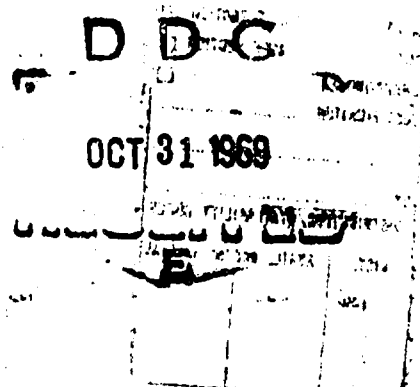


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FINAL REPORT ON A RESEARCH GRANT*

Submitted to the Air Force Office of Scientific Research

AN ANALYSIS OF ODOR INDUCED ACTIVITY IN THE OLFACTORY SYSTEM.



Prepared by Dr. David G. Moulton, Principal Investigator
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INTRODUCTION

The major activities supported by this grant were as follows:

- (A) Analysis of single unit activity in the olfactory epithelium and bulb of the rat and goldfish.
- (B) Development of telemetry techniques for recording neural impulses from freely moving animals.
- (C) Development of behavioral and olfactometric techniques to be used in conjunction with (B).

Since the progress in these areas has been largely documented by publications this report will concentrate on research that has not yet appeared.

(I) SINGLE UNIT RECORDINGS FROM THE OLFACTORY BULB OF THE GOLDFISH. (Crassius auratus).

In fish, many behavioral patterns critical for survival are triggered by compounds present in the environment in extreme dilution. These are the stimuli with the greatest biological significance for feeding, mating, avoiding predators, etc. It is a reasonable assumption that the olfactory system has evolved a sensitivity, and a means of processing this type of information in the presence of considerable background "noise". The high concentrations of stimuli used by most experimenters are in no way an approximation of the natural conditions of stimulation and may, in fact, block discovery of the underlying coding mechanisms. For example, inhibition is often reported to be a dominant feature of bulbar response. But is this degree of inhibition normally elicited by natural stimuli, acting in concentrations at which they are found in the environment?

Responses were characterised by two features:

- (1) Low level, or occasional absence, of inhibition.
- (2) Selective responsiveness of units.

Thus in over 200 units studied there was no evidence of inhibition in response to food extract - only excitation. With the other compounds tested varying degrees of inhibition were recorded but never exceeded 20%. This contrasts with the far higher levels of inhibition previously reported for fish.

Selectivity in responsiveness was seen in the large number of units which responded to only one or two of three compounds presented.

The conclusion from these findings is that if low concentrations of appropriate stimuli are used, inhibition is less prevalent in the olfactory

bulb of the goldfish than studies with higher concentrations of inappropriate stimuli might suggest.

(II) SINGLE UNIT ACTIVITY IN THE OLFACTORY EPITHELIUM AND BULB OF THE RAT

The basis of odor quality discrimination is poorly understood. Single unit analysis of odor-induced responses in the olfactory epithelium have so far failed to produce any evidence that odorants can be classified into a relatively small group of compounds or "primary odors." A study was therefore made of rat olfactory epithelial and bulbar units in an attempt to provide further information concerning the problem of odor quality discrimination. The rat is of particular interest because no recordings have so far been reported from the olfactory epithelium of any mammal, and because it is a species for which behavioral information is available. (The results of this study are presently being prepared for publication).

The main features of the rat olfactory bulb as elucidated by longitudinal section are shown in Figure 1. The zone of mitral cells - from which recordings were made - lies deep to the zone of glomeruli. The primary neurones converge on the surface of the anterior pole of the bulb.

