AD-777 274

FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

D. G. Moulton

Pennsylvania University

Prepared for:

Air Force Office of Scientific Research

November 1973

FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

Interim ~~Final~~ Report, November 1973

Prepared for the Air Force Office of Scientific Research

Grant No. 73-2425

D. G. Moulton
Principal Investigator
Monell Chemical Senses Center
University of Pennsylvania
Philadelphia, Pennsylvania 19104

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFOSR - TR - 73 - 2337	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG		5. TYPE OF REPORT & PERIOD COVERED Interim 5. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) D. G. Moulton		8. CONTRACT OR GRANT NUMBER(s) AFOSR-73-2425
9. PERFORMING ORGANIZATION NAME AND ADDRESS Monell Chemical Senses Center University of Pennsylvania 3500 Market Street Philadelphia, Pennsylvania 19104		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102F 9777 681312
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Office of Scientific Research (NL) 1400 Wilson Boulevard Arlington, Virginia 22209		12. REPORT DATE November 1973 13. NUMBER OF PAGES 42
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Detection curves for alpha-ionone for four dogs were derived using an automated odor-choice apparatus. Factors critical for maximizing, stabilizing, and evaluating performance of both dog and odor delivery system were identified. These include (a) use of a descending and not ascending concentration series, (b) minimizing decrements between successive dilution steps, (c) use of criterion of stability of performance based on achieving low variance. Six human subjects were tested on the same apparatus. The dog could detect alpha-ionone at 3-4½ log units lower than could human subjects. Detection curves

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT (Continued)

consist of two segments with markedly differing slopes. The discontinuity occurs at high performance levels suggesting the possible presence of a dual process at the receptor level. In cyclic females rats trained to detect cyclopentanone performance shows a high (.99 correlation coefficient) correlation with levels of circulating estrogens. Bilateral cervical sympathectomy did not eliminate the cyclicity in performance of these females. However, performance showed a significant elevation on the day of ovulation.

TABLE OF CONTENTS

SUMMARY

This report is in three parts: Part I concerns odor detection in dogs, Parts II and III concern odor detection in rats.

Part I summarizes results obtained with an automated behavioral apparatus for investigating odor detection in dogs and men. Detection curves for α-ionone in four dogs show discontinuities at relatively high performance levels. Below these performances decline to chance over 1 - 2 log units of concentration. Above, asymptotes are reached gradually over 2½ - 3 units of concentration. Such breaks in odor detection curve may imply a dual process at the receptor level.

Detection thresholds for α-ionone for 4 dogs and 6 human subjects differ by 3 - 4½ log units of concentration. For dogs the threshold range is 4.5×10^7 - 4.5×10^6 molecules/ml. One dog showed a lower threshold between 4.5×10^5 and $4.5 \times 10^{4.5}$ molecules/ml. In man preliminary results show threshold values from $4.5 \times 10^{9.5}$ - $4.5 \times 10^{8.75}$ molecules/cc.

Several factors, critical for maximizing, stabilizing and evaluating the dogs performance and ensuring the accuracy of measurement techniques have been identified. These include (1) Use of descending and not ascending concentration series. (2) Minimizing decrements between successive dilution steps tested. (3) Use of a criterion for stability of performance based on achieving low variance.

In Part II fluctuating performances of cyclic female rats in detecting the odor of cyclopentanone are correlated with changes in circulating plasma levels of estrogen.

The high correlation coefficient obtained (.99) demonstrates the

close dependence of the rats performance on ovarian hormones.

Part III describes an investigation of a possible level at which hormonal control of performance might be exerted - namely by cyclic changes in access of odorant molecules to receptors determined by alterations in the degree of engorgement of the nasal mucosa. Since this control is exerted by way of the sympathetic supply to this region the performance of normal cyclic females was compared with that of bilateral cervical sympathectomized females. The cyclicity of performance persisted in the experimental animals but showed a significant elevation in performance on the day of ovulation.

GENERAL INTRODUCTION

This is a report of a continuing series of inquiries into the factors which influence the performance of dogs, rats and (in one study) men on odor detection tasks. The overall aims are to identify certain of these factors, provide - where feasible - a quantitative estimate of their significance, consider the possible levels within the organism at which they originate, and explore their relevance to an understanding of the mechanisms of odor detection and recognition. The background to these studies, including a full description of the major techniques used and more extensive bibliographies, is given in an earlier summary (Final Report on Contract No. F44 620-60-C-0110, October, 1972 prepared for AFOSR by D. G. Moulton). However, points essential for understanding the present series of studies are summarized here.

The report is divided into three parts. Part I deals with the performance of dogs and men in detecting α-ionone under the same carefully controlled laboratory conditions over a range of concentrations. It explores variables that contribute to attaining maximum performance including the curious individual differences in susceptibility to the action of these factors seen especially in the performance of dogs at low concentrations of the test stimulus.

Parts II and III continue the analysis of cyclicity in performance of female rats on an odor detection task and its correlation with levels of circulating hormones. The possibility that this correlation might arise from the action of hormones on the sympathetic supply to the nasal mucosa is examined in Part III in which a study of bilateral cervical sympathectomy on performance yields an unexpected result.

PART I

DETECTABILITY CURVES FOR α-IONONE IN DOC AND MAN

Introduction

The experiments to be described relate to three different but interrelated problems.

(1) What is the absolute sensitivity of the dog to the odor of α-ionone and how does it compare with that of man ?

(2) What is the form of the detection curves for α-ionone in man and dog?

(3) What factors maximize the performance of dogs in this task?

The first question relates to the long-standing assumption that dogs possess olfactory powers far exceeding those of man. Such capacities have three main components concerning detection, discrimination and localization of odors. While some correlation among those abilities doubtless exists it does not necessarily follow that a highly developed ability to identify a complex mixture of odors or resolve it into its constituents is associated with considerably lower thresholds for odors. Consequently we must evaluate each capacity separately.

In this report we are concerned with the measurement of performance in an odor detection task. In the case of the dog quantitative information on this point is essentially restricted to three main groups of laboratory studies: those of Neuhaus (1953, 1954, etc.); Becker and his associates (1962); and Moulton et al. (1960).

These studies gave widely differing results. Thus Neuhaus (1953) reports that the dog is up to 100,000,000 times more sensitive to the odor of butyric acid than is man. Moulton et al. (1966) however, found the dog to be 100 - 1000 times more sensitive to this odor than is man. But one difficulty with these studies is their use of human data derived from the work of others using quite different types of apparatus and methodology. To obtain an accurate comparison it is important to test both human and canine subjects under as identical conditions as possible. In only one study has this been attempted: Becker et al. (1962), using a method for determining odor thresholds in a free ranging laboratory environment, found that the dogs ability to detect eugenol was greater than man's by a factor of only one log unit. Unfortunately this study lacked several desirable controls such as olfactometric control of stimulus variables.

The present study attempted to overcome the limitations of previous studies and provide more reliable quantitative information on canine and human thresholds by testing both in the same apparatus under as closely comparable conditions as possible and with careful control of stimulus variables (temperature, odor flow rate, concentration, purity and odor species).

Method

The apparatus and techniques used for testing dogs were described in detail in an earlier report (Final Report on Contract F44 620-70-C-0110, October 1972). However, some aspects were not previously reported in detail or have been modified since the original report. These are covered more fully below.

The experiments were conducted in a laboratory isolated from other buildings except on one side and continuously ventilated by a powerful wall fan which drew air from over a grass and tree-shaded area. The air was free from any contaminants detectable by man. This source of ventilation provided only the ambient room air through which subjects passed, or - in the case of human subjects - remained, between sessions of testing. The experimental apparatus and olfactometer were housed in a controlled environment chamber kept at 25°C which was also flushed continuously with room air by roof fans although otherwise isolated from the surrounding laboratory. Programming equipment and air purification stages of the olfactometer were housed outside the chamber in the main section of the laboratory.

Behavioral test apparatus

The behavioral testing apparatus was designed to accommodate both canine and human subjects. It consists essentially of a main chamber at the front of which are three small bays for sampling flowing air or odor. The exterior of the apparatus is constructed of wood and (apart from a glass door and observation panel in each sampling bay) is lined with aluminum foil surfaced with teflon. The dimensions of the main chamber are 120.6 cm long x 74.9 cm high and 90.2 cm wide. The sampling bays are spaced equally apart and can be separated from the main chamber by glass doors. The doors are hinged at the top and swing down to block the bay entrance or swing back to align against a one-way glass observation panel at the exterior surface of each bay. The dimensions and height of each bay and its height above the floor of the main chamber are such that it will accommodate the slightly lowered head of an erect, adult German shepherd.

