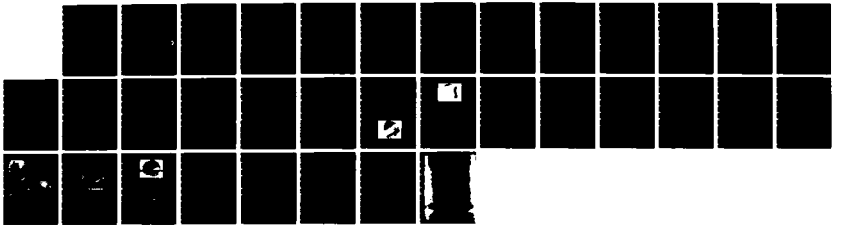
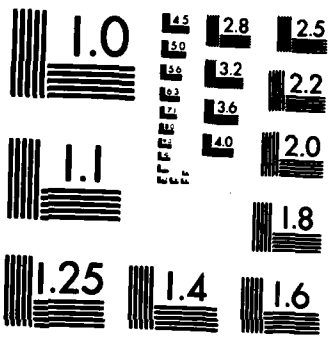
AD-A140 969 INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION 1/1
(U) PENNSYLVANIA UNIV PHILADELPHIA CLINICAL SMELL AND
TASTE RESEARCH CENTER R L DOTY ET AL. 01 FEB 84
UNCLASSIFIED ARO-18609.1-LS DAAG29-81-K-0128 F/G 6/16 NL





ARO 18609.1-LS (2)

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: DRXRO-IP-X-18609-LS DAAG29-81-K-0128	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION		5. TYPE OF REPORT & PERIOD COVERED: FINAL Aug 1, 1981 -- Jan. 31, 1984
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Richard L. Doty & David A. Marshall		8. CONTRACT OR GRANT NUMBER(s): DAAG29-81-K-0128
9. PERFORMING ORGANIZATION NAME AND ADDRESS: University of Pennsylvania, School of Medicine, Clinical Smell & Taste Res Center, 5 Ravdin Blg Hosp U of PA, 3400 Spruce St., Phil. PA 19104		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Research Office, Post Office Box 12211, Research Triangle Park, NC 27709		12. REPORT DATE: February 1, 1984
		13. NUMBER OF PAGES: 31
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.

DTIC ELECTE MAY 14 1984 S A D

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

NA

18. SUPPLEMENTARY NOTES

The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

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

Olfaction, Septal Organ, Odor Psychophysics, Odor Detection

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

Using a computer-controlled automated 3-choice test apparatus, a series of rat odor detection experiments were performed to establish the influences of an i.p. injected odorant upon olfactory sensitivity to that odorant. In addition, electrophysiological studies of surface potentials (electro-olfactograms) from (a) septal olfactory tissue and (b) septal tissue from the Organ of Masera were performed. No statistically-significant changes in odor detect- performance were found on successive daily tests following injection of the two test odorants (pentyl acetate and cynamic aldehyde). However, significant

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

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

84 05 11 0 0

AD-A140 969

DTIC FILE COPY

increases in detection performance were noted across test days in both the experimental (odor injected) and control (saline injected) animals. It was demonstrated for the first time that the septal organ of Masera is differentially responsive to odorants. ~~In general,~~ (a) the response boundary perimeters from one animal to another are consistent and in agreement with histologically-defined septal organ regions; (b) responses are largest at recording sites near the center of the organ; and (c) lower concentrations were needed to elicit a response from the organ than from olfactory epithelial tissue located posteriorly on the septum.

INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION

FINAL REPORT

RICHARD L. DOTY, PH.D., DAVID A. MARSHALL, PH.D.

FEBRUARY 1, 1984

U.S. ARMY RESEARCH OFFICE

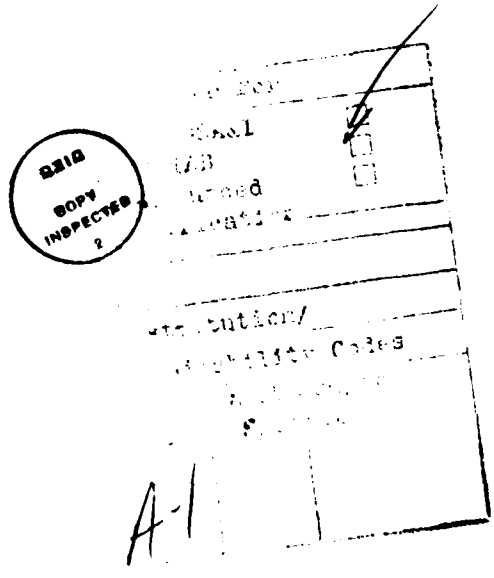
CONTRACT DAAG29-81-K-0128

University of Pennsylvania
Clinical Smell and Taste Research Center
Philadelphia, PA 19104

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED

FORWARD

The present work represents the completion of a series of experiments initially proposed by the late David G. Moulton to the Department of the Army, U.S. Army Research Office. Without his interest and guidance, this research would not have been possible.



INTRODUCTION

This report summarizes the findings of an extensive research effort designed to explore the possible influence of injected odorants upon mammalian olfactory sensitivity. It has long been believed that changes in the internal milieu of mammals influences their olfactory function, possibly via efferent projections to granule cells within the olfactory bulb, although few sound demonstrations of this phenomenon have been performed. The present work stemmed from observations of Neuhaus (1958), who reported that the odor detection threshold of the dog was altered following ingestion of specific odorants.

Neuhaus reported, in a 3-choice testing situation, that detection thresholds of a dog for butyric acid and alpha-ionone underwent characteristic biphasic shifts following ingestion of the homotypical odorant contained in gelatin capsules. For example, the apparent olfactory threshold for butyric acid slowly elevated from a preingestive baseline of 9.0×10^3 molecules/cm^3 of air to about 1.0×10^4 molecules/cm^3 of air within a few hours after ingestion of a butyric acid-containing capsule. After 24 hours, the threshold was considerably elevated (1.1×10^5 molecules/cm^3), following which time it decreased over the subsequent three days to a low point of 3×10^3 molecules/cm^3. The thresholds remained at below base-line levels for approximately two weeks, although they monotonically increased daily across this period. Neuhaus reported this phenomenon to be relatively stimulus specific; namely, ingestion of butyric acid resulted in subsequent performance shifts greatest for the odor of butryic acid and, although of the same form, of lesser magnitude and shorter duration for the odors of propionic and caprylic acid. Olfactory thresholds for alpha-ionone were reportedly not similarly influenced by the ingestion of butyric acid, suggesting that degree of generalization of the postingestive phenomenon was related to the chemical similiarity among the ingested and smelled odorants.

The series of experiments reviewed in this report sought to demonstrate this phenomenon in the rodent and to determine if these psychophysical changes were accompanied by alterations in electrophysiological measures of receptor activity, as measured by surface potentials from the olfactory epithelium (i.e., the electro-olfactogram or EOG; see Ottoson, 1971). The data collected by Neuhaus (as well as some unpublished canine data collected subsequently by Moulton and Marshall) were based upon only one or two subjects, and a demonstration of the phenomenon in the rodent would allow (a) meaningful statistical analyses of the changes using experimental designs employing relatively large numbers of subjects and appropriate control groups, (b) practical direct comparison between electrophysiological and psychophysical data in the same species, and (c) the development of a model for enhancement of olfactory function which could be practically used in the exploration of the chemical and physiological bases of this effect.

The rat psychophysical studies utilized a 3-choice computer-controlled operant testing system developed in our laboratory. This system, analagous to that described for the dog by Moulton and Marshall (1976), consisted of a stainless steel testing chamber enclosed in a sound-attenuated box coupled to a multi-stage dynamic air-dilution olfactometer. The olfactometer directed odorized air to one of three sampling ports, and non-odorized air to the remaining two. The position of the odor port was varied randomly from trial to trial, and the rat was trained to push a lever by the port containing the odorized airstream. Correct performances were reinforced by a water reinforcement. After training to a criterion of close to 100% correct detection performance at a moderately high odorant concentration level, the odorant concentration was gradually decreased until a performance level within the 50-70% correct range was obtained. Changes in performance from this baseline level were then observed following injection of target substances or control materials.

These psychophysical studies represented the first thorough evaluation of the so-called "sensitization" effect in rodents. In this work, great care was taken to insure that the experimenters involved in the psychophysical testing were not aware whether a given animal was from an experimental or control group. Furthermore, relatively large numbers of subjects were tested in paradigms which allowed for accurate statistical analysis of the results. Although we were unable to document this phenomenon in rats using three different odorants, several differences existed between our procedures and those employed by Neuhaus, Moulton and Marshall in the dog studies. First, we directly injected the odorants into the peritoneal cavity, whereas ingestion was the route of administration in the dog work. Second, we did not repeatedly test the same animals to the degree that was done in the dog studies. Third, all of the subjects of this study were males, whereas the few dogs used in the studies of Moulton and Marshall were females (Neuhaus does not indicate the sex of his subject). Whether these differences account for the differences between the rat and dog data requires further investigation. Interestingly, as discussed below, the most extensive of our psychophysical studies revealed a significant increase in performance across the days of the testing in both the experimental and control groups, demonstrating the need for caution in interpreting data from studies not incorporating adequate controls.

Despite our failure to document the so-called "sensitization" phenomenon in rats, our development of the first mammalian preparation for directly recording from the olfactory epithelium of a mammal allowed us to complete some unique studies of general importance to olfactory physiology. Thus, as indicated in more detail later in this report, we have shown that the the septal organ of Masera is responsive to air-borne chemicals. Furthermore, in accord with one of the primary aims of this study, we have mapped the patterns of responsiveness of selected odorants across the surface of the olfactory epithelium of the rat using a procedure similar to that used in amphibian forms (e.g., Kubie & Moulton, 1979).

The detailed description of most of our studies are contained in the progress reports attached in Appendix A. The studies completed since the last progress report are discussed in the next sections of the paper.

Psychophysical Studies

As indicated in our earlier progress reports, we found no statistically-significant effects of intraperitoneal injections of cynamic aldehyde or amyl acetate on the odor detection performances of rats. However, there was a trend in the expected direction in the the data from the study using amyl acetate (see progress report covering period from July 1, 1982 through December 31, 1982). Thus, we subsequently performed a more extensive and definitive study using this substance.

Twenty-four rats were trained to criterion on a series of descending concentrations and the experiment was begun. A computer failure occurred which necessitated retraining of these animals after a delay period. Following this period, only 18 of the animals regained stable performance and were thus continued in the study. These 18 rats were subsequently divided into two matched groups on the basis of their average baseline performances. One group received an i.p. injection of .1 cc saline, whereas the other received an injection of .1 cc amyl acetate after a minimum of 8 consecutive days of stable performance. The subjects were tested for 13 days following the injections.