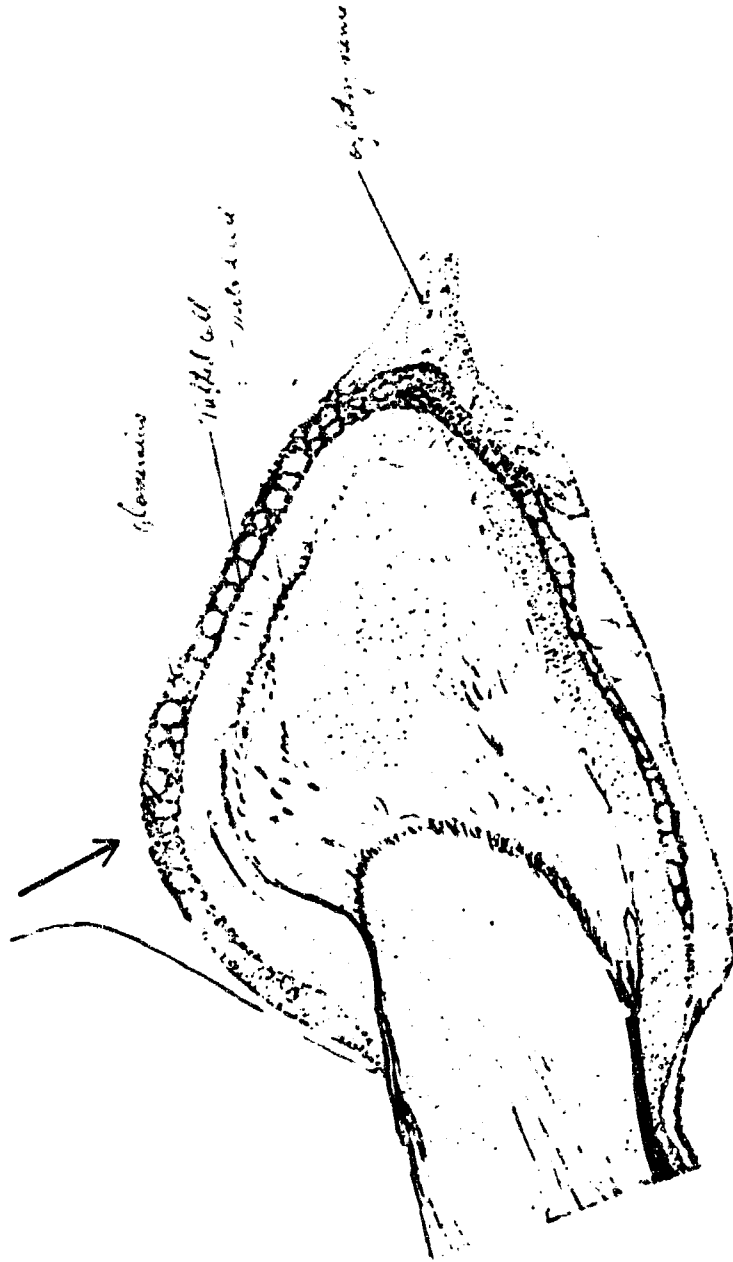
Materials and Methods

The animals used in these experiments were adult male albino rats 400-500 g. in weight. They were anesthetized with "Equised" (chloral hydrate 30%; magnesium sulfate 25%; chlorobutanol 0.25% and distilled water) administered intraperitoneally. The initial dose was 0.5cc per 400 g. body weight followed by 0.05 cc at intervals until the desired degree of anesthesia was reached. After securing the animal in a stereotaxic instrument, the skin and skull were removed sufficiently to expose the olfactory bulb and a portion of the epithelium. As a precaution against drying, the surgically exposed areas of the preparation were bathed in a mixture of 0.9% NaCl solution and mineral oil, maintained at 37°C. After the operation only preparations showing a regular breathing rate of 55± 5/min. were used. Recordings were made with glass microcapillary electrodes having a tip resistance of about 30 megohms and a tip diameter of less than 0.5 microns.

In recordings from the olfactory epithelium the surface of the tissue was observed with a reflecting microscope to ensure that the characteristic cilia movements were evident. The indifferent electrode was a small platinum wire in contact with the skin of the head. These electrodes were connected to a high impedance probe and Grass preamplifier (P5, A.C.). For observation and recording, the output of the preamplifier was connected to a dual beam oscilloscope and photographed with an oscilloscope camera. In some experiments tape recordings were also made.

The olfactometer used in these experiments was constructed entirely of glass and teflon. A source of compressed air was first purified by

FIG. 1: BULBUS OLFACTORIUS IN RAT
(THIS DRAWING IS PART OF THE TRANSVERSE
SECTION OF RAT OLFACTORY BULB)



passage over silica gel and activated charcoal and then split into three streams of known flow rate. The first stream was bubbled through the test odorant to provide a saturated vapor channel. The second was used to provide a first step dilution; and the third was used both as a third step dilution and as a wash line directed to the animal between stimulus presentations. Both the diluted odorant and the wash flows were delivered to a teflon face mask fitted tightly to the rat's muzzle. An exhaust valve led to the exhaust line.

In the main series of experiments four odorants were used: amyl acetate (A.A.), novovial alpha (N.A.), organum oil extract (O.O.) and alpha ionone (A.I.). Each was presented in each of three dilutions: 0.5; 0.5×10^{-2} , 0.5×10^{-5} of appropriate vapor saturated at $22 \pm 2^\circ\text{C}$. In the experiments on the olfactory bulb these odorants were presented successively in pairs according to a randomly determined sequence. After the first odorant of a pair was delivered to the preparation, and the nature of any response that occurred had been noted, the second odor was presented and the response of the same units was recorded. However, because of technical difficulties it was not always possible to present all four stimuli, or to present them in each of the three concentrations. The period of stimulation and intervals between tests were adjusted to give constant resting activity and no accommodation to the odorant or fatigue of the preparation was observed.