Odor or air enters through a perforated teflon disc on the floor of each bay while water can be delivered to a small glass petri dish immediately in front (close to the dog) of the odor/air disc. When the dog inserts its head into the bay, it interrupts a light beam directed at a photocell. This has no consequences unless the beam is interrupted for five seconds continuously - a signal that a choice has been made. (Longer intervals did not appear to aid performance.) This sampling interval was made constant at each bay independent of odor position.

Opening of the bay doors is initiated by pressure on a treadle at the rear of the main compartment. The treadle cannot be activated during the 25 second intertrial interval. The switching of air and odor inputs to the sampling bays is controlled by teflon solenoid valves. These solenoids may generate noises (certain of which might be heard by the dogs and not the investigator). To prevent this from providing a differential cue two control solenoids were inserted at opposite sides of the chamber. They deliver no flow but are activated each time air and odor positions interchange (or at the corresponding time in trials where no change in position occurs).

In addition white noise is delivered to three small speakers, one in each bay recessed in the wall directly above the point of stimulus delivery. These auditory masking devices are intended to eliminate not only solenoid noise but also any differences in the sound of air and odor streams that might arise from some undetected differences in the respective lines.

Olfactometer and odor/air delivery

The olfactometer was described in detail in the previous report. It is a six-stage continuous dilution system constructed with 3/8" pyrex glass tubing, joined with teflon tubing.

Although located inside a controlled environment chamber maintained at 23°, the two odor saturator bottles are independently maintained at 23° by a temperature controlled water bath. While this ensures saturations at a known stable concentration, its main advantage is the stability of flowmeter settings that ensues from eliminating significant temperature variations. (Relatively small temperature changes compound to produce a relatively large error when each of the six stages of the olfactometer is set to yield a maximum dilution.) The olfactometer has an effective dilution capacity of 10^{-9} of vapor saturation and delivers 9 l/min of odor to any bay.

The apparatus is controlled by a solid state programming system which provides for presentation of a randomly-determined sequence of odor/air delivery from the olfactometer.

Air is delivered to two of the three bays in the behavioral apparatus and odor to the third. The thirsty dog is trained using a water reward to investigate each bay in turn and indicate - by interrupting the light beam for 5 seconds - which bay is associated with the odor. If the dog is correct it receives water delivered to the dish in the floor of the bay. If incorrect, the bay door closes to eject the dog's head from the bay. (In practice, the dog soon learns to retract its head when the door starts to close.)

Experimental animals

The experimental animals were four female German shepherds. Two of the dogs were siblings and a third dog had the same sire. The fourth dog was unrelated to any of the remainder. Training began when the dogs were about 8 months old.

Procedures for deriving detection curves for α-ionone in dogs

α-ionone was chosen for testing because Neuhaus (1954) had previously published threshold data for dogs and men tested on this compound. It has no known biological significance for either dog or man and has an odor reminiscent of violets. Detection curves for α-ionone were derived in two main series of experiments corresponding to two descending series of concentrations. Each series consisted of eight test concentrations. The first series comprised 27,300 total trials for 4 dogs. The second series comprised 16,000 total trials.

Testing with α-ionone began at a concentration of 10^{-3} of saturated vapor. (This corresponds to 4.54 x 10" molecules/cc, or 7.53×10^{-10}M or 14.5×10^{-5} mg/L). The procedure used throughout the first descending series is described below. With minor modifications, the same procedure was used in the second series of trials.

At each concentration each dog was tested over a minimum of 10 testing sessions. Each session comprised 50 trials thus giving a total of 500 trials per animal. Once a minimum of 10 sessions was attained trials were continued on a given concentration until the dog's performance showed no more than a one percentage point change from the rolling average derived over three consecutive sessions. Performance calculations were based on the last 10 sessions at each concentration. In practice the total number of concentrations needed to reach criterion rnaged from 15-20. The testing of subjects to a criterion of a small and stable level of variability was adopted to maximize the probability that no further improvement in performance at each concentration was likely.

Before testing began on any concentration the olfactometer was cleaned by heating the controlled environment chamber to 60° and blowing first methanol vapor and then dichloromethane vapor through the dilution stages, delivery system and solenoids, for 24-48 hours, on higher concentrations, and up to a week for the lowest concentrations. After cleaning the olfactometer a blank session was run on each dog to ensure that there was no contamination (detectable by the dog) remaining from the previous concentration. "Blanks" also

established that no other extraneous cue had become a factor influencing performance. Blank sessions were identical to experimental sessions except for the absence of any deliverately introduced odor stimulus. Consequently, the maximum socre that a dog could obtain was chance (or 26-40% on a single session due to the random occurrence of correct positions), assuming no contaminants were present.

After satisfactory "blanks" were obtained, the stimulus concentration was lowered by one log unit of saturation on the first series and by 1/2 log unit on the second series. This procedure was followed until dogs reached threshold on the first series or passed the point on the detection curve where discontinuities of slope appeared on the first series.

During the initial periods of data collection at high concentrations these control sessions presented little difficulty. The animals worked on what was close to a 100% reinforcement schedule, and extinction was rapid as would be expected. However, at low stimulus concentrations the problem of securing a good blank became much more difficult. Trace amounts of odor are given off by the olfactometer and stimulus delivery system. These traces of stimulus molecules can be extremely difficult to eliminate particularly if they are of a high molecular weight such as is α-ionone (192.24). In these cases the procedure for cleaning outlined above was not always effective and it was necessary to remove solenoid valves and glass tubing connections from the olfactometer to the behavioral testing apparatus, and clean them separately by hand.

After the olfactometer had been cleaned and stimulus production was resumed, it was necessary to allow for a period of time until the olfactometer reached equilibrium again. Stimulus molecules adsorb onto the inner surfaces of the olfactometer and delivery system resulting in a lowering of the concentration being presented to the animals. We have found that at 10^{-7} of saturation, 15 hours of running time of the olfactometer gave scores from our best dog no better than 64%; at 17 hours scores rose to 90% and held around this level. When producing a concentration of 10^{-8} it required 20 to 24 hours of stimulus production to approach equilibrium. The performance of our best dog was used in making this determination. During the first 20 to 24 hours the mean performance of this dog was 44%. After this point the animal's mean rose to 71%. At 10^{-9}, about 57 hours were required before scores began to rise from a maximum of 38% up to about 50%.

After thresholds were established, the second descending series was begun starting at $10^{-3.5}$ of saturation. In this series concentrations were tested at 1/2 log unit intervals until threshold concentration was again reached. The criterion for shifting to the next test concentration in the second descending series was 10 sessions per animal with the last 5 sessions being used for performance calculations. The purpose of this second descending series was to investigate the reversal of the slope of the stimulus response curves which were particularly noticeable for two of the dogs.

Detection curves for α-ionone in human subjects

Since this phase of the experiment is still in progress and the results are preliminary, we shall reserve a full description of the experiment for a later report. In brief, human subjects were first familiarized with the odor by sniffing a series of dilutions in benzyl benzoate. They were then tested in the same testing apparatus as the dogs over a descending series of concentrations. Initially, successive dilutions were spaced at one half log unit apart but at lower concentrations the amount was decreased to 1/4 log steps. The subjects received a 10¢ reward for a correct response. The subjects were two women and four men. Each subject was provided with a teflon covered nose cone, one end of which was pressed against the odor/air outlet part.

Results

(1) Maintenance of odor control of performance

At concentrations at or below threshold and during "blank" sessions the dogs were required to perform when reinforced only once in every three trials. In contrast, at higher concentration levels, the frequency of reinforcement approached 100%. This raises the question of whether performance at lower concentrations, and during blank sessions showed any change either by a tendency to extinguish or by a transition in the dog's strategy from one of active searching to random choice (in view of the marginally greater frequency of reinforcement that they could achieve at these levels). This point is particularly important in assessing the validity of threshold scores.

In fact, at successively lower concentrations the dogs developed successively higher resistance to extinction. In other words, they showed a gradual transition to performances of partial reinforcement schedules. They continued to respond when rewarded on the average of one out of three trials.