The mean performances of the nine rats in each group are shown in Figure 1 in 2-day long blocks for both pre- and post-injection periods. A repeated-measures analysis of variance performed on the arcsin transformed mean performance values found no significant influences of saline vs. amyl acetate injection ($F = 0.00$). However, a significant pre- vs postinjection effect was present ($F = 15.58$, $df = 1/16$, $p = .003$), as was the effects of days ($F = 2.40$, $df = 7/112$, $p = .025$), indicating that both groups increased their performance across the stages of the experiment. As can be seen in Figure 1, no injection type by day interaction was present ($F = 0.81$, $df = 7/112$, $p = .531$). Thus, this rather definitive study fails to statistically demonstrate a change in performance specific to the injected substance. However, it does clearly show that the animals increased their performance across the days of the testing.

Electrophysiological Studies

Early observations by Moulton and colleagues (e.g., E. White and Moulton, unpublished data, 1970) suggested the possibility that injection of alpha-ionone interperitoneally results in an increase in the average multi-unit dischrages elicited by vapor phase concentrations of alpha-ionone drawn through the nose. These observations were not followed up by systematic work at the time, but were viewed as a suggestion that the presence of a blood-borne odorant might influence responses to the same odorant delivered via the normal air-borne route.

Subsequently, Moulton and co-workers examined the response properties of the amphibian olfactory receptor sheet; these studies suggested distinct differences in responsiveness of the underlying receptor sheet depending upon the region stimulated (e.g., Kauer & Moulton, 1979; Kubie & Moulton, 1979; Mackay-Sim, Shaman & Moulton, 1982).

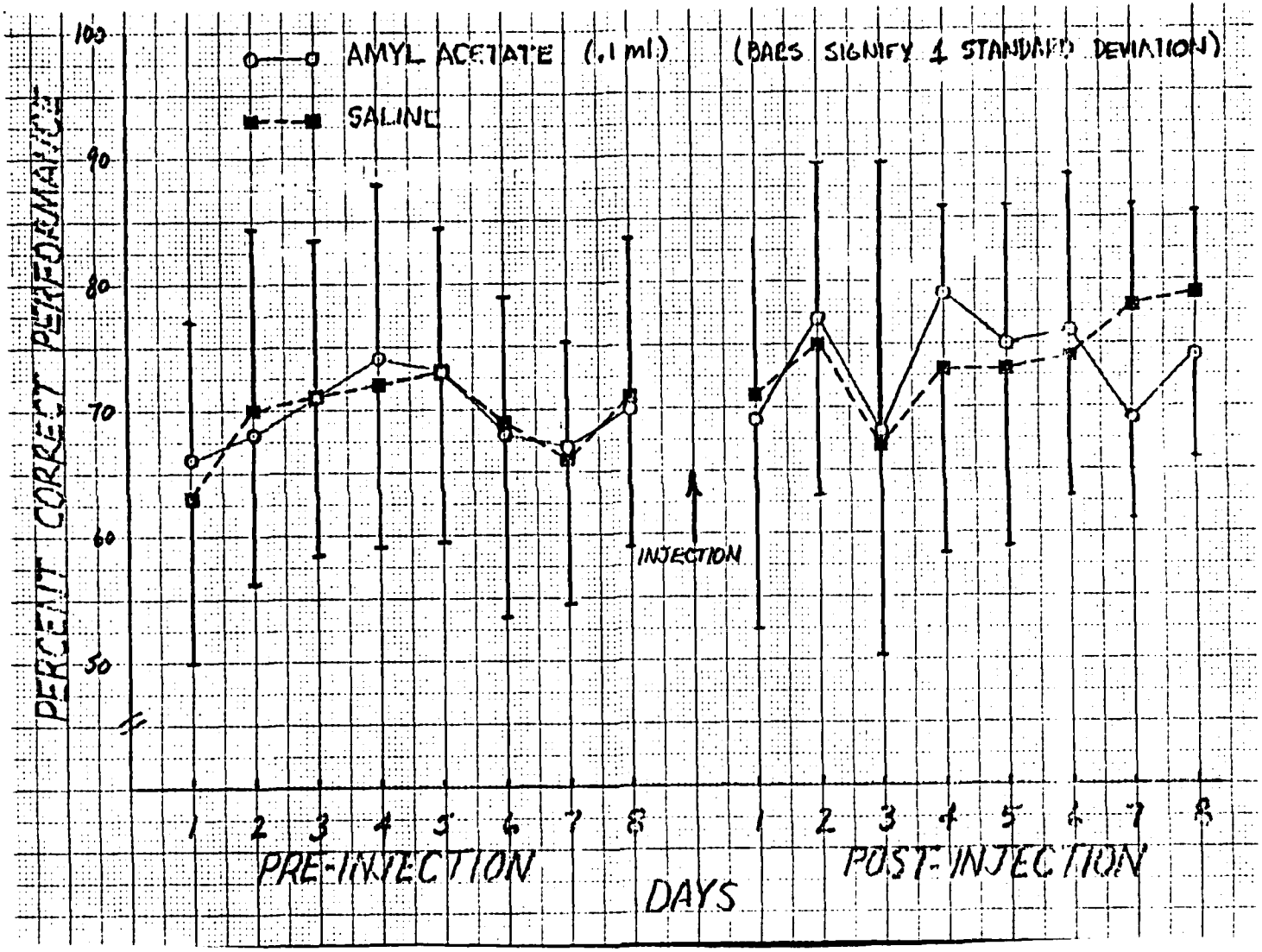


Figure 1. Percent correct performance in 18 trained rats for the detection of the odor of amyl acetate in a 3-choice test situation before and after an interperitoneal injection of either 0.1 ml amyl acetate or 0.1 ml saline.

From this background, a major goal of our project was to ascertain whether the spatial responsiveness of the rat olfactory epithelium was influenced by "sensitization" effects. Because our initial experiments did not clearly demonstrate this phenomenon, we focused our attention on changing odorants and increasing the sample sizes of our psychophysical studies. We felt it prudent to clearly demonstrate the phenomenon before we searched for underlying electrophysiological correlates. In preparation for the latter work, however, we developed a means for recording surface potentials from the olfactory epithelium of the rat (i.e., the electro-olfactogram). Details of the steps undertaken in the development of this preparation are presented in the previous progress reports (Appendix A).

The first of the olfactory epithelial mapping studies utlized amphibia, whose olfactory epithelia are relatively flat and accessable to recording electrodes and punctate stimulus presentation. In mammals, large sectors of the olfactory epithelium occupy complexly enfolded surfaces on ethmo- and endoturbinate structures. In order evaluate spatial responsiveness in a rat preparation, we found it necessary to record from the olfactory epithelia located on the posterior septum. Our preparation allowed us to record the responsiveness of receptors on the septal organ of Masera, as well on the septal segment of the olfactory region per se, to the same stimulants. The receptor region of the septal organ is located directly in the main respiratory flow path anteior to the olfactory epithelium.

The main septal olfactory epithelium demonstrated a relatively uniform response to pentyl acetate at 17 recording sites, as indicated a representative preparation (Figure 2). A series of recordings revealed that the septal organ differed somewhat in its responsiveness from that of the olfactory epithelium at the lower concentrations, but appeared remarkably similar at the higher concentrations (Figure 3).

Since the last progress report, septal organ responses have been recorded from 15 rats. These pioneering studies have led to several salient findings: (a) the response boundary perimeters from one animal to the next are reasonably consistent and in agreement with the histologically-defined regions that have been described; (b) no responses to either odor or to blank air pulses have ben obtained beyond the margins of the septal organ; (c) responses to all test odorants are largest at recording sites located near the center of the septal organ epithelium; and (d) the lowest concentrations to which these sites respond appear to lie well below the lowest that we have tested, and below those effectively eliciting responses from sites on the main olfactory epithelium.

Although we have been unable to confirm, in the rat, the existence of the so-called sensitization phenomenon, we have demonstrated, for the first time, that the septal organ of Masera is responsive to a broad range of chemicals, has an apparently lower threshold for such stimulation, and likely serves as an important intranasal chemosensory system in addition to the classic systems of CN I, CN V, and the vomeronasal organ.

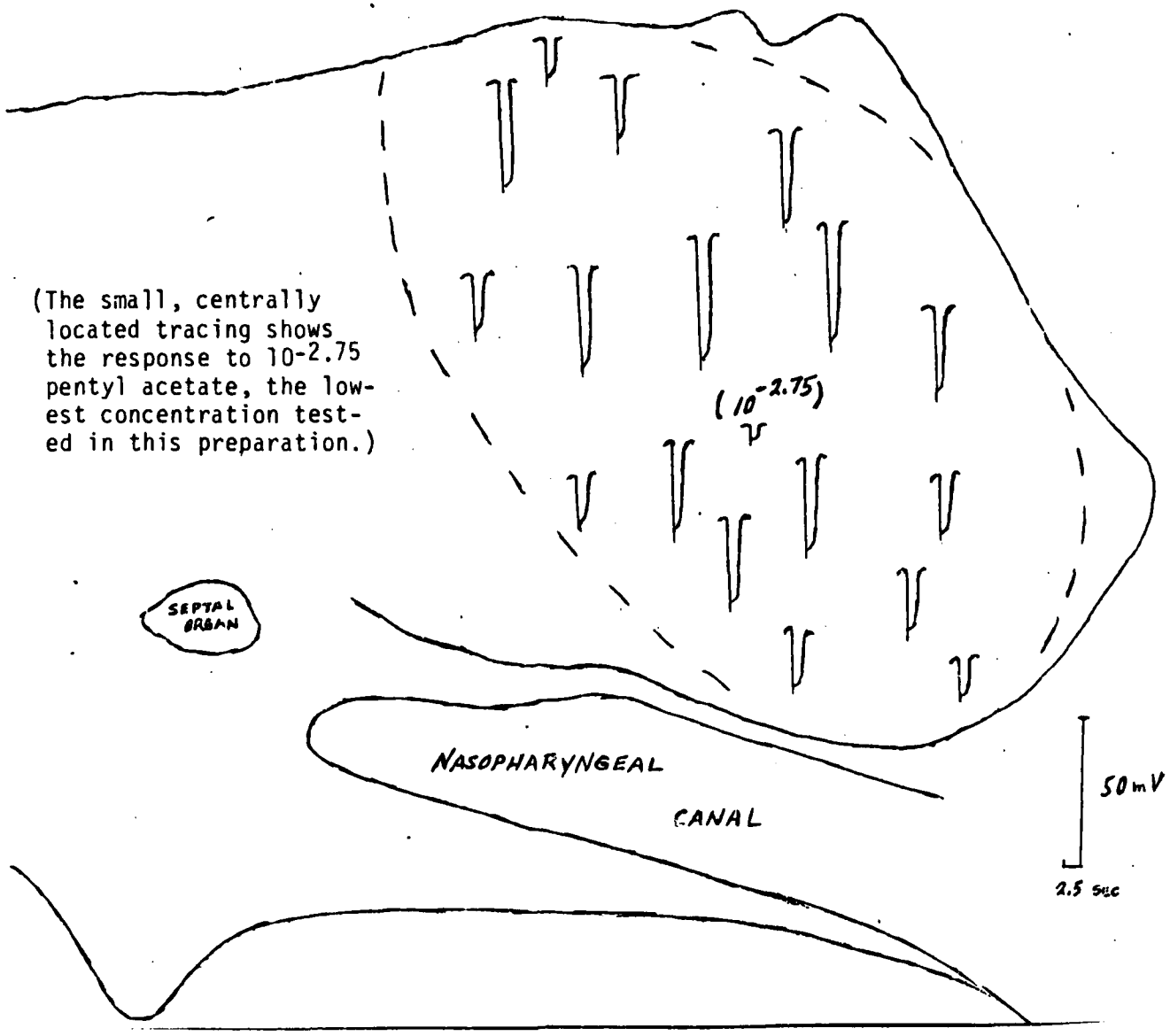


Figure 2. Schematic sagittal view of the main septal olfactory area showing superimposed responses to 10^{-1} amyl acetate at a sampling of recording sites. Responses are traced from penwriter records.