Results

a) Olfactory Epithelium

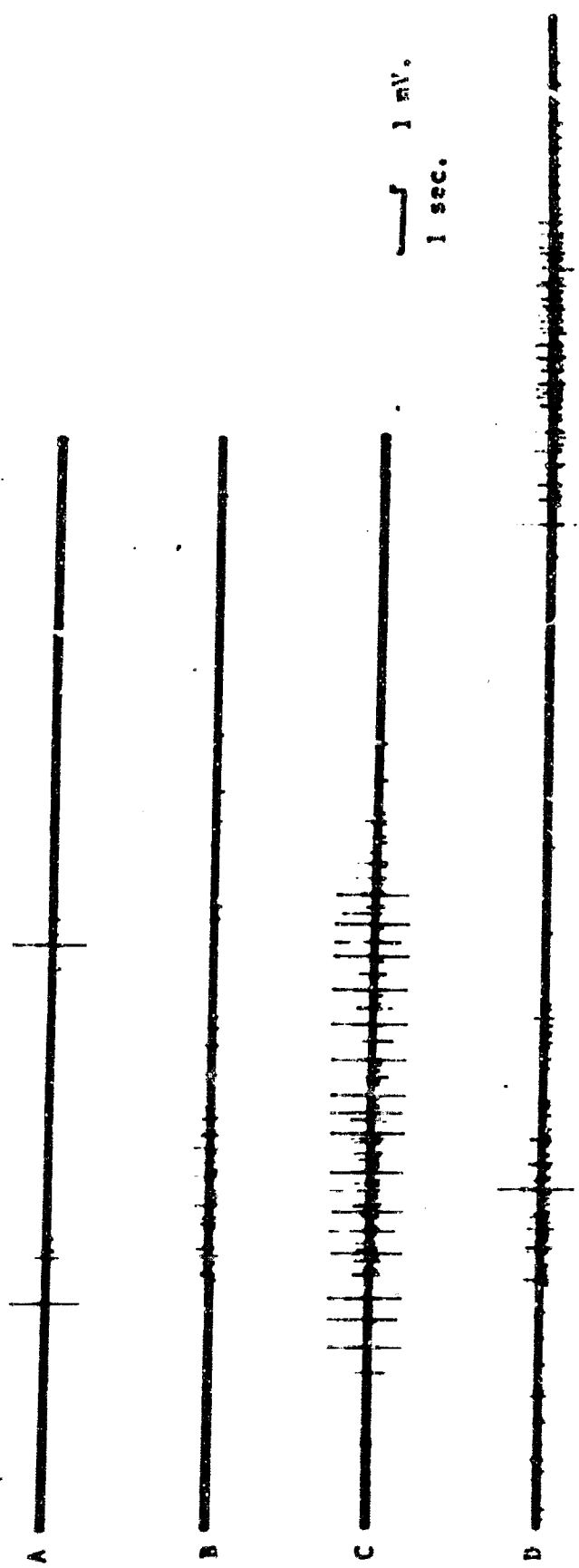
When the micropipette is slowly lowered into the olfactory epithelium the first spike discharges can generally be recorded at a distance of about 100 μ from the surface. Typically there is a low resting discharge showing marked increase in frequency with the appropriate odor stimulation, and having an amplitude of about 2 mV or more (Fig 2, A, B, C).

Successive presentations of the same stimulus elicited the same response pattern on each occasion. However, it was difficult to hold the same unit for prolonged periods (over 30 minutes) apparently because the electrode tip was moved by pulsation of blood capillaries. The electrode tip frequently broke soon after a unit was picked up.

Amyl acetate was a more effective stimulus than the others used. When filtered air was passed over the preparation a resting discharge was observed. In some cases this activity disappeared during odor stimulation. For convenience we shall refer to this as "inhibition" without implying an interpretation of the underlying mechanism.

In units which were excited by odor stimulation an increase in concentration led to a proportional increase in the rate of firing. However, differences in excitation patterns elicited by different odorants were not sufficiently distinct to distinguish them from differences in discharge patterns elicited by different concentrations of the same odorant. Because of the technical difficulties, a more extensive analysis of single epithelia units was not attempted (Fig 3).

FIG. 2a UNITARY DISCHARGES IN OLFACTORY EPITHELIUM
(AMYL ACETATE STIMULATION)