Evidence that this performance was based on active searching rather than random choice comes from two directions. The first is seen in the performances of all but our poorest performing subject during

blank trials. In particular, the best subject (whose threshold falls nearly one and a half log units below the others) showed consistently decreasing scores paralleling removal of residual odorant from the system. Other subjects also showed this effect between concentration reductions at higher levels. In no case did blank session scores fall immediately to chance levels. In other words, when a detectable amount of odor was present, the subject worked to maximize performance scores. Moreover, typical searching patterns, involving sampling of each bay, persisted to the point where a subject ceased responding.

A second line of evidence concerns the performance at concentrations bracketing a threshold value and for two half-log unit steps below, including blank sessions. During "blanks" dogs continued to search for the stimulus by repeated sampling from each presentation bay. In the poorest performing subject this searching pattern broke down only after trials were continued at a concentration about one and one-half log units below its threshold. At this point it developed a position preference and would sample only one bay. (Consistent choice of a single position is the best strategy for maximizing reward frequency at an average of 33%, given the stimulus position sequence used in this study). This shows a loss of stimulus (odor) control and a shift to reinforcement control of performance.

Position preferences did not develop in other instances - sampling patterns persisted even at near-threshold concentrations. These facts, together with the generally decreased variability of the second descending series, support the conclusion that the final curves for three of the four dogs represent the best detection performance.

(2) Factors influencing attainment of maximum stable performance

The study identified several factors that determine the maximum performance reached, its degree of stability or both. The evidence for each factor will be considered separately.

(a) Magnitude of concentration change

This experiment was not designed in advance but its need became apparent during the course of the study. It demonstrates that the maximum performance attainable is a function of the concentration decrement. (Assuming that the performance has stabilized at a higher concentration initially)

Dogs were initially trained on α-ionone at a concentration of 10^{-3} of vapor saturation. After 200 trials the mean performance for 4 dogs reached 95% correct. A further 2,750 raised

this score to 98% (S.D. = 3%). It was assumed that a stable maximum performance had been attained and blank sessions were run prior to testing a lower concentration. The blanks were successful and a new concentration level was established at 10^{-5} of vapor saturation - a drop of two log units. After 200 trials (50 trials/dog) the mean score at 10^{-5} was 38%. After a further 2,950 trials the mean rose to 71%. Performances at this level were highly variable although showed little tendency to improve. This raised the possibility that the concentration decrement had been too large. It was therefore decided to train the dog at a concentration level only one log unit higher than 10^{-5} before retesting at 10^{-5}.

A concentration of 10^{-4} was established. After 200 trials the mean rose to 95%: with a further 1850 trials, 99% was reached. Again the system was cleaned, a successful blank obtained, and the stimulus concentration lowered to 10^{-5} for retesting. In marked contrast to the earlier finding, the mean now rose to 98% after 200 trials. Testing continued through 3200 trials to yield a stable performance of 96% (S.D. = 3%).

In other words, there was an overall increase in performance of 25 percentage points (and decline in standard durations by 14 points) between each testing on 10^{-5}. But even more striking is the comparison between the first 200 trials of each testing. The increase here is 60 percentage points.

As a result of this experience, subsequent decrements in the first series were held to one log unit. On the second series half log unit decrements were chosen and in most cases some gain in performance is evident. In addition, the time required to reach a stable performance level was reduced. (In studies with human subjects the magnitude of concentration decrements was also found to be a limiting factor. Consequently, decrements on lower concentrations were held to quarter log steps.) Once a decline occurred, however, a large number of trials did not improve performance or reduce variance.

(b) Direction of concentration change

It should be evident from the above data that no meaningful information, if any, would have been achieved by beginning with an ascending concentration series. It might be argued, however, that once a descending series had been completed an ascending series should have been run. In fact, this is precisely what was attempted. But while the best performing dog appeared to achieve performances that were extremely close to the curve delineated on the descending series, the remaining dogs, although initially performing effectively at or below threshold, rapidly switched into reinforcement control of

performance and did not achieve scores significantly above chance. Consequently, to reestablish performance levels it was necessary to return to higher concentration levels and descend down to the desired concentration. This accounts for the second descending series.

(c) Sampling time

Both human and canine subjects were allowed to sample the test bays for as long as they required. In the case of dogs there was, in theory, an upper limit of 25 seconds on sampling time but in practice this was only reached when the dogs ceased to perform.

Dogs typically reached a decision within a few seconds regardless of the concentration. In contrast, however, human subjects sometimes took up to 10 minutes to make a choice at concentrations below their ability to achieve 95-100% success. At least one subject found when a lower concentration was presented in the range of 10^{-3} - 10^{-4}, the quality of the odor appeared to have changed. Consequently, some of the initial trials at a given concentration were primarily concerned with determining what represented the stimulus. When this was done, performance improved markedly. Subsequent to this, however, performance was clearly maximized by prolonged sampling time. In several subjects the improvement effected was equivalent to increasing the concentration by about 2 log units.

(3) Detection curves for α-ionone

The detection performances for each of the four dogs is given in Tables 1 and 2. Figure 1 summarizes this data in the form of detection curves. Several points are apparent.

Firstly, at concentration levels below 10^{-7}, three of the dogs show performances that are closely similar. The fourth dog is clearly superior. Secondly, the dynamic range of the curve extends over 3.5 - 4.5 log units. Thirdly, all curves can be considered as consisting of two segments. One segment corresponds to performances on concentrations below $10^{-6.5}$ - 10^{-7} of vapor saturation. The upper segment corresponds to higher concentrations. The lower limb falls steeply to threshold while the upper limb ascends more gradually to the asymptote of the curves. Furthermore, the upper limb shows one or more discontinuities of slope while the lower is relatively uncomplicated.

Of the discontinuities in slope, one is particularly well defined and occurs in all dogs. It develops within the range $10^{-5.5}$ - $10^{-6.5}$ of vapor saturation and in the two best performing dogs appears as an actual reversal in slope. No other reversal is seen consistently

CAPTION TO FIGURE 1

Fig. 1a Detection curves for two dogs tested over two series of sessions on α-ionone vapor. The performances of dogs 2 and 3 and the standard deviations for dog 4 in the main series are excluded for clarity of presentation but the data are given in Tables 1 and 2 (Appendix). Standard deviations are plotted for dog No. 1 in the main series. The two points lying between the the 60 and 80 per cent correct levels at 10^{-5} were those derived during initial trials following a drop in concentration of two log units. The remaining points at 10^{-5} were derived following retraining and a drop of only one log unit in concentration (see text). The curve for dog 1 (first series) includes two points at $10^{-7.5}$ and $10^{-8.5}$ which were derived in response to an ascending series of concentrations. All remaining points represent trials derived on a descending series.

Fig. 1b Mean performances of four dogs in detecting α-ionone ± standard deviation. The isolated point at 10^{-5} was derived following a two log unit drop in concentration. All other points were derived following a one half or one log unit drop in concentration. The data for all dogs on ascending and both descending series of concentrations are included in this figure with the exception of the second descending series for dog 4. This curve showed a marked departure from that of the remaining dogs and from that of the same dog obtained during the first descending series and it is assumed that some unknown factor lowered the dogs performance to an unrepresentative level.