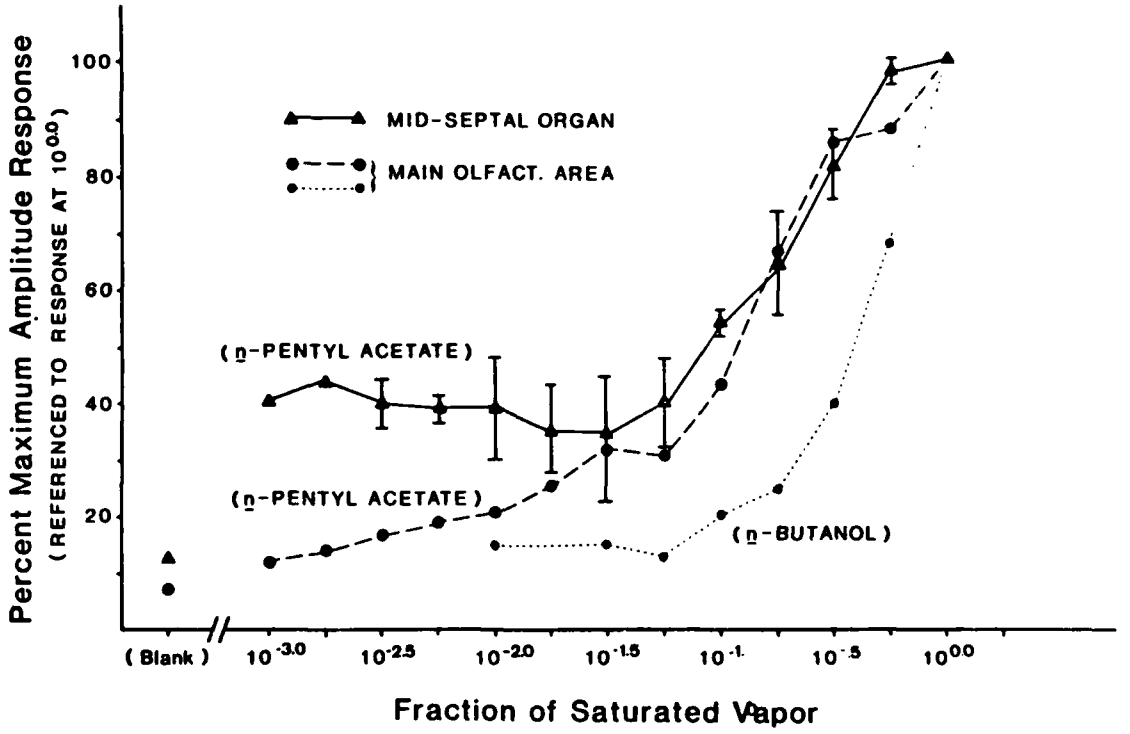


Figure 3. Electro-olfactograms from the septal organ and from the main olfactory region of the rat. The filled circles are responses to amyl acetate and butanol from the same main olfactory site in one animal, and the triangles are septal organ responses from the same preparation averaged together with septal organ responses from one other animal.

Publications Resulting from this Work

1. Doty, R.L. Odor guided-behavior. Experientia, in preparation, 1984. (An invited review in which some of the psychophysical data will be presented from this project)

2. Marshall, D.A. & Maruniak, J. The septal organ: What the "Advanced Sentinal" sees. Submitted to Science, 1984. (Presentation of physiological data from the septal organ)

3. Silver, W.L., Mason, J.R., Marshall, D.A. & Maruniak, J.A. Rat trigeminal, olfactory, and taste responses after capsaicin desensitization. Submitted to Brain Research, 1984. (Research that was in large part stimulated directly by the current grant and utilized equipment designed for the present grant)

BIBLIOGRAPHY

Kauer, J.S. & Moulton, D.G. Response patterns of olfactory bulb neurons using odor stimulation of small nasal areas in the salamander. J. Physiol. (London), 1974, 243, 717-737.

Kubie, J.S. & Moulton, D.G. Regional patterning of response to odors in the salamander olfactory mucosa. Neuroscience Abstracts, 1979, 5, 129.

MacKay-Sim, A., Shaman, P. & Moulton, D.G. Topographic coding of odorant quality: Odorant specific patterns of epithelial responsivity in the salamander. J. Neurophysiol. 1982, 48, 584-596.

Moulton, D.G., & Marshall, D.A. The performance of dogs in detecting alpha-ionone in the vapor phase. J. Comp. Physiol., 1976, 110, 287-306.

Neuhaus, W. Die veranderung der riechscharfe des hundes durch orale duftstoffgaben. Z. Vergleich. Physiol., 1958, 41, 221-241.

Ottoson, D. The electro-olfactogram. In L. Beidler (Ed.), Handbook of Sensory Physiology, Vol. IV, Chemical Senses, Part 1, Olfaction. New York: Springer-Verlag, 1971, pp. 95-131.

ACKNOWLEDGMENTS

We are particularly grateful to Mr. Steven Applebaum and Mr. Michael Moran for their technical assistance during the project period. Without their keen attention to detail and painstaking concern about accuracy, this work would not have been possible.

APPENDIX A

PROGRESS REPORT

(TWENTY COPIES REQUIRED)

1. ARO PROPOSAL NUMBER: DAAG29-81-K-0128

2. PERIOD COVERED BY REPORT: August 1, 1981 to December 31, 1981

3. TITLE OF PROPOSAL: INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION

4. CONTRACT OR GRANT NUMBER: DAAG29-81-K-0128

5. NAME OF INSTITUTION: University of Pennsylvania

6. AUTHOR(S) OF REPORT: Richard L. Doty, Ph.D.

7. LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

None

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

Richard L. Doty, Ph.D., Principal Investigator, 10% effort
David A. Marshall, Ph.D., Co-Principal Investigator, 50% effort
Joel A. Maruniak, Ph.D., Research Associate, 50% effort

No degrees were awarded during this reporting period.

BRIEF OUTLINE OF RESEARCH FINDINGS

We have made significant progress during this initial project period in both the psychophysical and electrophysiological phases of our work. Earlier data from our laborartory suggested that the olfactory detection performances of dogs increased following ingestion of test odorants. This performance enhancement was relatively specific to the odorant under evaluation, in that ingestion of a chemically-unrelated odorant had no influence on the olfactory detection of a particular target odorant.

During this initial period, we extended the aforementioned test series by examining the odor detection performance of a dog for the test compound pentyl acetate after ingestion of the following compounds: pentyl acetate, alpha-ionone, and d-limonene. The mean baseline detection scores (established over a number of days) did not increase following the ingestion of either alpha-ionone or d-limonene. However, a peak in sensitivity occurred 7-10 days after the ingestion of the pentyl acetate, supporting our previous findings.

We have now initiated the electrophysiological pilot studies to establish if mapping of the rat olfactory epithelium for odorant sensitive regions is feasible. To familiarize ourselves with recording electroolfactograms in rats, we have obtained recordings of the EOG from the cribriform plate (through which the olfactory receptor nerves pass from the epithelium to the bulb). Following this pilot work, we have conducted studies to establish the best way of exposing the epithelium directly to allow for recording from it. In a recent series of preparations, the nasal and some of the maxillary bones overlying the nasal cavity of 6 adult male rats were removed under urethane anesthesia. Because of the traumatic aspects of this procedure (thermal cautery and bone wax were liberally used to control bleeding, and Ringer's solution was used to clear debris and clotting), these pioneering attempts failed in five of the six cases. However, in the sixth rat, we were able to obtain recordings from the dorsal part of the epithelium. This was made possible by driving an electrode through the roof of the lining of the nasal cavity in small increments until EOGs appeared. EOGs to both amyl acetate and butanol were obtained using this procedure. These data, to the best of our knowledge, represent the first EOG directly recorded from the epithelium of the adult rat. Hopefully, in the next six months, our procedure will be perfected so that extensive mapping of the adult rat epithelium can be accomplished. If these attempts fail, we will continue our sensitization work using non-mammalian preparations where epithelial mapping is less problematical (e.g., salamanders).

To date, progress in our psychophysical studies with dogs has been hampered by the small number of subjects that can be tested in our single test chamber and the relatively long time periods required to establish stable performance levels. For this reason and because of our pioneering electrophysiological work on rats, we have now set up several automated rat operant olfactory chambers and have trained a group of rats in a forced-choice testing paradigm directly comparable to that we use with dogs. After screening a number of rats, 12 relatively good performers have been selected and stable baseline performances have been established for them using the odorant cynamic aldehyde. We are currently initiating a test series to establish the influences of single dose effects of this odorant on their detection performance. Following the determination of this information, we will evaluate dose level effects and repeated administrations of the ingested odorant at various time intervals.

PROGRESS REPORT

(TWENTY COPIES REQUIRED)

1. ARO PROPOSAL NUMBER: DAAG29-81-K-0128

2. PERIOD COVERED BY REPORT: January 1, 1982 to July 1, 1982

3. TITLE OF PROPOSAL: INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION

4. CONTRACT OR GRANT NUMBER: DAAG29-81-K-0128

5. NAME OF INSTITUTION: University of Pennsylvania

6. AUTHOR(S) OF REPORT: Richard L. Doty, Ph.D., David Marshall, Ph.D., Joel A. Maruniak, Ph.D.

7. LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

None

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

Richard L. Doty, Ph.D., Principal Investigator, 10% effort
David A. Marshall, Ph.D., Co-Principal Investigator, 50% effort
Joel A. Maruniak, Ph.D., Research Associate, 50% effort

No degrees were awarded during this reporting period.

Dr. R. L. Doty 18609-L
Dr. David A. Marshall
University of Pennsylvania
Smell and Taste Clinical Research
Center
Philadelphia, PA 10104

BRIEF OUTLINE OF RESEARCH FINDINGS

The initial data from both our psychophysical and electrophysiological studies were analyzed during this period and are presented in this report. These are summarized below.