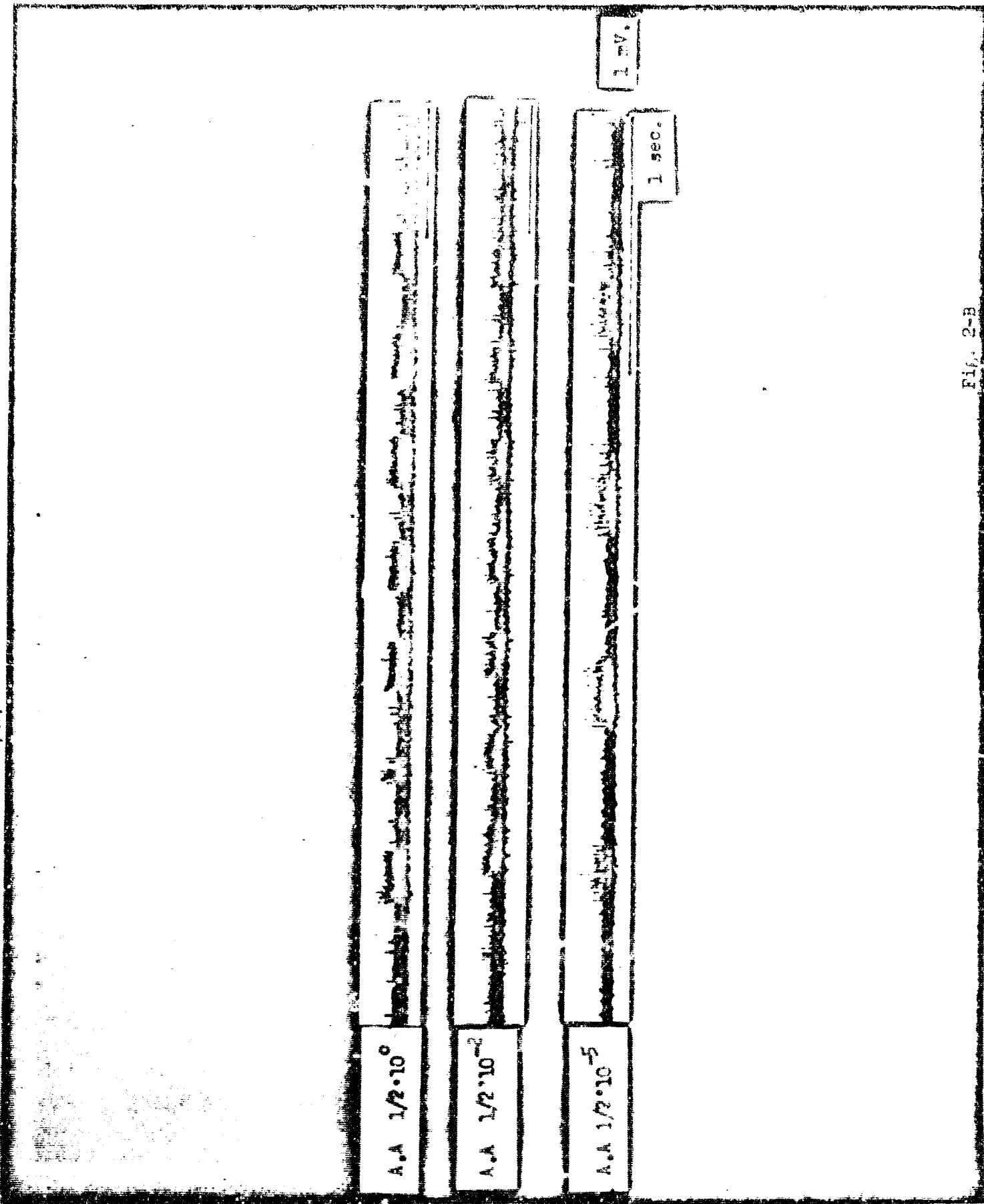


Fig. 2-B