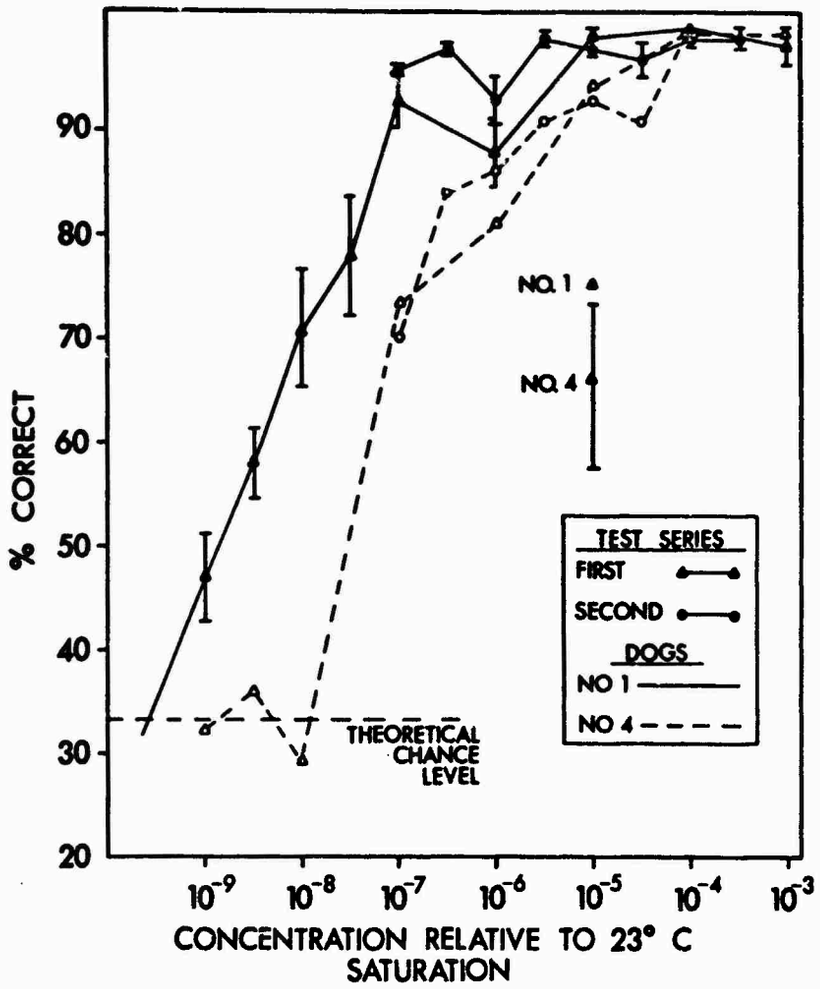
% CORRECT
90
80
70
60
50
40
30
20
NO. 1
NO. 4
TEST SERIES
FIRST
SECOND
DOGS
NO 1
NO 4
THEORETICAL CHANCE LEVEL
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3}
CONCENTRATION RELATIVE TO 23° C SATURATION

Fig. 1a

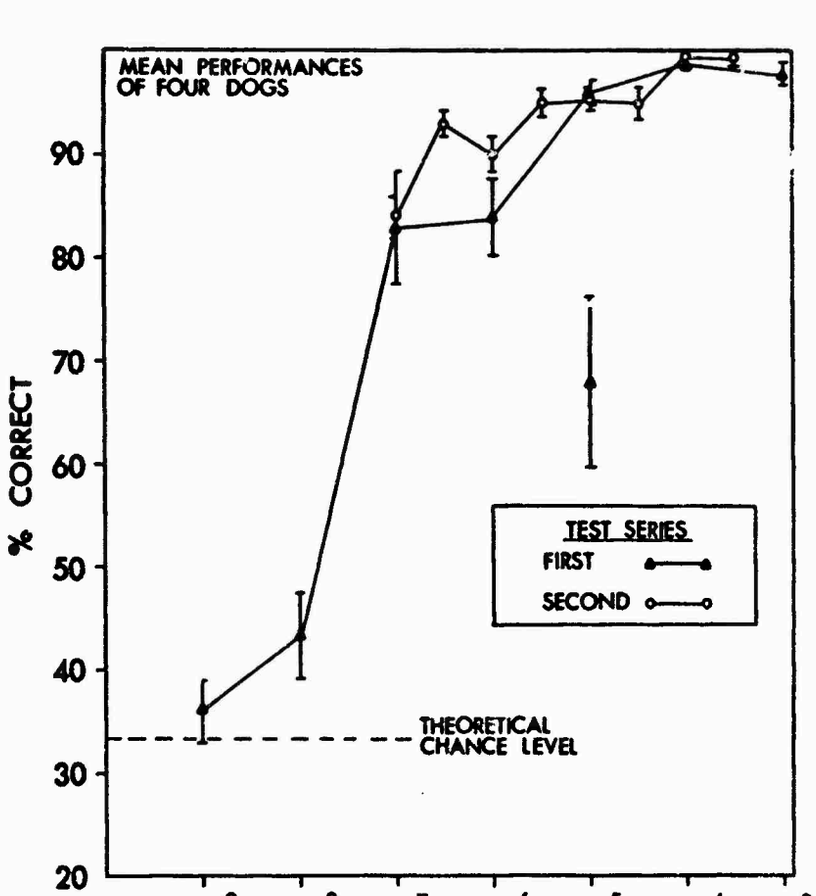
MEAN PERFORMANCES
OF FOUR DOGS
% CORRECT
90
80
70
60
50
40
30
20
TEST SERIES
FIRST
SECOND
THEORETICAL
CHANCE LEVEL
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
CONCENTRATION RELATIVE TO 23°C
SATURATION

Fig. 1b

on both the ascending and descending series. The conformation of the reversals seen in the first series came about one year later. The thresholds for the curves fall within the range $10^{-9.5}$ - $10^{-7.5}$ of vapor saturation (or $4.5 \times 10^{4.5}$ - $4.5 \times 10^{6.5}$ molecules/cm^3). The performance of the most effective dog is thus superior by 1-1/2 - 2 log units to that of the poorest performer. The mean threshold for the four dogs was 10^{-8} - $10^{-8.5}$ of vapor saturation. The standard deviations of the mean for each concentration tested tend to increase with progressively greater dilutions. The standard deviation for points in the first descending series tend to be greater than the corresponding points for the second series.

(4) Performance of human subjects

In preliminary trials human thresholds (2 women and 4 men) for α-ionone ranged from $10^{-4.5}$ to $10^{-5.5}$ of saturated vapor. (This is equivalent to $4.54 \times 10^{9.5}$ - $4.54 \times 10^{8.5}$ molecules/cc.) The mean human threshold was $10^{-5.0}$ of saturation. Sex differences were not significant.

DISCUSSION

The main body of quantitative evidence concerning odor detection in dogs is in the series of studies by Neuhaus (1953, 1954). This work is widely cited in the liaterature with the implication that it is the definitive statement in this area. Close inspection of his work, however, raises a number of disturbing questions that concern the validity of his methods and his findings. More generally they point to problems that are of wider significance in considering the validity of much detection data in the field of olfaction regardless of species. Since these questions, together with others generated by a further study on dogs (Moulton et al, 1960), were in mind when the present study was designed, it is pertinent to consider the possible sources of difficulty in Neuhaus' work and compare it with the present findings. This may provide, in part, the background necessary to understand the need for the present intensive study and to assess the significance of our results.

(a) Sorption difficulties

In describing his method of stimulus delivery, Neuhaus (1953) was clearly aware of the necessity of achieving equilibrium conditions. (Economically, his ingenious method for obtaining odor dilutions was ideal for determining the actual odorant quantities introduced into the delivery system.) He states (1953, p. 531), however, that adsorption time could not be measured, and he makes no mention of having flushed out and resaturated his olfactometer between concentration changes. He states that he allowed 10 minutes

running time between concentration changes to establish equilibrium. The trial times given in sample protocols, however, (Neuhaus 1953, 1954) show few intervals as long as 10 minutes and many were 5 minutes or less. We have clearly demonstrated in our studies that periods of up to a week may be necessary to rid lines of α-ionone and gain acceptable "blanks". Consequently, we must conclude that the actual concentration of α-ionone delivered to the dog in Neuhaus" (1954) study were probably greater than the values given.

(b) Flow rate

A major limitation in the olfactometer which Neuhaus' used was the low useable output volume/min. The reason for this - which is in general true for capillary olfactometers - lies in the small odorant quantities available from the evaporative surface in the capillary containing the odorant. A large flow volume is necessary for reasons unrelated to the accuracy of measured odorant concentration. Rather it relates to sniffing behavior. In the dog sniffing appears to maximize the quantities of odorant entering the nose. Neuhaus (1953, p. 535) recognizes that this creates a potential problem in his own study. His flow rate was no more than 3 - 4 cm^3/sec (.18-.24 l/min). His subject, however, showed large differences in the time and intensity of sniffing at high and low concentrations. At low concentrations the dog pressed its nares against the outflow apertures and sniffed with sufficient force to increase flow rate measurably over a 20-30 sec period. At high concentrations the dog makes a choice with an easy pass of its nose 1-2 cm from the output. It is true that this does not change the odor concentration in a given volume of air. But the volume of air changes so the number of odorant molecules entering the nares must also change.

In the present study, this difficulty has been avoided by providing for an odor flow rate of 9 l/min to each of the three sampling bays - over 40 times the volume provided by Neuhaus to his dog.