Psychophysical Work

During this funding period we completed the test series, mentioned in the last report, on the influences of intraperitoneal injections of cynamic aldehyde on odor detection performance. This work represents the largest study ever undertaken on this topic, as well as the first to introduce double-blind controls into the procedure. As indicated in the last report, we have put considerable emphasis on rat psychophysics, since the electrophysiological work we are doing presumes that the sensitization phenomenon occurs in this species. Surprisingly, however, few data are available to document this presumption and ours is the first to carefully examine this proposition.

Odor detection performance was evaluated in a 3-choice test apparatus encorporating air-dilution concentrations of the target stimulus cynamic aldehyde. Performance was repeatedly assessed before and after injection of either (a) 0.075 ml cynamic aldehyde or (b) 0.075 ml saline solution. The preinjection data were used to roughly assign animals into the two treatment conditions on the basis of similar performance levels (six experimental and six controls) during the latter days of the preinjection testing. The twelve rats were tested daily or bidaily over a test period of six weeks. Because of calibration problems with the olfactometer during the early days of the testing period, only the data from ten days before the injections and ten days after the injections were subjected to detailed statistical analysis. Following an arc sin transformation of the % correct performance means, the data were subjected to a three factor analysis of variance with repeated measures on two factors (i.e., a 2 (cynamic aldehyde, saline) by 2 (preinjection period, postinjection period) by 5 (2 day trial blocks during both periods = 10 total days). The results of this statistical analysis are presented in Table 1.

Table 1. Summary of Analysis of Variance Performed on Odor Detection Data

Source	Sum of Squares	df	Mean Square	F value	P value
Total	11.296	119	----	----	
Between Ss	6.230	11	----	----	
Groups	0.928	1	0.928	1.750	ns
Error	5.302	10	0.530	----	
Within Ss	5.066	108	----	----	
Injection	0.213	1	0.213	1.507	ns
Trials	0.160	4	0.040	1.542	ns
Group x Inj	0.255	1	0.255	1.797	ns
Group x Trials	0.062	4	0.016	0.596	ns
Injection x Trials	0.456	4	0.114	3.674	.025
Gp x Inj x Trials	0.221	4	0.055	1.782	ns
Error 1	1.416	10	0.142	----	----
Error 2	1.041	40	0.026	----	----
Error 3	1.241	40	0.031	----	----

The major finding from this work is the unexpected one of no significant influence of the injection of cynamic aldehyde on the odor detection performances of the rats. However, as indicated by the injection by trials interaction, the animals did perform significantly different across the 2-day blocks of trials before the injections than after the injections, regardless of the nature of the injections. This effect is demonstrated in Figure 1.

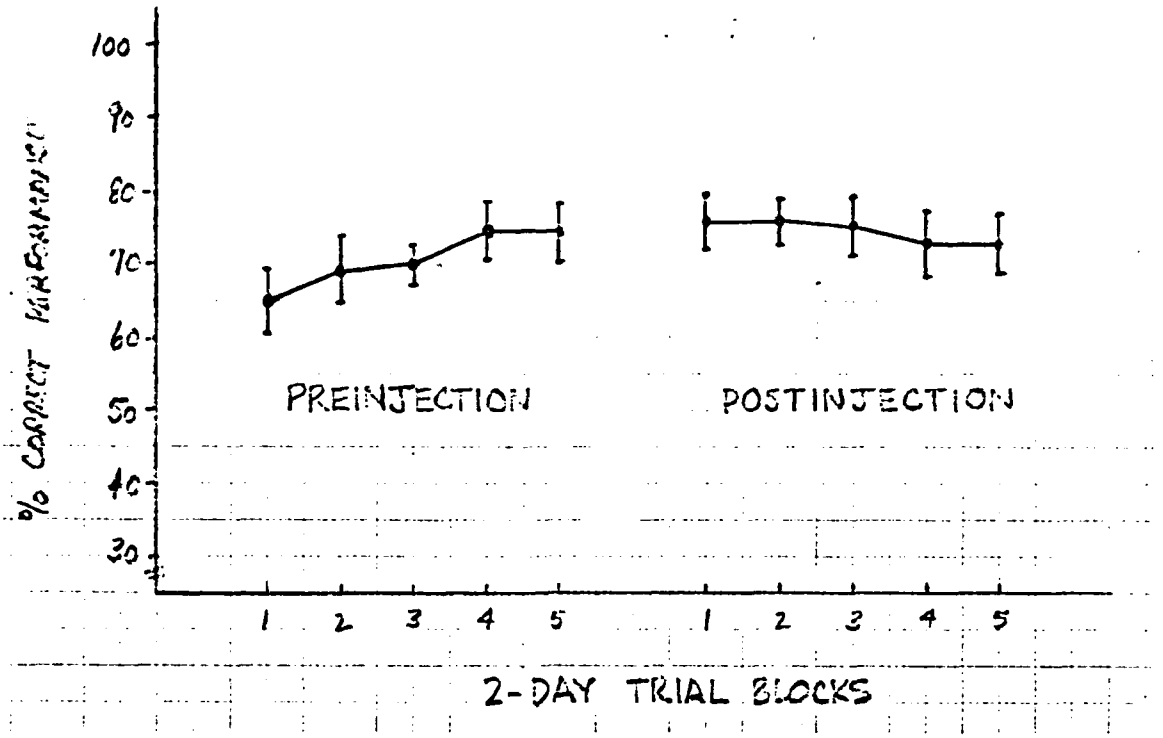
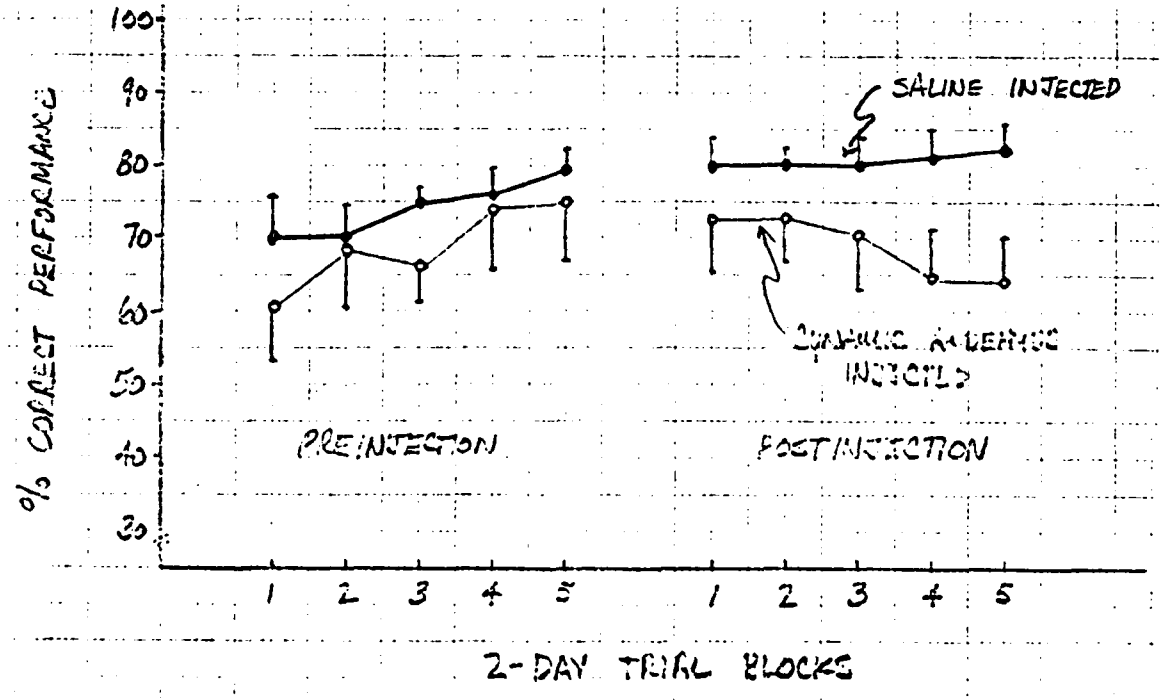


Figure 1. Mean (± 1 SEM) correct performance of 12 rats across 2-day trial blocks (50 trials per block) before and after injection of either cynamic aldehyde (6 rats) or physiological saline solution (6 rats).

Although not statistically significant, it is interesting to note that the means of the controls did not decrease following injection of the saline, whereas the means of the animals injected with cynamic aldehyde tended to do so -- a result opposite from what we had expected to occur (Figure 2). More data are needed, however, to clarify whether this is a meaningful phenomenon, which would be reflected by a significant 3-way interaction between trials, injection period, and experimental group. Clearly, in the present sample, considerable variance was present in the cynamic aldehyde injected group.



.Figure 2. Mean (+ 1 SEM) correct performance of 12 rats across 2-day trial blocks as a function of experimental or control group and pre- and postinjection period.

The lack of an enhancement of performance due to intraperitoneal injections of cynamic aldehyde may be due to a number of factors, including the following: (a) the sensitization phenomenon does not occur in rats; (b) the sensitization phenomenon does not occur for cynamic aldehyde; (c) the sensitization phenomenon does not occur for the dosage level of cynamic aldehyde used in this study; and (d) the effects of sensitization may have been suppressed by other factors, such as adaptation of the receptors through the blood-borne route.

We plan, during the next funding period, to repeat this double-blind experiment using a different compound, alpha-ionone, for which we have reason to believe produces sensitization in dogs. Clearly, it is important to demonstrate the sensitization effect in rats before expecting to see physiological changes in mucosal regions due to such injections.

Electrophysiological Work

After considerable difficulty, we have now developed a preparation that allows for at least rudimentary electrophysiological recording from the rat olfactory epithelium. The complex geometry and rich vascular supply of the nasal chamber presents major difficulties in exposing sufficiently large areas of the nasal cavity for placements of the punctate odor stimulator and recording electrode. By painstaking step by step operations designed to provide time for drainage and cessation of seepage of blood and interstitial fluids (which cannot be allowed to contact the recording area for optimal recording), we have been able to record from the rat olfactory epithelium. Our approach is pictured in Figure 3. This figure shows a lateral view of the posterior-dorsal septal receptor sheet through an opening produced by removal of the lateral ethmoidal turbinals. A recording electrode is shown with its tip positioned above the mucosa. Figure 4 shows the punctate odor stimulator positioned over a photograph of the septal mucosa onto which has been superimposed a circular area representing the approximate spread of a single odor stimulus. The stimulator consistsof an outer pipette, connected to a suction source, and an inner pipette about one half the diameter of the outer one through which odorant is driven by a syringe pump.