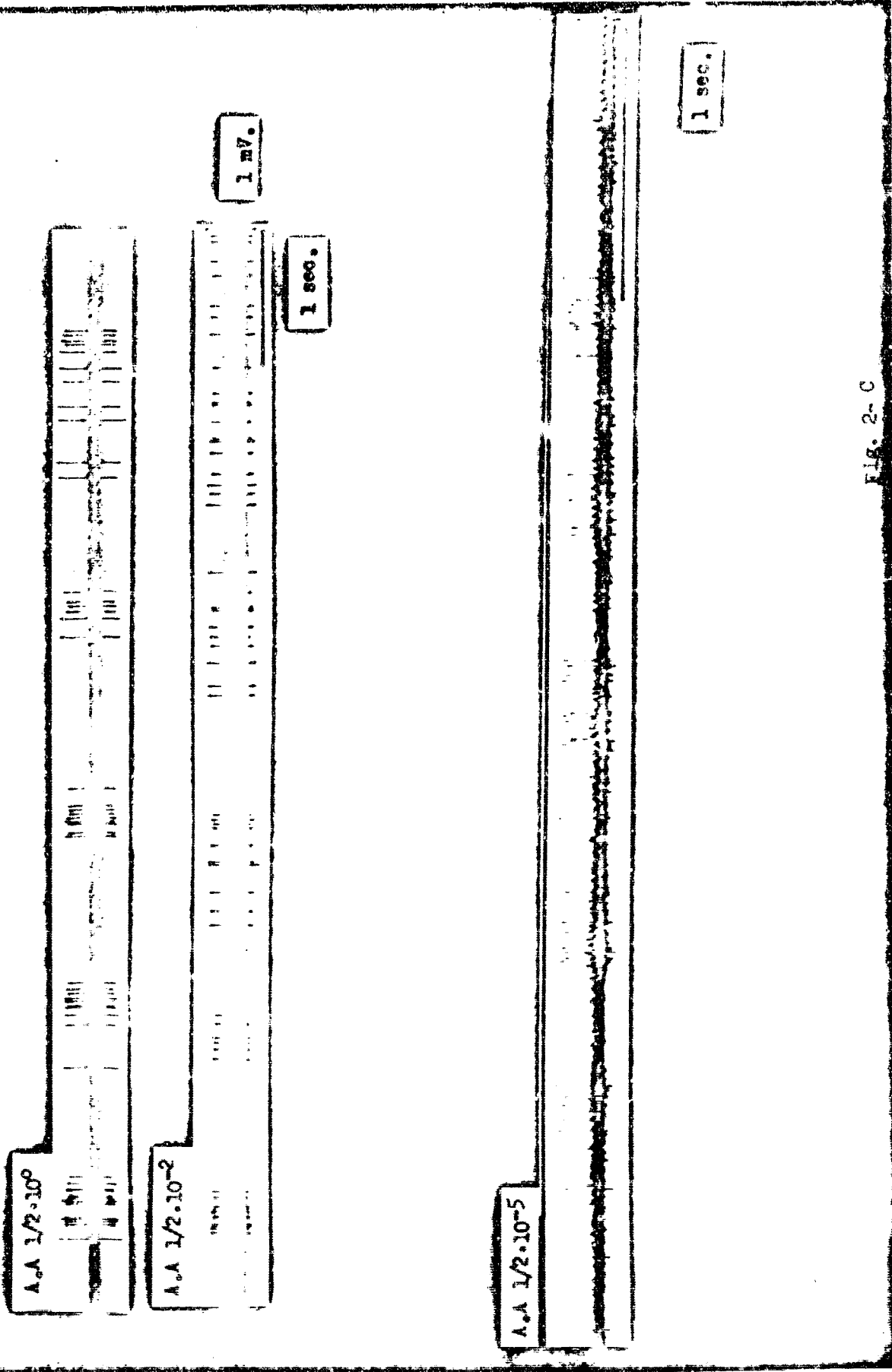
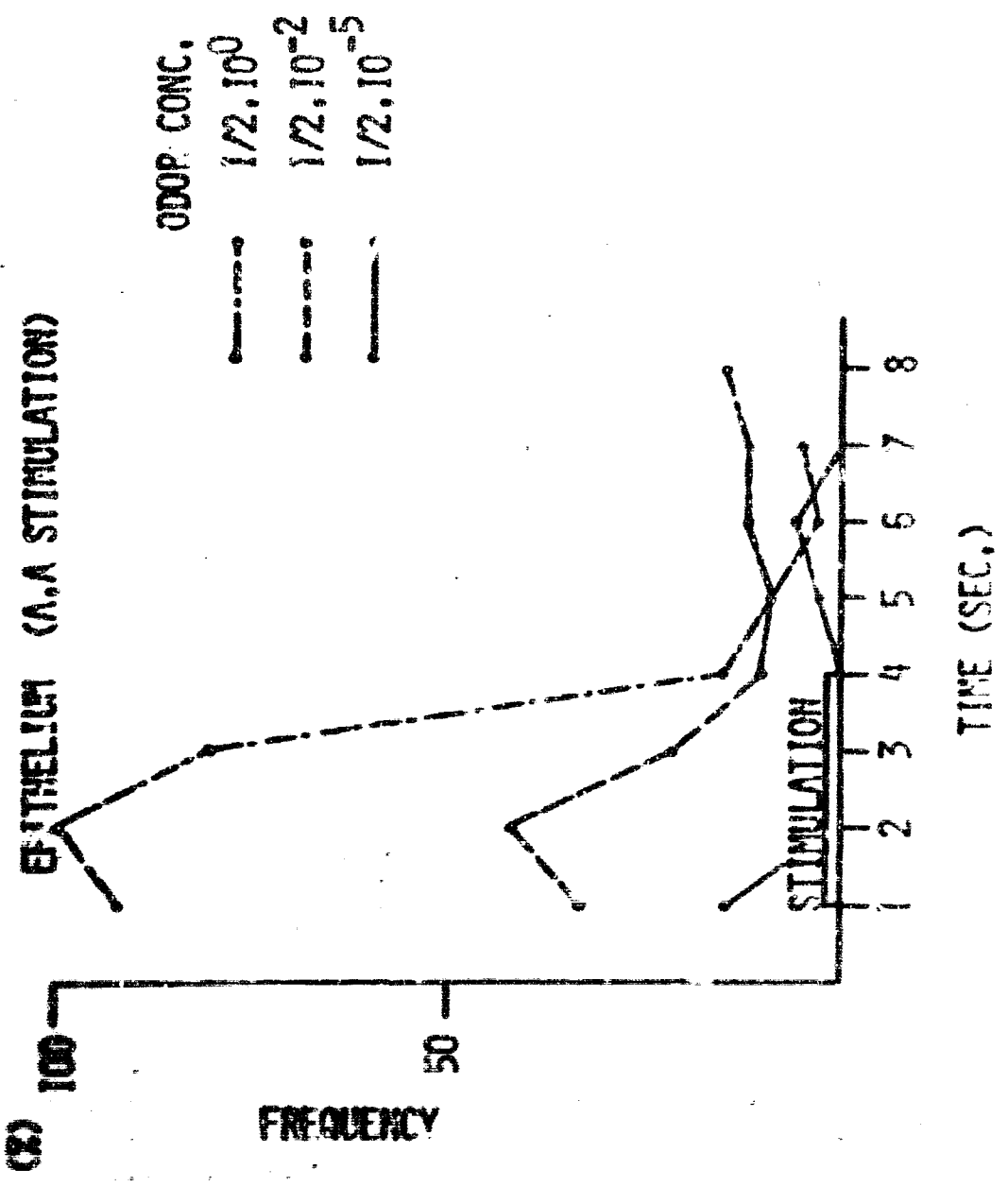