(c) Stability of performance

In Neuhaus' (1953,1954) work, the procedure and behavioral technique used would not have allowed the animal to maximize its performance. He reports that the dog required retraining daily at high concentrations in order to maintain performance at low concentrations. If this was not done the dog failed to perform after non-reward trials. These difficulties precluded the possibility of running blank trials and necessitated switching from high to low concentrations within a testing session. In addition, only 5-10 trials were run at any test concentration. This procedure should result in spuriously high threshold values.

The present results emphasize the problem of maintaining adequate performance levels when dogs are working at or near threshold concentrations and demonstrate the ease with which it is possible to obtain "false" thresholds if adequate precautions are not taken. For example, the initial 200 trials (50 trials/dog) which we ran on 10^{-5} of α-ionone produced a score of 38%. By Neuhaus' criteria this would have been more than an adequate number of trials. Over the next 2950 trials, however, scores rose to 71%. In addition, variance was reduced to very low levels for some concentrations at least. Neuhaus, however, gave no data that would allow adequate assessment of variance, and it is our experience that a reduction in the variance of session-to-session performance is the key to a valid assessment of olfactory capabilities. As a result of the procedures used, the present study yields performances that appear both reliable and repeatable even after a several months gap.

These three main sources of error in Neuhaus' work tend to act in two opposing directions. The first error - that of contamination carry-over between trials on different concentrations - leads to overestimates of sensitivity. The second and third errors lead to underestimates of sensitivity. The net result might thus be to place the reported threshold closer to its real value than would be the case if any one of these errors was operating alone.

In this context, it is not surprising to find that Neuhaus reported threshold values of 1×10^5 molecules/cc for α-ionone which falls within the threshold range of the best performing dog in the present study - $4.5 \times 10^5 - 4.5 \times 10^{4.5}$ molecules/ml.

Comparison between human and canine thresholds

The preliminary estimate of mean threshold levels for α-ionone seen in this study was 4.5×10^9 molecules/cc for human subjects as compared with a mean of $4.5 \times 10^{5.5}$ for dogs. This difference of about 3.5 log units is close to that of 3 log units estimated by Neuhaus on the basis of the data he obtained on dogs and Moncrieff obtained (with a mixture of α and β-ionone) on human subjects. However, these comparisons should be viewed with caution since there are a variety of factors which cannot be equally matched in such studies. For example, there is no way of assessing the motivational equivalence of water and money rewards for dog and man. Also the human subject is at a slight disadvantage in having to use a 10" nose cone.

Odorant

Both canine and human subjects appeared to have difficulty in transferring performance from higher to lower levels of concentration on α-ionone. In human subjects at least this might be described as difficulty in remembering and identifying the quality of the odor, particularly at lower levels of concentration. Thus in comparison with lower saturated fatty acids, for example, α-ionone could be characterized as lacking "salience". One manifestation of this is the "false" thresholds attained at higher concentration levels (described in the previous section). When concentration steps are reduced by small enough steps subjects do not "lose track" of the stimulus.

Comment on the use of a "restricted operant" procedure

The behavioral technique used in this study can be classified as a "restricted operant" procedure: access to the stimulus presentation bays is under pre-set program control with each test trial separated by a constant interval. In contrast, in a "free operant" testing situation, the subject is able to perform the desired response at any time. This response is usually pressing a treadle, movement of a lever-bar, or for pigeons, pecking a key. One of the most sensitive measures of free operant behavior is a change in response rate; alterations both of stimulus and reinforcement conditions may first show up in a response record as such a change. Under certain experimental conditions, for example, one can accurately predict cessation of responding (extinction) from fluctuant decreases in response rate occurring well before a last response is emitted.

Using a restricted operant procedure such as ours, a response rate measure is not possible due to the necessity for intertrial intervals. A finding from free operant studies which applies in an important way to our procedure, however, concerns effects of the frequency with which a subject receives a reward. It is typically found, for example, that when a subject is rewarded on each trial, termination of the reward is quickly followed by cessation of responding. When a reward is delivered periodically, for every n-th response or every n seconds, the subject will generally continue to respond for some multiple of n depending upon the size of n.

In testing a series of successive odor dilutions, the subject initially experiences close to a one-to-one ratio of rewards to responses; performance at 95% and better means that a reward is received on nearly every trial. Upon completing a series of sessions at a relatively high concentration, this ratio being nearly one,

the subject is thus maximally vulnerable to extinction when stimulus concentration is lowered and rewards become fewer. This is particularly true when blocks of blank trials (no stimulus present) are inserted after completing each concentration. Extinction may also be a problem as test concentrations approach threshold values. In Neuhaus' work (1953, 1954), for example, a dog's failure to perform after non-rewarded trials made it necessary to retrain the animal each session by starting at a high concentration and shifting concentrations downward to a level where the animal ceased responding.

Conclusions

(1) In training dogs to detect low concentrations of α-ionone optional stable performances were achieved only when the following conditions (among others) were met:

(a) Training is based on achieving criterion on a descending and not ascending concentration series.

(b) Decrements between successive concentrations tested were kept low (½ log unit steps appear sufficient).

(c) A criterion for stability of performance based on achieving low variance was vigorously applied (despite the large number of trials that this might dictate).

"False" performance levels some 30 percentage points below those later achieved by the animal can easily be attained if the above criteria are not applied.

(2) Detection curves are valuable in evaluating response stability, in providing a broad basis for assessing individual differences and in defining the dynamic range of performance.

(3) Detection curves for α-ionone have a dynamic range of about 3-3.5 log units in the dog and about 1-1½ log units in man.

(4) Detection curves for dogs show a marked discontinuity of slope at a relatively high performance level. Below this level performances decline steeply to chance over ½-1 log unit of concentration. Above, asymptotes are reached gradually over 2½-3 log units of concentration. Such breaks may imply a dual process at the receptor level.

(5) Detection thresholds for 4 dogs and 6 humans differed by 3-4 log concentration units.

(6) The detection threshold for dogs is 4.5×10^{7}-4.5×10^{6} molecules /ml. In man thresholds range from $4.5 \times 10^{.95}$- $4.5 \times 10^{8.5}$ molecules /ml.

BIBLIOGRAPHY

Moulton, D.G., Ashton, E.H. and Ea , J.T. (1960). Studies in olfactory acuity. IV. Detectability of n-aliphatic acids by the dog. Anim. Behav. 8, 117-128.

Neuhaus, W. (1953). Über die Reichschärfe des Hundes fur Fetsäuren Z., Vergl. Physiol., 35, 527-552.

Neuhaus, W. (1954). Die Reichschwellen des Hundes für Jonon und Äthylmercaptan und ihr Verhältnis zu anderen. Reichschwellen bei Hund und Mensch. Z. Natur-forsch., 9b, 560-567.

PART II

RELATION BETWEEN GONADAL STEROID LEVELS AND PERFORMANCE OF FEMALE RATS ON AN ODOR DETECTION TASK.

Introduction

In the final report on contract No. F 44 620-70-C-0110 (AFOSR October, 1972) we described a series of experiments concerning the hormonal influences on odor detection performance in rats. These studies established that--in contrast to males--the performance of female rats on an odor detection task undergoes cyclic variations that are associated with the phases of the estrous cycle. The cyclicity was non-specific to the odor tested, maximal around ovulation and eliminated by ovariectomy as the induction of pseudopregnancy. Androgen-tested ovariectomized rats showed a significant dose-dependent increase to performance levels comparable, or superior, to that of normal males.

In brief, these studies show a general correspondence between altered hormonal levels and performance. It remained to determine how precisely--both in time and in magnitude--performance levels follow changes in specific ovarian hormone concentrations. To answer this question we first need to measure performance levels over a time scale of hours, rather than days, since hormonal levels alter relatively rapidly during certain stages of the estrous cycle. We can then compare these variations in performance with known variations in the activity of specific hormones, particularly estrogen.

Method

The odor-testing apparatus used was described in detail in the 1972 report on AFOSR Contract F 44 620-70-C-0110. In essence, it consists of a main chamber at the fromt of which are two vertical odor/air tunnels. Odor is delivered to one tunnel from an air dilution olfactometer while the other receives "blank" (filtered) air. These two flows are interchanged according to a randomly-determined sequence during testing sessions. To make a choice a thirsty rat inserts its head through a port into either tunnel for 5 seconds or longer, breaking a photocell circuit. If it chooses odor, 0.1cc water wells out from a spout in the tunnel as a door descends to block the blank air port. 15 secs later a door descends to close the remaining port. Both doors rise 60 secs later. If the incorrect choice is made, both doors descend immediately, and the rat is removed from the apparatus.