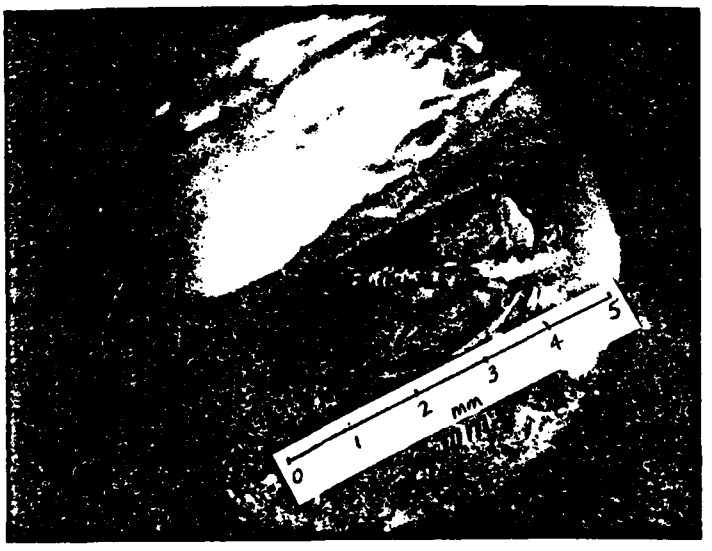


Figure 3. Opening into nasal chamber for electrophysiological recording. See text for details.

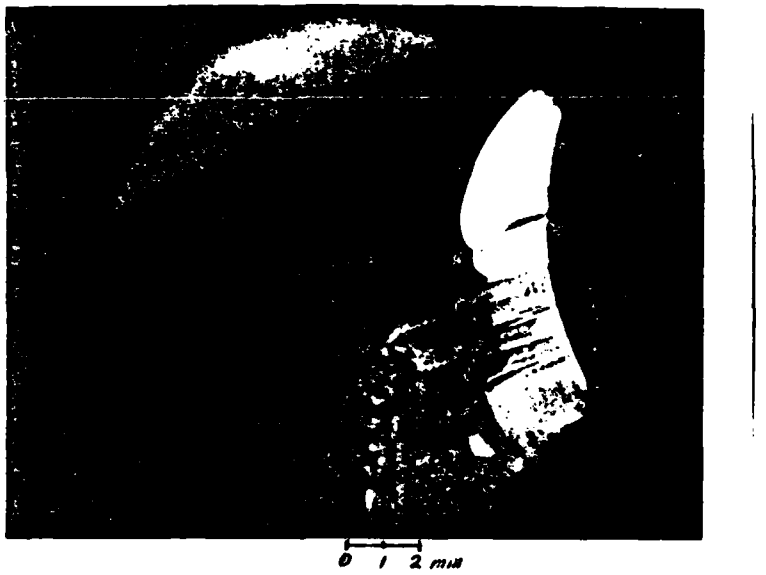


Figure 4. Picture of punctate odor stimulator positioned over a photograph of the septal mucosa. Note circular designation of approximate odorant spread from delivery system.

Examples of electroolfactograms (EOGs) recorded from the olfactory epithelium are presented in Figure 5 (next page). These EOGs were obtained from two different recording sites along an ethmoturbinate. The odorants used were saturated vapor phase limonene, pentyl acetate and butanol. While the use of such high concentrations of odorants must be viewed as a rough probe of epithelial sensitivity, the recordings at the two sites illustrated reveal differences in epithelial responses to the three odorants. The most remarkable difference is the different polarity of response observed for pentyl acetate at the two recording sites.

Although, obviously, more data need to be collected, these preliminary recordings suggest that the patterns of differential sensitivity to odorants reported for the salamander olfactory mucosa may also hold for the rat olfactory mucosa. We plan, during the next funding period, to explore this possibility in more detail and with a larger number of preparations.

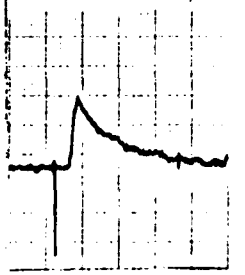
In summary, we have now developed our techniques to the point where meaningful recordings can be made. Nonetheless, collection of data is very slow, given the difficulties in obtaining appropriate recording preparations.

FIGURE 5

SITE 1
(ANTERIOR)

LIMONENE

PENTYL ACETATE

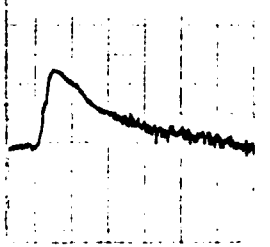


BUTANOL

SITE 2
(POSTERIOR)

LIMONENE

PENTYL ACETATE



BUTANOL

PROGRESS REPORT

(TWENTY COPIES REQUIRED)

1. ARO PROPOSAL NUMBER: DAAG29-81-K-0128

2. PERIOD COVERED BY REPORT: January 1, 1983 through June 30, 1983

3. TITLE OF PROPOSAL: INVESTIGATION OF MECHANISMS UNDERLYING ODOR RECOGNITION

4. CONTRACT OR GRANT NUMBER: DAAG29-81-K-0128

5. NAME OF INSTITUTION: University of Pennsylvania

6. AUTHOR(S) OF REPORT: Richard L. Doty, Ph.D., David Marshall, Ph.D.

7. LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS PERIOD, INCLUDING JOURNAL REFERENCES:

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

Richard L. Doty, Ph.D., Principal Investigator, 10% effort
David A. Marhsall, Ph.D., Co-Investigator, 50% effort
Michael Moran, B.A., 100% effort (from March to present)
Steven Applebaum, B.A., 100% effort (from March to present)

No degrees were reported during this reporting period.

Dr. R. L. Doty 18609-L
Dr. David A. Marshall
University of Pennsylvania
Smell and Taste Clinical Research Center
Philadelphia, PA 10104

BRIEF OUTLINE OF RESEARCH FINDINGS

This report summarizes primarily our ongoing electrophysiological studies. The large-sample odor sensitization study was planned to be completed by the end of this reporting period. However, a break-down of the computer delayed progress on this project by about two months. Before the computer failure, 24 rats had been trained to criterion on a series of descending concentrations and the end-point concentration had been reached. However, some retraining will now be necessary to stabilize the rats at the appropriate concentration. We anticipate completing this project in September or October and will include its results in the final progress report.

During this period we applied the mammalian septal recording preparation we developed during the last funding period and, additionally, explored the septal organ -- a patch of receptive tissue located on the septum proper. This system have never been recorded from before, and we are quite proud to have demonstrated it to be chemically sensitive to a broad array of chemicals.

The details of this recording work follows on the subsequent pages.

Response Mapping of the Septal Olfactory Organ

Olfactory receptors occupy the mucosa covering a well delineated dorsal and posterior region of the nasal septum in most mammals. A prominant identifying feature of this region (and of olfactory epithelium in general) is the presence of secretory Bowman's glands. Except for a small grouping of Bowman's glands and cells closely resembling olfactory receptors — a separate island isolated from the dorsal olfactory region — the remainder of the septum is covered with respiratory epithelium. This 'island', surrounded by respiratory tissue, lies near the base of the septal wall, a few mm anterior to the opening of the nasopharyngeal duct, and in this position is directly exposed to the main respiratory airstream. It was first described by Broman (1921) in newborn mice, and from its more recent description in the opossum by Rudolfo-Masera (1943) is sometimes called the "organ of Masera", or more generally, the septal olfactory organ (for recent reviews, see Bojsen-Møller, 1975; Katz and Merzel, 1977).

Its function has not been investigated previously, although an obvious suggestion of what this might be was first noted by Rudolfo-Masera and later acknowledged and elaborated on by others (e.g., Tucker, 1971; Bojsen-Møller, 1975). With its histological likeness to known olfactory receptor tissues, the course of its innervating nerve bundles to the caudal olfactory bulbs (Bojsen-Møller, 1975), and its position placing it directly in the pathway of all respiratory flows, it clearly could serve in a primary role to alert the animal to the presence of odors — prior to, and possibly as the basis for, initiation of active odor sampling. Other possibilities might include a role in providing 'reference' signals, due to its favored position with respect to airflow, or special sensitivity to certain biologically relevant odorants. It is clear, however, from the odor responses we have recorded in the present series of functional mapping experiments, that the sensitivity of septal organ receptors to a standard test odorant, n-pentyl acetate, is no less than (and possibly may exceed) that of receptors located in anterior portions of the main septal olfactory mucosa.

Methods in brief

For these functional mapping preparations we have used mature male and female Long-Evans strain rats (300-400 g), anesthetized with urethane (1.5 g/kg), tracheotomised, and maintained at 37° by a heated operating platform and insulating blanket. The septum is exposed by excision of the anterior portion of the zygoma, enucleation, removal and retraction of underlying tissues, and finally, a careful removal of lateral skull and the intervening turbinate structures. EOG (electro-olfactogram) potentials are recorded using an 80–100μ agar/saline filled pipette (0.7 % NaCl) mounted mounted jointly with a pair of punctate odor stimulator pipettes (100 – 150μ tips) on a holder that closely positions all three tips in a triangular pattern ($\approx$ .5 mm on a side). The stimulators receive metered flows at known concentrations from an arrangement of syringe pumps and over-the-surface vapor saturators. The stimulator (Fig. 1, D.) permits the delivery of precisely-timed odor (or blank air) pulses to a restricted circular area with the recording electrode tip at its approximate center. The odorants for which we have recorded septal organ responses (and have used in recording from the main septal olfactory area) include, in addition to n-pentyl acetate, d-limonene, pinene, and n-butanol.

Our procedure for mapping the septal organ region (as for the olfactory area) starts with an 'instant' photograph (a Polaroid print) taken through the operation microscope at a magnification of 10 — 15x. Branches of an anterior capillary arborization wind densely throughout the area of the septal organ; their unique shapes

and turnings provide useful and easily distinguishable landmarks. These features permit recording sites not only to be plotted accurately on the photograph, but to be re-recorded from with an estimated position error of about one electrode tip diameter (< 100μ). Test stimuli are delivered to a recording site in 2.5 sec pulses, at a flow rate of 1.4 ml/min, and are spaced in time at intervals of at least 1 min. EOG potentials are fed through a unity-gain electrometer, and are amplified and directly recorded by a penwriter.

Our aims in this first series of mapping experiments were: 1) to establish the response boundary perimeter using a single test odorant at known and fixed concentration and flow-rate; 2) to determine whether responses to this stimulus vary systematically at different recording sites; 3) to measure the dynamic response range at the most sensitive sites using a graduated series of concentrations; and 4), to establish the degree of sensitivity at sites responsive to odor to airflow alone, using as a control, matching blank flows.

The main findings to date in summary

The centralmost sites of responsiveness, relative to a response boundary perimeter, appear to be the most sensitive both to odor and blank airflows. Toward the periphery, both odor and flow responses decline; flow responses often decline first and may be absent at the more distal sites where small odor responses reliably occur. We have used pentyl acetate as a standard test odorant for most of these initial experiments; one reason, because of the widespread responsiveness of the main septal olfactory mucosa to this odorant, is the availability of response data for comparisons. The responsiveness of septal organ sites to butanol and limonene appears to be slightly less than to pentyl acetate; we plan to examine responsiveness to these and other odorants systematically in the continuing series of experiments.