FIG. 2- C

Fig. 3c Frequency variation in unitary discharges in olfactory epithelium



Results

a). Olfactory Epithelium

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b). Olfactory bulb

A glass micropipette was lowered in steps of 5 or less into the olfactory bulb until the mitral cell layer was reached. At about 200 μ from the surface of the bulb, single units were recorded extracellularly. The largest units had an amplitude of about 1 mV or more. The units disappeared if the electrode tip was moved 5 μ . Because such movements occurred naturally (apparently due to pulsations of blood vessels and respiration) a small teflon sheet was placed on the surface to reduce this source of difficulty.

Unitary discharges from the bulb could be divided into the following groups:

- (1). Units which show a high frequency level of response to odor stimulation (Figs. 4 & 5)
- (2). Units with some low level of "resting" discharge with a larger spike amplitude than (1). The frequency of this discharge was always decreased by odor stimulation (Fig. 6).
- (3). Units with some low level of "resting" discharge which was not influenced by odor stimulation (non-responsive units Fig. 10).

The units of type (1) appeared with increasing frequency with each successive inspiration of the odorant. The relation of frequency of discharge to amyl acetate concentration for such a unit is shown in Fig. 4. The initial response was a burst of several units. After stimulation stopped the active units returned to the "resting" level of firing. When a second

FIG. 4: UNITARY DISCHARGES IN OLFACTORY BULB
(MYL ACETATE STIMULATION)

