock (control trials). Twenty trials consisting of 8 to 10 odor trials and 10 to 12 control trials were presented in each session.

The components of an odor trial were as follows: a 20-second "pre" period in which lever presses were counted was followed by a 20-second period during which the odor was presented and responses counted. At the offset of the odor valve, a shock was presented for duration of 0.5 seconds. Shock intensity ranged from 1 to 10 milliamps (mA) depending upon the subject's response stability, and physiological conditions such as foot pad moisture or other variables affecting conductivity.

Trials were initiated manually on a variable schedule (average once every 30 sec).

Responses made during an odor presentation (D) were compared to responses in a comparable period of pre-odor time (P). Suppression ratios were generated using these terms (P-D)/(P+D) as devised by Dinc and Smith [6]. Suppression ratios could range from -1.0 (complete facilitation) to 1.0 (complete suppression).

A mean daily suppression ratio of 0.9 or better for five consecutive days at the first three concentrations of 10⁻³, 10⁻², and 10⁻¹ vapor saturation was the criterion for determining that odor suppression training had been established. A descending series of odor concentrations was used across sessions beginning with 10⁻³ vapor saturation. Only one odor concentration was used within each session. The decrements of odor concentration progressed in one half to one log, unit steps until a concentration of 10⁻⁰ was ob-
FIG. 1. Diagram of the experimental chamber accompanied by a view of the interior air flow of the breathing chamber.

FIG. 2. Schematic diagram of olfactometer.
TABLE 1

COMPARISON OF THRESHOLD VALUES WHICH WERE DETERMINED BY FOUR METHODS FOR ALL SIX SUBJECTS

<table>
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<tr>
<th>Method</th>
<th>Mean</th>
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<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
<th>Dog 5</th>
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<td>Mean</td>
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RESULTS

Mean Suppression ratios as a function of odor concentration were plotted for each dog. Suppression to the odor and to baseline trials is illustrated in Fig. 3 for Dog 3 in Experiment 3. From such curves threshold values were calculated in three ways for purposes of comparison, since "threshold" can be defined in several ways.

In Method 1 the threshold was defined as the lowest concentration at which odor and baseline control trials differed significantly from each other (t-test); threshold values appear in Row 1 of Table 1.

In Method 2 threshold values were determined by noting the concentration at which the mean suppression ratios for odor trials were equal to or less than 0.333. This numerical value (0.333) denoted that the animal lever pressed twice as many times in the "pre" period as in the "during" period. These threshold values for each of the six dogs are reported in Row 2 of Table 1.

In Method 3 (see Row 3) the median of the baseline trials was found and then used as a focal point, such that suppression ratios for odor trials which were equivalent to or above this median were considered positive odor detections. The lowest odor concentration at which the number of positive detections exceeded 0.05 significance level, using the chi-square test, was designated as threshold.

The threshold values calculated by the above three methods were based on the last test session for each of the dogs.

An additional approach to the computation of threshold values utilized several days of testing at each concentration as well as several replications of the curve for each subject. The mean suppression ratio for a particular concentration was based on the last five sessions across all replications. The threshold values of Method 4 (see in Row 4 of Table 1) used the same criterion level as Method 2, i.e., when mean suppression ratios were equal to or lower than 0.333.

DISCUSSION

This experiment demonstrated that the conditioned suppression technique can be used to determine olfactory thresholds for dogs. Several methods of defining these threshold values were examined for comparative purposes with all methods yielding essentially the same results. The coefficient of concordance among the methods was 0.78 (p<0.05).

EXPERIMENT 2: HUMAN OLFACTORY THRESHOLDS FOR AMYL ACETATE

Using a breathing chamber of the same design, the olfactometric arrangement described above was used to determine human olfactory thresholds.

METHOD

Subjects

Ten subjects, three females and seven males, participated. Ages ranged from 21 to 60 years. Two of the subjects were heavy cigarette smokers. Data from only eight of the subjects were evaluated since one subject developed a severe head cold and the second complained of headaches caused by inhalation of the initial high concentrations of amyl acetate.

FIG. 3. Suppression performance of Dog 3 to amyl acetate for the last session of data collection and baseline control trial curve.
DETERMINATION OF OLFAC TORY THRESHOLDS

Apparatus

A separate breathing chamber of identical design, and materials as the one described above was constructed and mounted on a tripod. Tubing from the olfactometer was disconnected from the dog testing chamber and affixed to the human breathing chamber. In all other respects the olfactometric equipment was the same as in Experiment 1.

Procedure

The subject was seated in front of the breathing chamber and blindfolded. The following instructions were given to the subject by the experimenter who remained in the testing room throughout:

1. Place your nose into the breathing chamber.
2. Indicate your readiness for a trial by raising your index finger.
3. A trial will be presented for 20 seconds during which a breathing pattern should be established to maximize your detection of odor.
4. The experimenter will then ask you to reply “yes” or “no” to the question: “Was an odor detected?”
5. A period of two minutes will elapse before the next trial is initiated.

A trial consisted of a 20-second exposure to either odorized or nonodorized air. This procedure continued for each subject until the response “no” was elicited on all trials at two successive concentrations. A series of descending concentrations was presented within a session beginning with 10−5 vapor saturation and decreasing in 0.2 log unit steps. The entire procedure was repeated within a few days of the first session.

Threshold was defined as the concentration at which the subject responded “no” on fifty percent of the odor trials. This method of determining threshold differs from Experiment 1 since the suppression ratio (P−D)/(P+D) did not apply.

RESULTS

Threshold values ranged from 10−4.5 to 10−3.0 vapor saturation, with the modal threshold being 10−4.5. This value was approximately 2.6 log units greater than the mean threshold observed in Experiment 1 for dogs. These results are generally consistent with Moulton [15] and Marshall and Moulton [12] in which the dog was reported to be from two to four log units more sensitive than humans.

GENERAL SUMMARY

This investigation has compared threshold values of humans to those of the dog. The chemical stimulus, the olfactometer, and the breathing chambers were identical in both experiments.

The odor delivery system and methods of behavioral quantitation are thought to be effective in determining olfactory sensitivity in dogs and humans. It is hoped that this work addresses the differences reported in the literature, not only with respect to canine olfactory thresholds but to the canine-human comparisons as well.

There are several probable causes for the anomalies which exist in the literature. If the studies are compared with a species or between species the experimenter must consider the chemical stimuli being used and their purities, the means of odor delivery, behavioral methodologies, effects of adaptation, the levels of concentrations tested, and finally the potential of the odorants for stimulating trigeminal or vomeronasal receptors rather than olfactory receptors per se.

Briefly, addressing a few of these considerations, Engen’s [7] work implied that some odorants which yield concave upward functions as opposed to a power function produce different odor qualities at high concentrations than at low concentrations. The question then arises: Is the shift in quality due to concentration increases of olfactory origin or are they a result of low concentrations stimulating olfactory receptors and high concentrations stimulating a composite of olfactory-trigeminal receptors? This composite stimulation could result from the odorants themselves (Ashton et al. [2]) or from the levels of concentration [1, 11, 15]. As stated by Cain [4] the rate of growth seen in a power function will vary from one substance to the next but how and why perceived magnitude vary with concentration is a complex issue. Obviously then, considerations such as these should be of utmost importance when making any olfactory sensitivity comparisons.

REFERENCES