Figure 1. shows lateral views of the exposed septal epithelium at three increasing magnifications. In A., the inset dot mark represents relative response magnitude at a dorsal anterior site for comparison with the septal organ responses mapped in C. In B., the recording electrode is positioned at a site in the septal organ area (the pictured scale is in mm). The device pictured in D. is similar to one that we use: O_1 and O_2 deliver stimuli (odor or blank air); V_1 and V_2 are vacuum line connections, normally drawing stimulus flow to exhaust, and switched off by timed activation of solenoid valves for stimulus delivery; E is the recording electrode, H the holder, and M the mucosal surface.

Figure 2A. shows penwriter tracings of responses from a different preparation to the indicated fractions of saturated pentyl acetate vapor recorded at the site indicated in B. In C. (taken from Katz and Merzel, 1977), an overview of the rat nasal septum shows the septal organ (OM) as determined by histological criteria (primarily the presence of Bowman's glands).

Figure 3. shows, for the same preparation as in Fig. 2, a map of responses to $10^{-1.0}$ pentyl acetate (B.), including tracings from the penwriter records for each site. The cross-hatched portions of the response tracings indicate responses at there sites to blank air.

According to our initial aims, it can be said from the results for five preparations (in each of which from 10 to 23 sites were mapped) that: 1) the response boundary perimeters, from one animal to the next, are reasonably consistent and are in agreement with the histologically defined regions described by Bojsen-Møller (1975), and by Katz and Merzel (1977); 2) the responsiveness of septal organ sites clearly differs, increasing to a maximum toward the center of the area – implying, possibly, an increasing density of receptors; 3) the range of concentrations to which the most sensitive (central) sites respond appears to extend well below the lowest concentration of pentyl acetate that we tested, which was $10^{-2.5}$; and 4),

the sensitivity to blank airflows partially paralells that to odor. In one very recent preparation, however, the more peripheral sites, responding about one-half maximally to odor, showed little or no response to blank airflows. If in fact responses to blank air represent artifacts, they do appear to correlate with odor sensitivity, and none occur at recording sites that are well beyond the septal organ boundaries in respiratory epithelium.

References

Bojsen-Møller, F. Demonstration of terminalis, olfactory, trigeminal and perivascular nerves in the rat nasal septum. J. comp. Neurol. 1975, 159, 245-256.

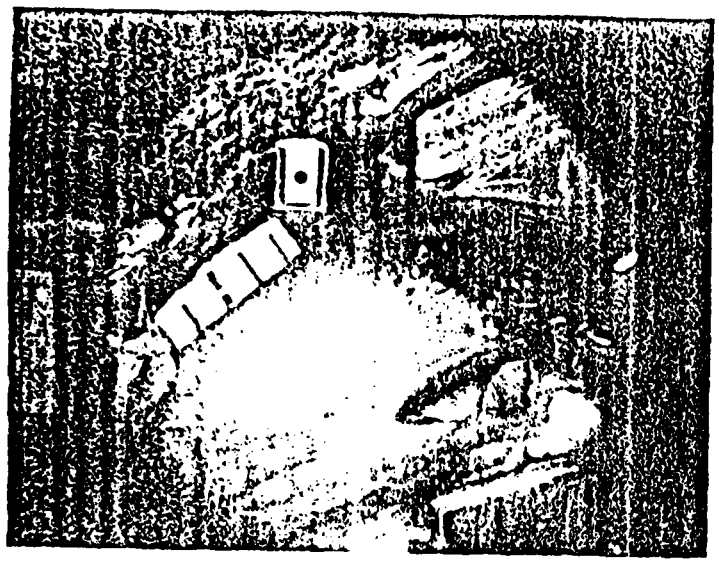
Broman, I. Über die Entwickelung der konstanten grössern Nasenhöhlendrusen der Nagetier. Z. Anat. EntwGesch. 1921, 60, 439-586.

Katz, S. and J. Merzel. Distribution of epithelia and glands of the nasal septum mucosa in the rat. Acta anat. 1977, 99, 58-66.

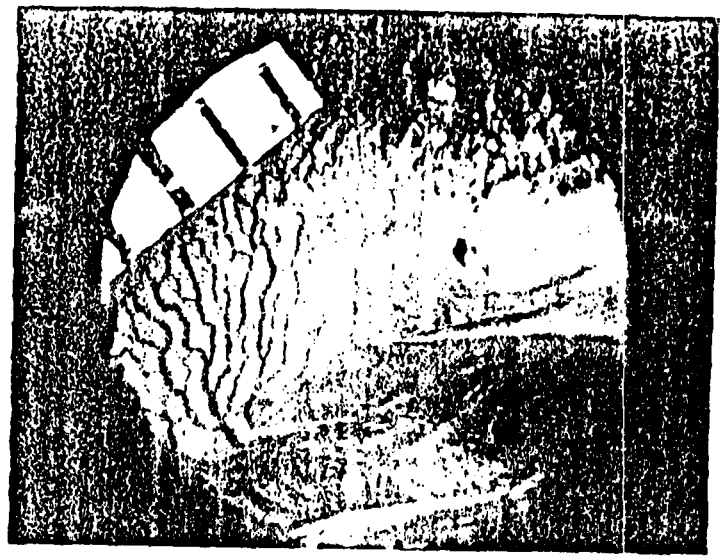
Rudolfo-Masera, T. Su l'esistenza di un particolare organo olfattivo nel setto nasale della cavia e di altri roditore. Arch. Ital. Anat. Embriol. 1943, 48, 157-212.

Tucker, D. Nonolfactory responses from the nasal cavity: Jacobson's organ and the trigeminal system. In L. Beidler (Ed.), Handbook of Sensory Physiology, IV. New York: Springer-Verlag, 1971.

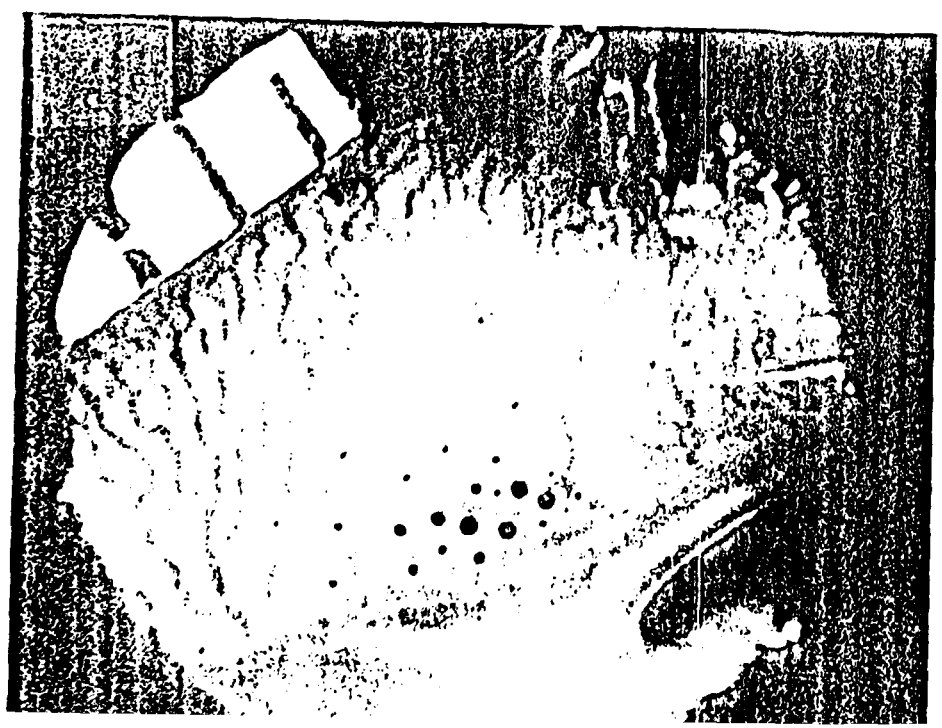
A.



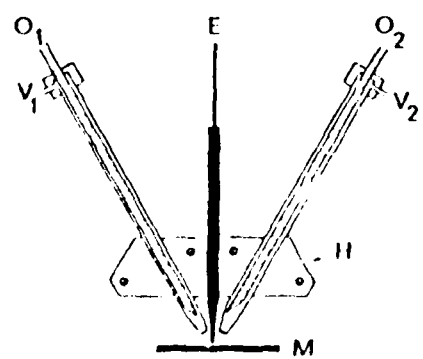
B.



C.



D.



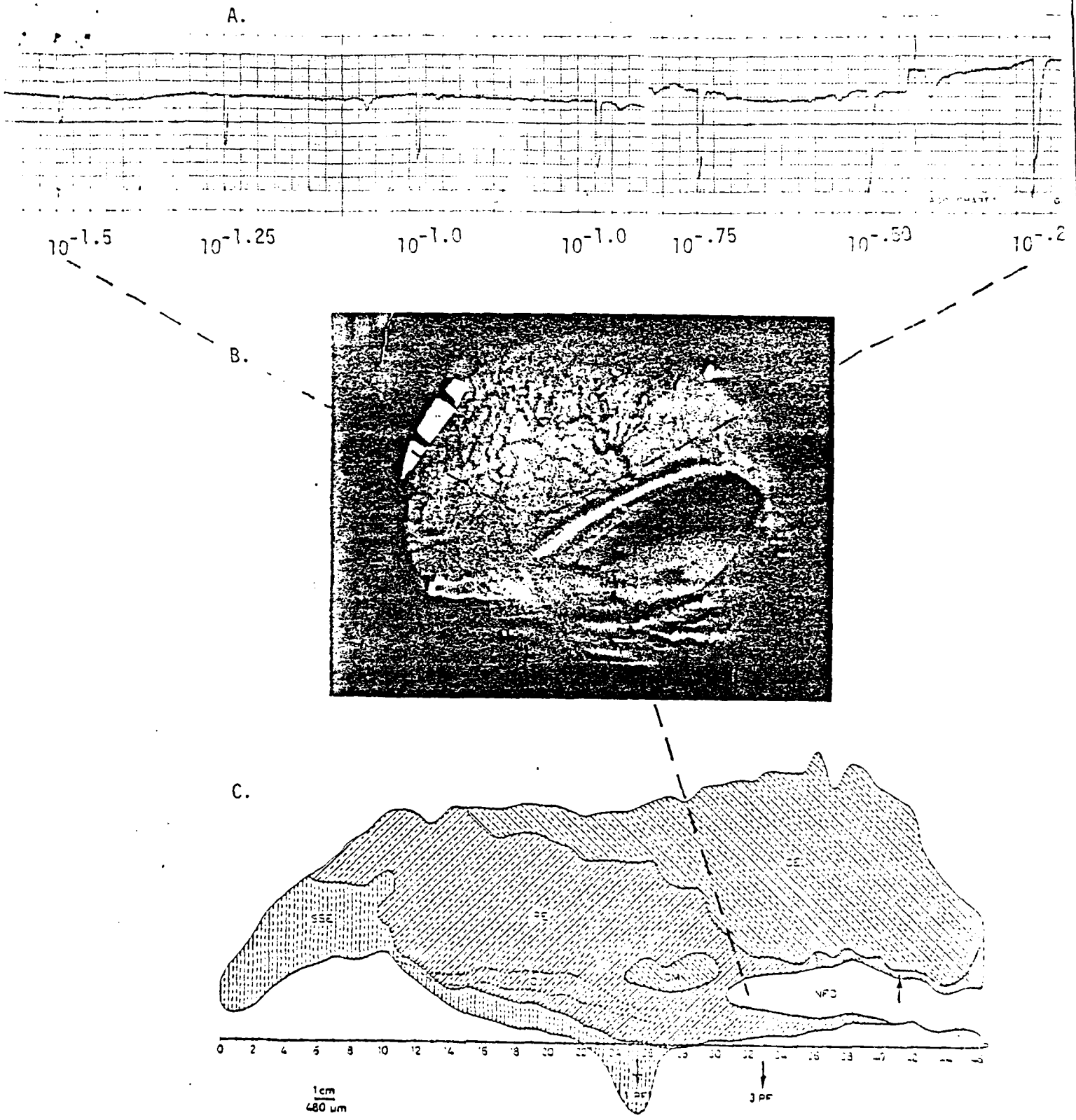
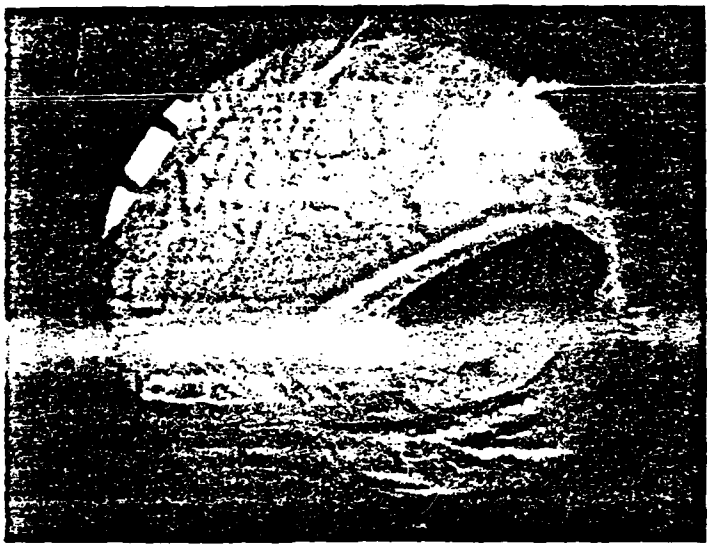