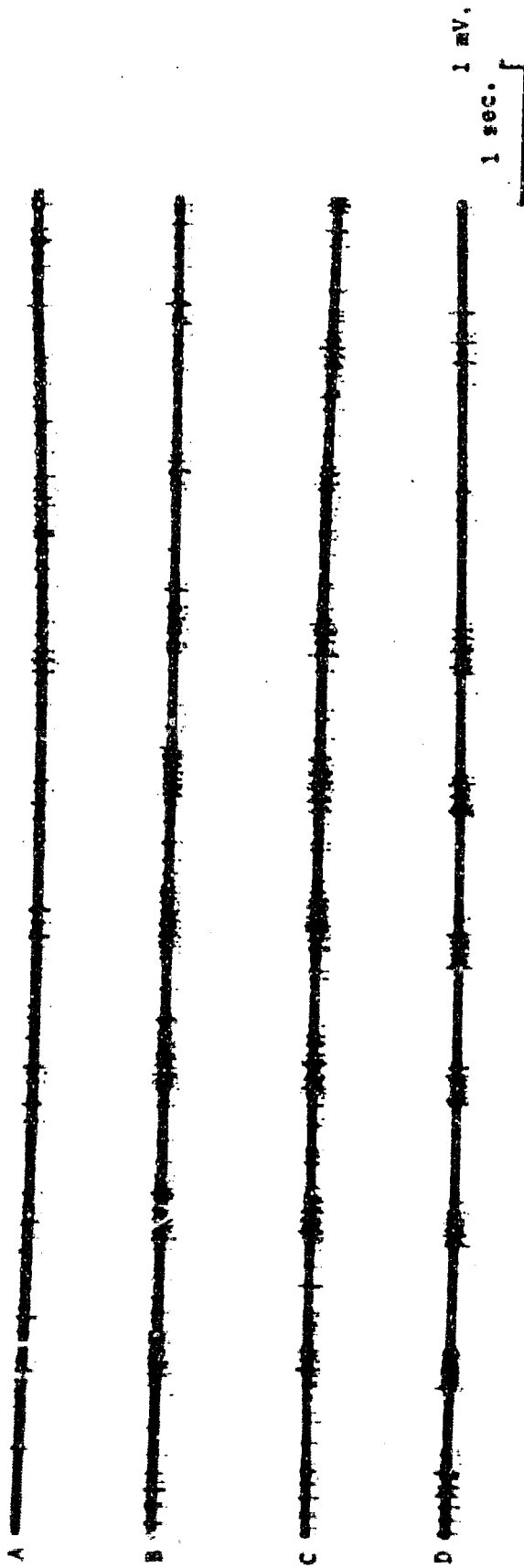


FIG. 5: UNITARY DISCHARGES IN OLFACTORY BULB
(A.A., H.A., O.O AND A.I. STIMULATION)

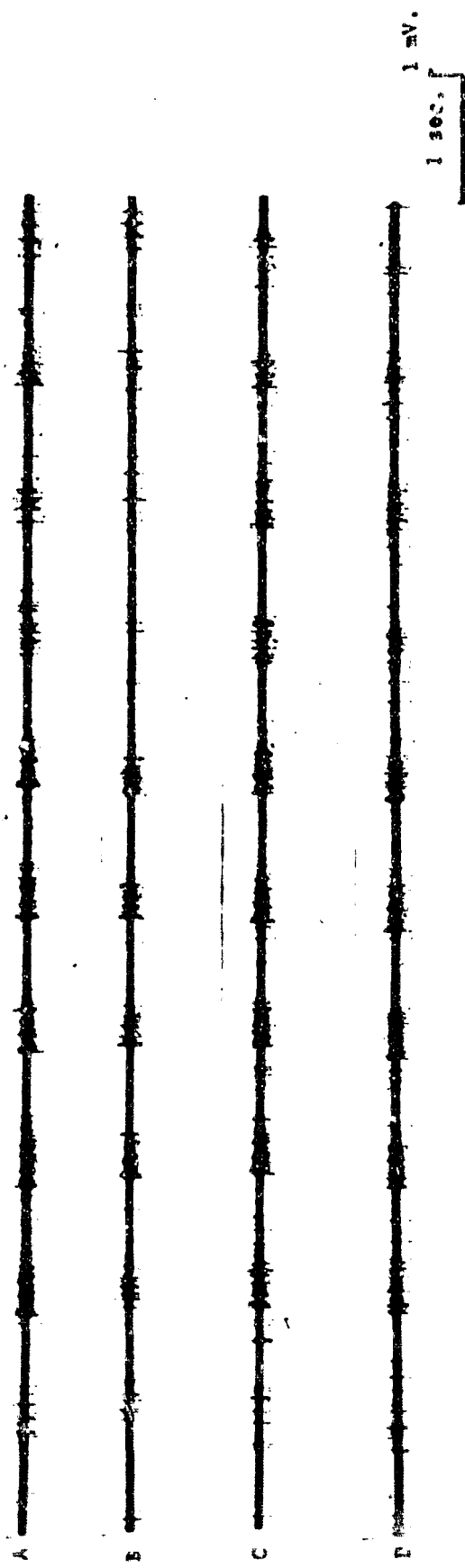
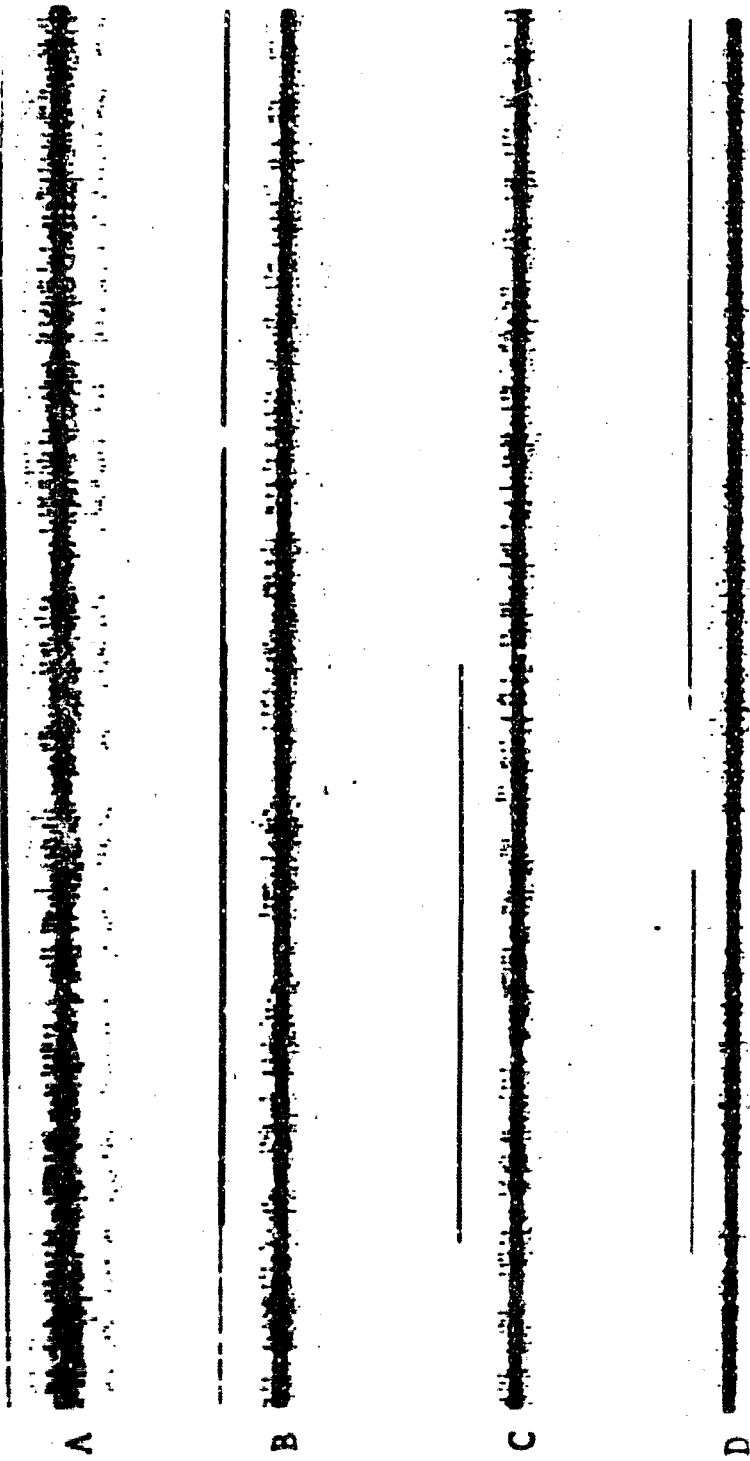