The subjects were six three-month old femals Long-Evans hooded rats (200-250 g). They were maintained in groups of 2-3 to a cage in the same room on a light/dark schedule of 14/10 hours. Temperature was maintained at 24-26° c, and humidity was relatively constant. Food was available ad lib,

but water was presented for only 30 minutes daily at the end of experimental sessions. All rats had regular four-day cycles as judged by vaginal smears.

To obtain the necessary performance data rats were tested on cyclopentanone (at 10^{-3} of vapor saturation) at 2:00, 3:00, 4:00, 9:00, 10:00 and 11:00 PM over a period of equivalent to two complete estrous cycles. Each rat received a total of 480 trials. The data for each cycle were then combined and plotted against published data for the estrogen concentration in ovarian venous plasma during the estrous cycle (Yoshinaga et al, 1969).

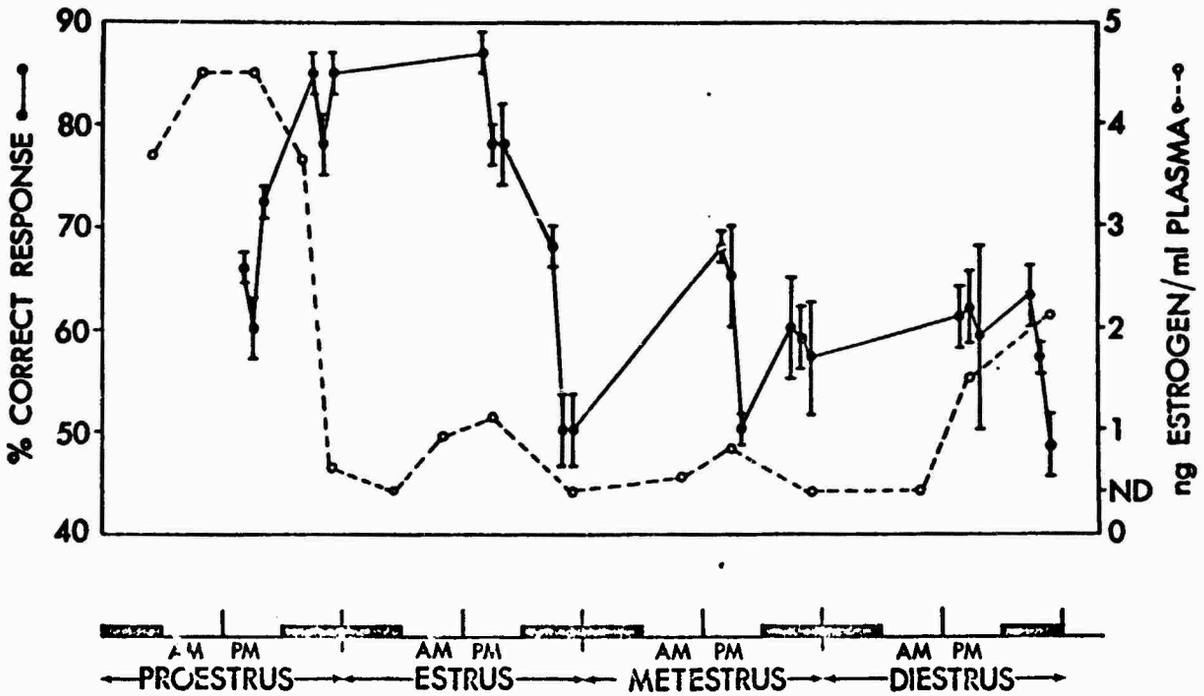

FIG. 2

Results

The results of the comparison are shown in Figure 2. Each point on the performance curve represents the mean ±SE for the group. The general correspondence in the shapes of these curves is immediately apparent. But first consider the behavioral data alone. The performance on the afternoon of the day of estrus was significantly higher than on the afternoon of proestrus ($p<.01$). However, note that there is no significant difference in odor detection performance between the afternoon of estrus and the evening of proestrus. From our own determinations and from previous studies (Everett, 1964; Yoshinaga et al, 1969) it is known that ovulation in rats occurs during the early morning hours of the estrous day. This the results clearly indicate that performance in the time around ovulation--from the evening of the proestrus to the afternoon of estrus--is significantly higher than that observed during the other portions of the sexual cycle ($p<.001$).

The performance data reveal a further important point. The correct responses on the afternoon of proestrous are significantly higher than those on the evening of diestrus ($p<.01$), but not significantly different from those during the hours of 2-4 PM on the day of diestrus. This rise in the level of response in the preovulatory period corresponds to the time period in which ovarian plasma concentrations of both estrogen and progestins are elevated (Yoshinaga et al, 1969; Goldman et al, 1969; Hashimoto et al, 1968). In addition, the level of response declines in the post-ovulatory period. This follows the cyclic decrease in the plasma concentrations of the gonadal hormones.

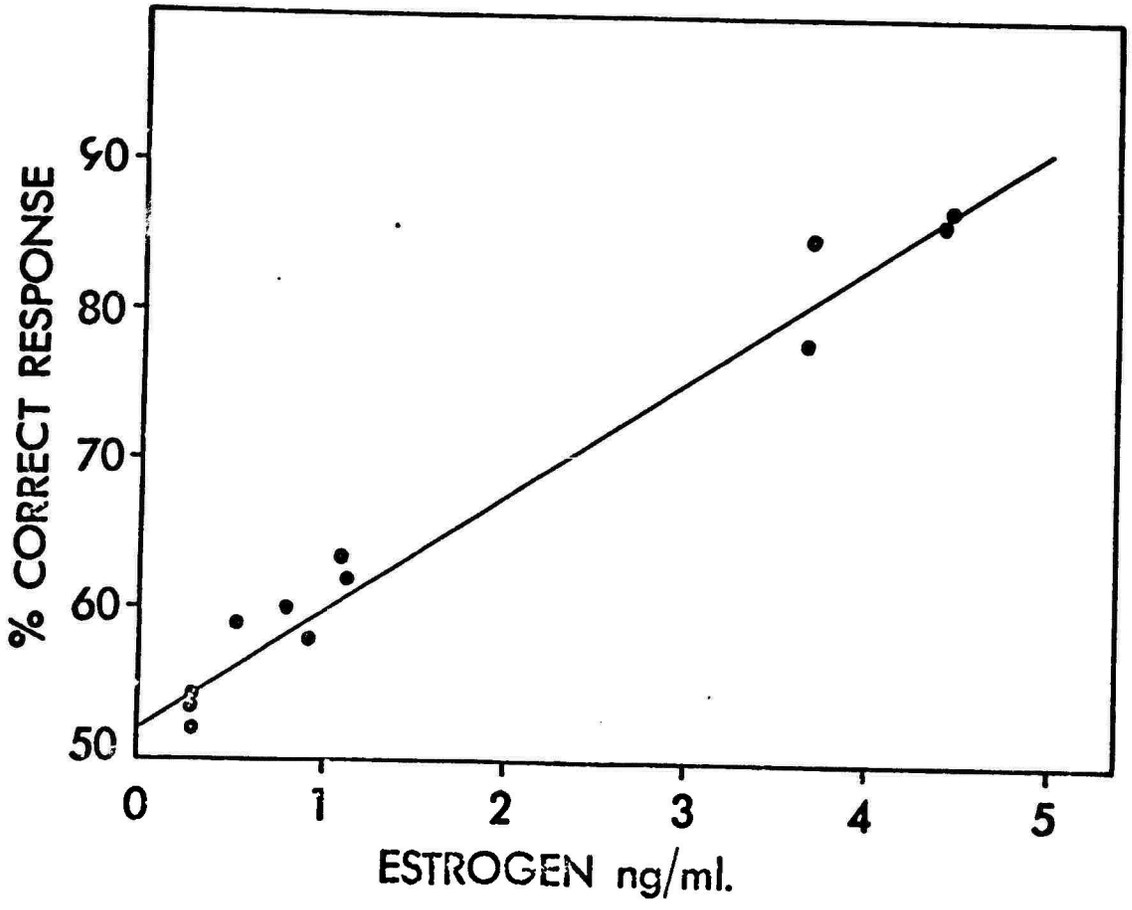

FIG. 3

When we compare these curves certain points of correspondence become evident. In particular, the peaks and depressions of the estrogen concentration curve are followed consistently by corresponding variations in the performance curve with an average latency of 19.8±1.8 hours. We therefore advanced the estrogen curve 19.8 hours on the time axis and plotted measured concentrations of estrogen in plasma against corresponding times (see Fig. 3) the linear correlation coefficient was 0.99 and the slope was 7.80.