Fig.2. Map of the septal mucosa showing the limits of the three different epithelia: stratified squamous (SSE), respiratory (RE) and olfactory (OE). The organ of Jacobson (OJ) is not part of the mucosa, since it is located inside an osseous cavity of the septum. The organ of Masera (OM) is lined with olfactory epithelium. The narrow indicates the area of the nasopharyngeal duct (NFD) separated from the septum by an osseous wall. 1PF = First palatine fold; 3PF = third palatine fold.

(Fig. 2., "C." above, is from Katz and Merzel, 1977)

Figure 2. Location maps of the septal organ (of Masera) and responses to n-pentyl acetate concentrations at one recording site. (See text,

A.



B.

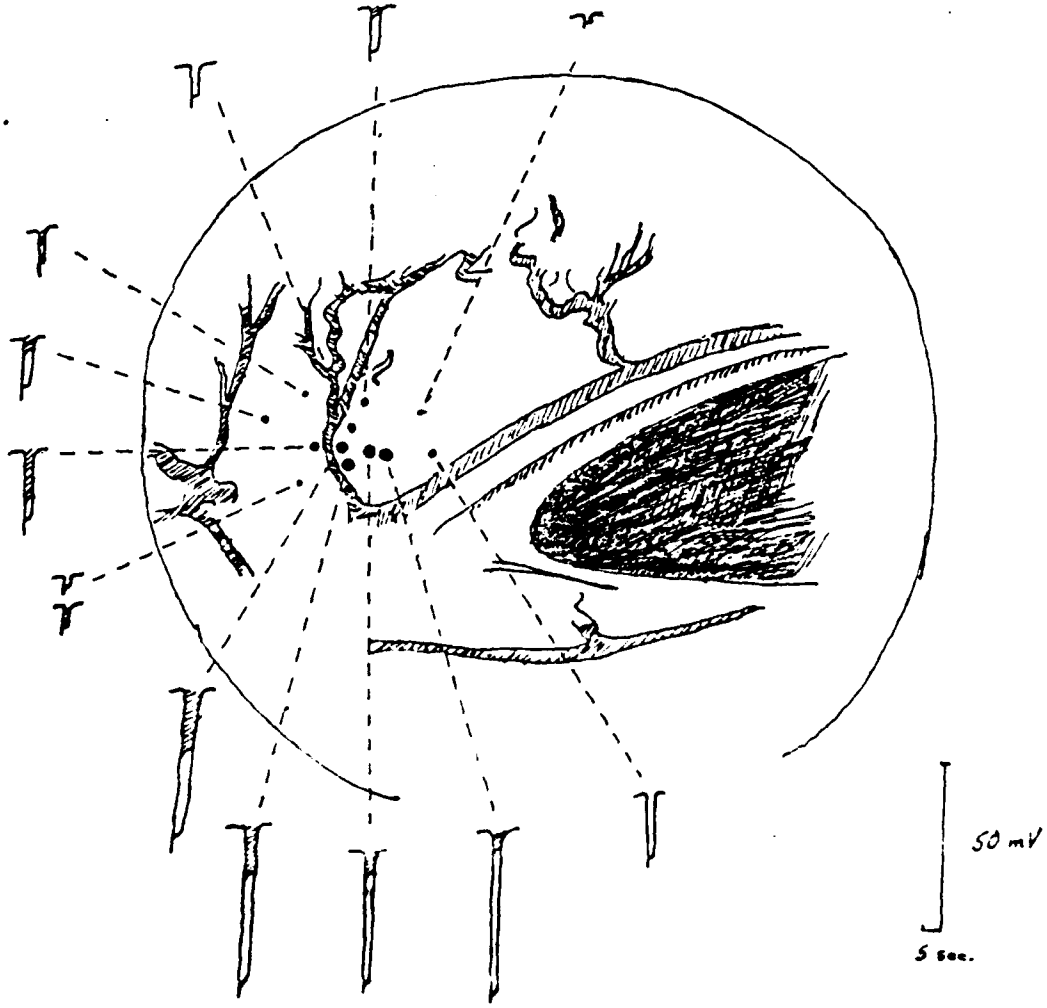


Figure 3. Septal organ mapping of responses to 10^{-1} n-pentyl acetate and a matched blank-air flow. (See text)

Response Characteristics of Rat Olfactory Epithelium

The amphibian olfactory epithelium — that of the tiger salamander, in particular — displays clear spatially differentiated sensitivities to a variety of odorants. Anterior regions give larger responses than do posterior regions to "anterior stimulants" such as butanol, trimethylamine, and cyclopentanone. Examples of "posterior stimulants" are limonene and camphor, whereas pentyl acetate, a "uniform stimulant", produces comparable responses from both areas (see Moulton, 1981).

Spatial coding of odor quality, as a general concept, was first proposed by Adrian (1950); evidence that this could be a functional principle in the rabbit was seen and discussed by Moulton (1965); a recent report of recordings in the rat from primary olfactory afferents, with their approximate topographic origins preserved in passage through the cribiform plate, confirms the existence of differing odor sensitivities among regions (Thomesen and Døving, 1979). Prior to the present studies, however, successful in vivo recording of responses directly from the mammalian olfactory epithelium has not been reported. The reasons for this appear to have been primarily technical, as we experienced many months of unsuccessful attempts before arriving at effective surgical and preparation maintainence procedures.

Our recording methods are essentially as described in the preceeding section (Septal Organ Mapping), the only major difference being in the means of locating recording sites on the mucosa and transfering these to a map. Unlike the septal organ region, with distinct capillary convolutions as landmarks, capillaries in this area tend to angle across in parallel, are less distinct, and as landmarks are generally unsatisfactory. We thus use X-Y coordinates read from drive axis scales of the micromanipulator on which the electrode/stimulator holder is mounted.

Findings to date

The area of the olfactory epithelium extends far anterior, in a narrow dorsal band, as well as ventral-posterior to near the base of the cribiform plate (see Fig. 2 of the preceeding section). The recordings we have made to date include neither the most anterior nor farthest ventral-posterior regions; in the continuing studies, these are to be explored. Our initial aim was to record from sites well within the boundaries of the olfactory area , to establish the concentration-response characteristics at these sites, and yet to cover a reasonable portion of the anterior-posterior dimension.

Figure 1. shows data for one representative preparation in which response curves for pentyl acetate are very similar at anterior and posterior sites; this appears to be the most consistent of our findings in the several preparations for which we have comparable data. Anterior responses to butanol are generally smaller than, but are parallel to, pentyl acetate responses. (A posterior butanol response series was not recorded in this preparation.)

Responses shown in Fig. 2, for a different preparation, indicate a large difference in the responses of an anterior site to pentyl acetate and limonene; this also appears to be a consistent finding from other preparations. The responses to pentyl acetate and limonene at the posterior site in this preparation may not be accurately representative, as they were obtained last at a time when the preparation appeared to have deteriorated.

No firm conclusions concerning the extent of spatially differentiated sensitivities are warrented from the limited number of data at hand. It does appear from what we have seen, however, that pentyl acetate qualifies as a "uniform stimulant", and that anterior responses to limonene are generally small, whereas those to butanol are relatively large. This appears, so far, to be in agreement with established results for the frog and salamander.