Discussion

Latencies of the order noted here (19.8 hours) are not without precedent. Thus the behavioral latency of the action of estrogens in the ovariectomized rat ranges up to 20 hours post-injection (Green et al, 1970; Ross et al, 1971).

It is also important to note that progestin secretion reaches peak levels several hours before ovulation (Hashimoto et al, 1968) and other studies have shown that behavioral estrous effects of profesterone occur after a time latency of several hours. Sufficient data to correlate progestin secretion with odor detection performance at corresponding intervals of time, however, was not available.

In conclusion, it is strikingly clear that the performance of cyclic female rats closely correlates with Venous levels of progesterone. This provides convincing evidence that performance on an odor detection task is a sensitive indication of the levels of this gonadal steroid and vice versa. However, it does not indicate at what level in the organism the steroid acts to produce this effect. This is the subject of Part III.

Bibliography

Everett, J.W. (1964). Central neural control of reproductive functions of the adenohypophysis. Physiol. Rev., 44, 373-431.

Goldman, B.D., Kamber, I.A., Siiteri, P.K., and Porter, J.C. (1969). Temporal relationship of progestin secretion; LH release and ovulation in rats. Endocrinology 85:

Green, R., Luttge, W. G., and Whalen, R. W. (1970). Induction of receptivity in ovariectomized female rats by a single intravenous injection of estradiol-17B. Physiol. Behav., 5, 137-144.

Hashimoto, I., Hendricks, D. M., and Anderson, L. L. (1968). Progesterone and Pregn-4-en-20α-ol-3-one in ovarian venous blood during various reproductive states in the rat. Endocrinol. 82: 333-341.

Ross, J., Claybaugh, C., Clemens, L. G., and Gorski, R. A. (1971). Short latency induction in estrous behavior with intracerebral gonadal hormones in ovariectomized rats. Endocrinol. 89: 32-38.

Yoshinaga, K., Hawkins, R. A., and Stocker, J. F. (1969). Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy. Endocrinol. 85: 103-112.

PART III

THE INFLUENCE OF BILATERAL CERVICAL SYMPATHECTOMY ON THE PERFORMANCE OF FEMALE RATES ON AN ODOR DETECTION TASK.

Introduction

We have shown that gonadal steroids influence the performance of rats detecting odors but the mode of hormonal action is unclear. In fact, target mechanisms abound between the point of arrival of odorous molecules in the nasal airways and the final efferent output. Of these, the first in sequence are the mechanisms controlling odor access to receptor sites. In particular, the patency (or degree of constriction) of the nasal airway has been identified as a significant variable (Tucker and Beidler, 1956) presumably because flow rate is a first order variable in the olfactory stimulation process (Tucker, 1963). Patency, in turn, is under control of the autonomic nervous system. In particular, sympathetic tone usually keeps the blood vessels of an organ constricted to about half their maximum diameter (Jackson, 1970). Thus stimulation of the sympathetic supply to the nasal mucosa results in a marked increase in the patency of the nasay airway - an increase which is associated with the enhancement in the response of thedprimary olfactory neurones to odors (Tucker, 1956).

The possibility that autonomic control may, in turn, come under hormonal control is supported by several lines of evidence of which the most immediately relevant are the reports that in women, the degree of engorgement of the nasal mucosa fluctuates during the course of the menstrual cycle - maximum congestion occurring at menstruation (Schneider & Wolf, 1960). Hypogonadal women also show changes in nasal mucous membrane function while receiving androgen and estrogen (Schneider et al., 1958).

Thus the cyclicity in performance of female rats on an odor detection task might be determined by variations in odorant access to receptors reflecting fluctuations in sympathetic activity. An experiment - involving elimination of the sympathetic supply to the nasal region - was designed to test this hypothesis.

Method

Eight female Long-Evans rats about 5 months old were used. They were divided into 2 groups - one with three rats served as sham operated controls while the remaining five formed the experimental group. Each member of this last group was anesthetized with equithesin and the cervical sympathetic ganglion exposed and sectioned bilaterally. It lies in the same sheath as the carotid arteries (Farris & Griffith, 1963; Zeman& Innes, 1964). Postoperative examination showed bilateral ptosis of the eyelids and miosis of the pupils (Cannon & Rosenbluth, 1949).

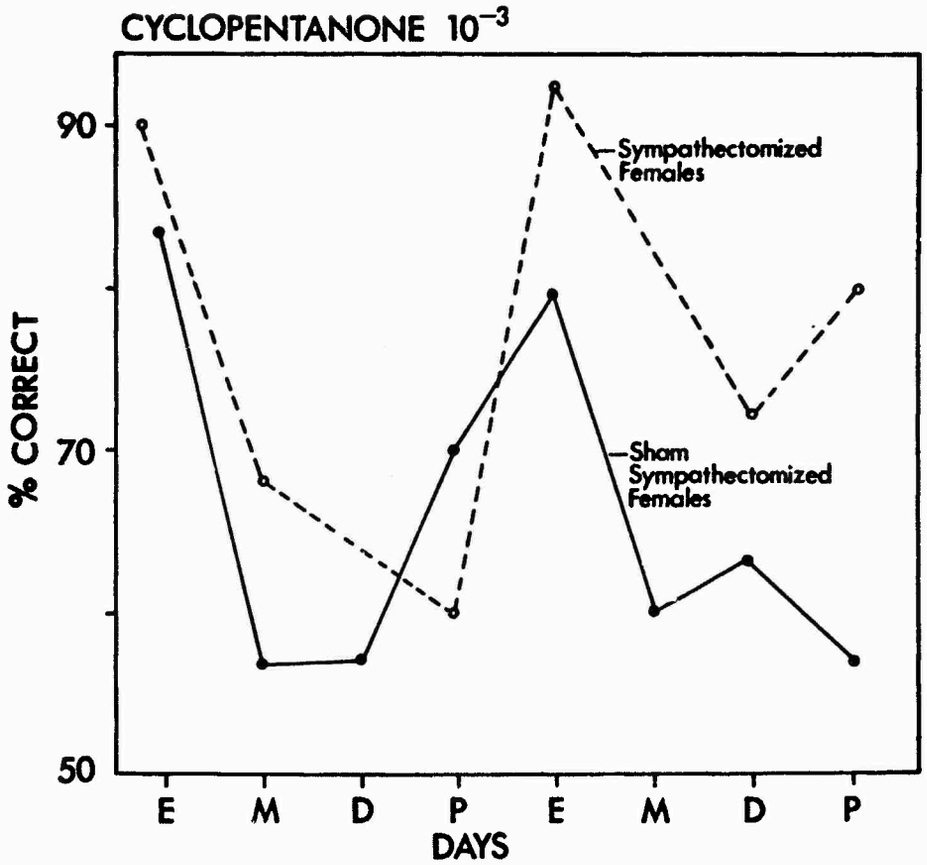
CYCLOPENTANONE 10^{-3}
90
70
50
% CORRECT
Sympathectomized Females
Sham Sympathectomized Females
E
M
D
P
E
M
D
P
DAYS

Figure 4

Members of the control group underwent the same operation except for the sectioning of the cervical sympathetic supply. Rats were allowed three days to recover. Testing was completed within 14 days of removal to ensure that no significant sprouting could have occurred from other sympathetic neurones in this region. The rats were presented with cyclopentanone (10^{-3} of saturation) and each was given ten trials per day for eight consecutive days (in the afternoon). Vaginal smears were taken on 16 consecutive days in the immediate postoperative period.

Results

It is clear from Figure 4 that - following sympathectomy - the cyclic fluctuations in performance during the course of the estrous cycle persist. The scores of the experimental rats do not differ significantly from those of the sham-operated controls. There is, therefore, no evidence that the basic cyclicity in scores is imparted by hormonal influences on sympathetic outflow to the nasal mucosa.

We cannot assume that this conclusion necessarily applies to other species. The rat is said to lack cavernous tissue in the nasal mucosa (Taylor, 1961) so there is less possibility that vasomotor changes could markedly alter nasal airway resistance. Nor is it clear to what extent a rat - as opposed to some other species - can compensate for increased airway resistance by increased intensity of sniffing.

On the other hand, the overall performance of the sympathectomized rats was superior to that of the controls. It is therefore possible that sympathectomy has some effect on the level of performance at least during certain stages of the estrous cycle. To examine this possibility data from another group of normal rats - that obtained in Experiment 1 - was compared with the data obtained in this experiment. The comparison shows that the performance scores of the sympathectomized rats were significantly higher than this normal group on the days corresponding to vaginal estrous ($p<.025$) and metaestrous ($p<.025$). This could be taken as offering some limited support for the view that sympathetic control of nasal mucus membrane function influences performance in an odor detection task. However, there is one difficulty with this interpretation. Stimulation of the cervical sympathetic nerve in the rabbit increases the diameter of the nasal airways (Tucker & Beidler, 1956). Sectioning of the nerve eliminates this capacity and presumably results in a sustained reduction in odorant access to receptors. Consequently, if the rat is similar to the rabbit in this respect, we would anticipate a lowering and not an enhancement of performance on an odor detection task. This raises

the possibility that sympathetic activation may be more significant for olfaction in decreasing nasal mucus secretion than in inducing vasoconstriction of nasal vessels. Since the effects are marginal, however, it is premature to speculate on the basis of this apparent anomaly, and further studies are, in any case, needed to establish the magnitude of the effect.

In the rat, postganglionic sympathetic axons arising in the superior cervical ganglion seem to provide the only innervation of the pineal gland and are necessary to maintain the response of the melatonin-forming enzyme - hydroxyindole-o-methyltransferase - to light (Moore & Rapport, 1971). However, despite this effect, the estrous cycles of the sympathectomized rats (which were kept on a diurnal light schedule) were kept on a diurnal light schedule) were not altered by denervation of the pineal as determined by examination of vaginal smears. This is consistent with the findings of Moore and Rapport (1971).

Bibliography

Cannon, W. B. and A. Rosenbluth. The Supersensitivity of Denervated Structures. New York: MacMillan, 1949.

Farris, E. J. and J. O. Griffith, Jr. (Eds.) The Rat in Laboratory Investigation. New York: Hafner Publishing Company, 1963.

Jackson, R. T. Pharmacologic responsiveness of the nasal mucosa. Ann. Otol. Rhinol. Laryngol. 79: 461-467, 1970.

Moore, R. Y. and R. L. Rapport. Pineal and gonadal function in the rat following cervical sympathectomy. Neuroendocrinology 7: 361-374, 1971.

Mortimer, H., R. P. Wright, C. Backmann, and J. B. Collip. Effect of oestrogenic hormone administration upon nasal mucous membrane of the monkey, Macaca mulatta. Proc. Soc. Exptl. Biol. Med. 34: 535-538, 1936.

Schneider, R. A., J. P. Costiloe, R. P. Howard and S. Wolf. Olfactory perception thresholds in hypogonadal women: changes accompanying administration of androgen and estrogen. J. Clin. Endocrinol. and Metab. 18, 379-390, 1958.

Taylor, M. An experimental study of the influence of the endocrine system on the nasal respiratory mucosa. J. Laryngol. 75: 972-977, 1961

Tucker, D. Physical variables in the olfactory stimulation process. J. Gen. Physio. 46: 453-489, 1963.

Tucker, D. and L. M. Beidler. Autonomic nervous system influence on olfactory receptors. Am. J. Physiol. 187. 637 (Abst.) 1956.

Zeman, W., and R. M. Innes. Craigie's Neuroanatomy of the Rat. New York: Academic Press 1964.

APPENDIX

		Dog 1	Dog 2	Dog 3	Dog 4
10^{-3}	X	98%	97%	99%	99%
	S.D.	3.1	1.1	3.0	1.3
10^{-4}	X	100%	99%	99%	99%
	S.D.	0.84	0.84	0.84	1.5
10^{-5}	X	99%	95%	95%	94%
	S.D.	2.1	3.1	3.3	2.8
10^{-6}	X	88%	84%	83%	81%
	S.D.	6.4	7.2	7.1	9.2
10^{-7}	X	93%	86%	80%	73%
	S.D.	5.5	12.4	13.9	10.7
10^{-8}	X	71%	38%	36%	29%
	S.D.	11.7	8.6	6.7	6.3
$10^{8.5}$	X	58%	30%	30%	36%
	S.D.	6.5	3.5	2.8	6.4
10^{-9}	X	47%	33%	33%	32%
	S.D.	8.5	4.4	4.9	5.7

Table 1: Performance of four dogs on descending series of concentrations of α-ionone: Series 1

Means (X) and standard deviations (S D.) of the mean are derived by grouping trials over 15-?0 sessions.

		Dog 1	Dog 2	Dog 3	Dog 4
$10^{-3.5}$	X	99%	100%	95%	99%
	S.D.	1.9	0.0	2.7	1.0
10^{-4}	X	99%	100%	98%	100%
	S.D.	1.0	0.0	1.7	0.0
$10^{-4.5}$	X	97%	98%	89%	91%
	S.D.	3.3	1.6	3.2	4.7
10^{-5}	X	98%	96%	71%	93%
	S.D.	1.4	2.1	10.1	2.8
$10^{-5.5}$	X	99%	96%	75%	91%
	S.D.	1.0	2.2	7.6	4.9
10^{-6}	X	93%	92%	63%	86%
	S.D.	4.5	2.9	9.5	2.4
$10^{-6.5}$	X	98%	98%	70%	84%
	S.D.	0.9	1.5	12.1	5.9
10^{-7}	X	96%	82%		70%
	S.D.	1.0	2.3		9.6

Table 2: Performance of four dogs on descending series of concentrations of α-ionone: Series 2. Means (X) of 15-20 sessions are given with standard deviations (S.D.)

Saturation	Molecules/cc	M	Mlg/L
$10^{-3.0}$	4.54×10^{11}	7.53×10^{-10}	14.5×10^{-5}
$10^{-3.5}$	$4.54 \times 10^{10.5}$	$7.53 \times 10^{-10.5}$	$14.5 \times 10^{-5.5}$
$10^{-4.0}$	4.54×10^{10}	$7.53 \times 10^{-11.0}$	$14.5 \times 10^{-6.0}$
$10^{-4.5}$	$4.54 \times 10^{9.5}$	$7.53 \times 10^{-11.5}$	$14.5 \times 10^{-6.5}$
$10^{-5.0}$	$4.54 \times 10^{9.0}$	$7.53 \times 10^{-12.0}$	$14.5 \times 10^{-7.0}$
$10^{-5.5}$	$4.54 \times 10^{8.5}$	$7.53 \times 10^{-12.5}$	$14.5 \times 10^{-7.5}$
$10^{-6.0}$	$4.54 \times 10^{8.0}$	$7.53 \times 10^{-13.0}$	$14.5 \times 10^{-8.0}$
$10^{-6.5}$	$4.54 \times 10^{7.5}$	$7.53 \times 10^{-13.5}$	$14.5 \times 10^{-8.5}$
$10^{-7.0}$	$4.54 \times 10^{7.0}$	$7.53 \times 10^{-14.0}$	$14.5 \times 10^{-9.0}$
$10^{-7.5}$	$4.54 \times 10^{6.5}$	$7.53 \times 10^{-14.5}$	$14.5 \times 10^{-9.5}$
$10^{-8.0}$	$4.54 \times 10^{6.0}$	$7.53 \times 10^{-15.0}$	$14.5 \times 10^{-10.0}$
$10^{-8.5}$	$4.54 \times 10^{5.5}$	$7.53 \times 10^{-15.5}$	$14.5 \times 10^{-10.5}$
$10^{-9.0}$	$4.54 \times 10^{5.0}$	$7.53 \times 10^{-16.0}$	$14.5 \times 10^{-11.0}$
$10^{-9.5}$	$4.54 \times 10^{4.5}$	$7.53 \times 10^{-16.5}$	$14.5 \times 10^{-11.5}$

Table 3: Conversion table for α-ionone at 23°C