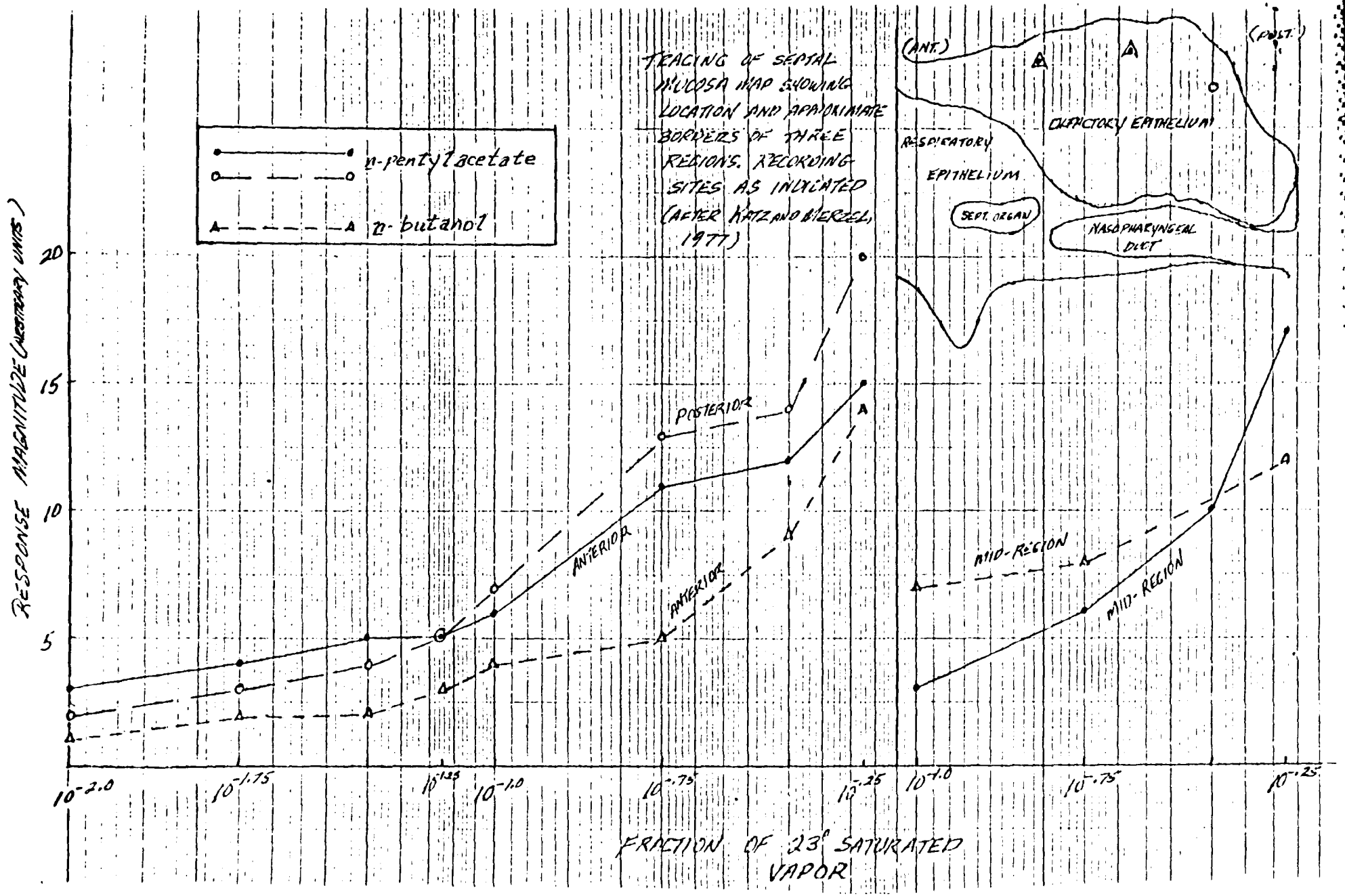


Figure 1. Concentration-response curves for three odorants recorded at anterior, middle, and posterior sites on rat septal olfactory epithelium.

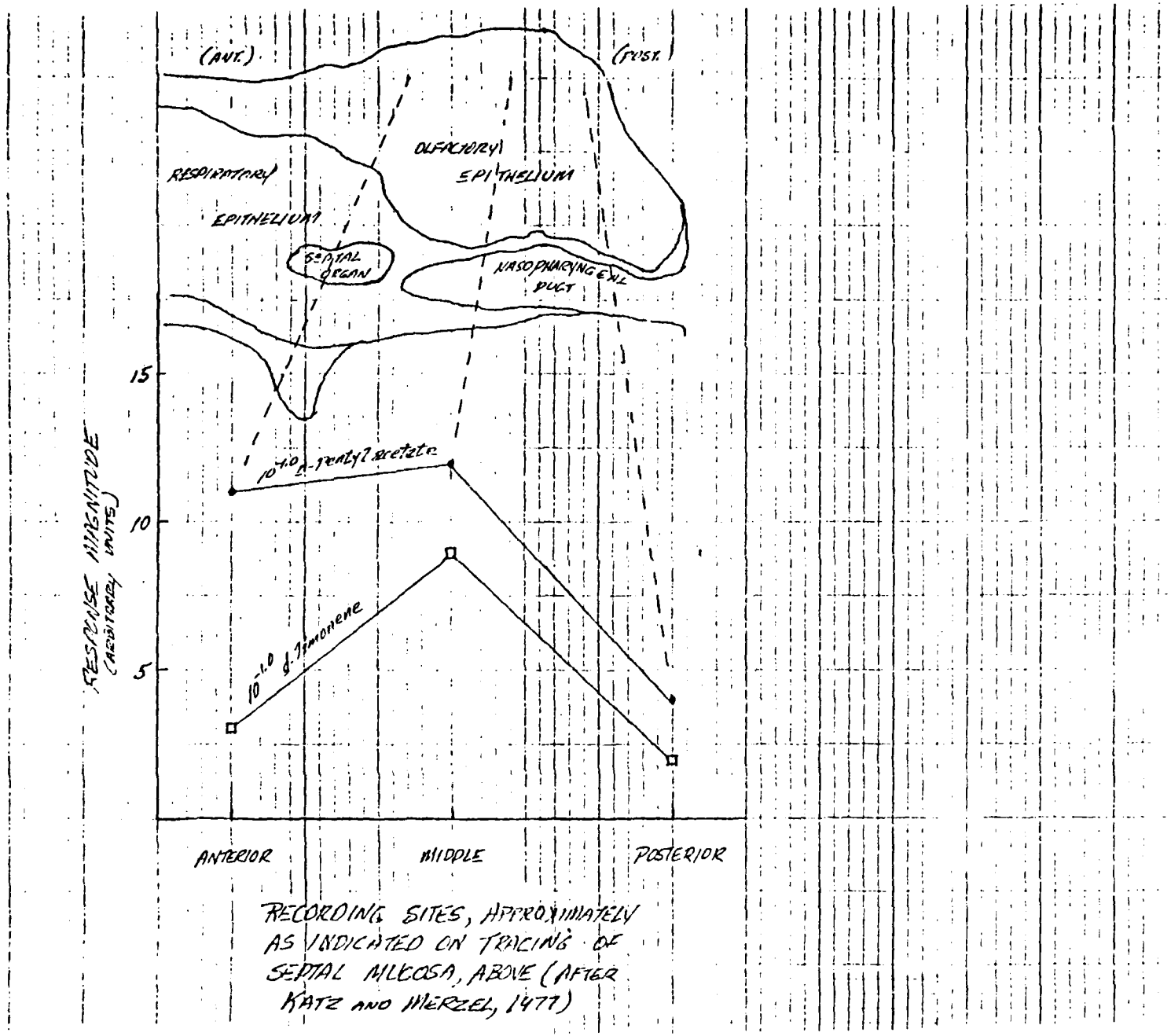


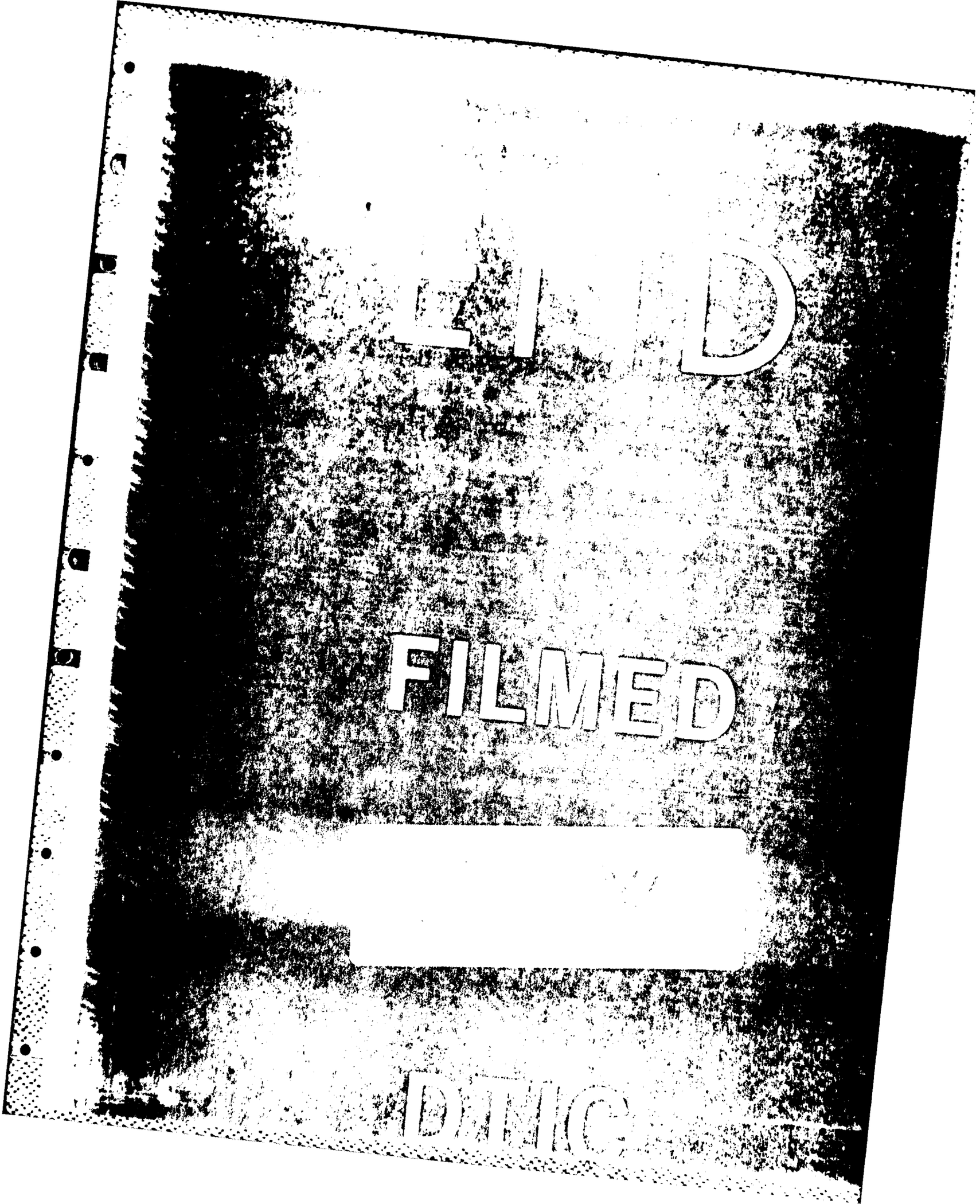
Figure 2. Responses to $10^{-1.0}$ concentrations of n-pentyl acetate and d-limonene recorded from anterior, middle, and posterior sites on rat septal olfactory epithelium.

Additional References

Adrian, E. D. Sensory discrimination with some recent evidence from the olfactory organ. Br. Med. Bull. 1950, 6, 330-333.

Moulton, D. G. Structure-activity relations in olfaction. In H. R. Moskowitz and C. B. Warren (Eds.) Odor Quality and Chemical Structure. American Chemical Society, Washington, D. C., 1981.

Thomesen, G. and K. B. Døving. Spatial distribution of the EOG in the rat: a variation with odour quality. Acta Physiol. Scand. 1979, 99, 270-280.


D
FILMED
DTIC

A.