FIG. E: UNITARY DISCHARGES IN OLFACTORY BULB
(INHIBITION PATTERN) A, A STIMULATION



┌ 1 mV.
└ 1 sec.

and different species odor was now applied, some units responded to the new odorant whilst others were unresponsive.

In one typical experiment in which all odorants were delivered at the same concentration, no significant differences could be detected in the resulting patterns of excitation (Figs. 8 & 9). In these figures units responding to a given odorant are defined as those with 40%, or more of the height of the units having the highest amplitude. Frequency variations of these units during each burst of activity (occurring during each inspiration of the stimulating molecules) can then be plotted as a percentage of the standard figure. This figure is the frequency in the period just before stimulation.

Thus it appears that the evoked potentials show a progressive activity change during odor stimulation. Such a change has a clear relation to the quantity of the odor and may have some relation to the quality of the odor. The threshold is apparently changed at an active site by an odorant and its change is closely linked to the coding mechanism for odor discrimination at this primary center (Figs. 7, 8, & 9).

Frequency counts may be reduced in magnitude in some instances by the application of the stimulus. Thus in Fig. 6 one large unit decreased in frequency during odor stimulation.

III. TELEMETRY TECHNIQUES

The overall aim of the entire research project has been to elucidate the mechanisms of odor quality discrimination not only through the acute preparation, but also in the unanesthetized animal. Direct wire methods of recording from freely moving animals impose restrictions on the quality of the record and on freedom of movement. This is an important consideration in behavioral tests designed to focus the attention of the animal on the test odor.

The problem can be overcome by the use of miniature amplifier-transmitters. However, commercially available systems are either too bulky, or inadequate for the transmission at frequencies characteristic of the nerve impulse. The solution has been to develop appropriate telemetry devices. These are described fully in several publications, and will not be considered in detail here (Skutt et al, 1967; Moulton, 1967a; 1968b; Marshall & Celebi in preparation; Moulton et al, 1970).

The resulting unit can be carried without apparent effort by a rat and 6 units can be mounted on a specially-designed head cap implanted on the head of a rabbit. Single unit activity has been successfully telemetered from the olfactory bulb of rats involved in an odor detection task. The activity is led from chronically implanted stainless steel electrodes

IV. BEHAVIORAL TECHNIQUES

The use of telemetry devices allows animals to be trained in odor detection or discrimination tasks which focus the animal's olfactory equipment on the test odor and allow the resulting odor-induced activity to be recorded, in the form of single units, from chronically implanted microelectrodes. Techniques applicable to both rats and rabbits have been developed and described in detail (Moulton 1968a, 1968b; 1970).

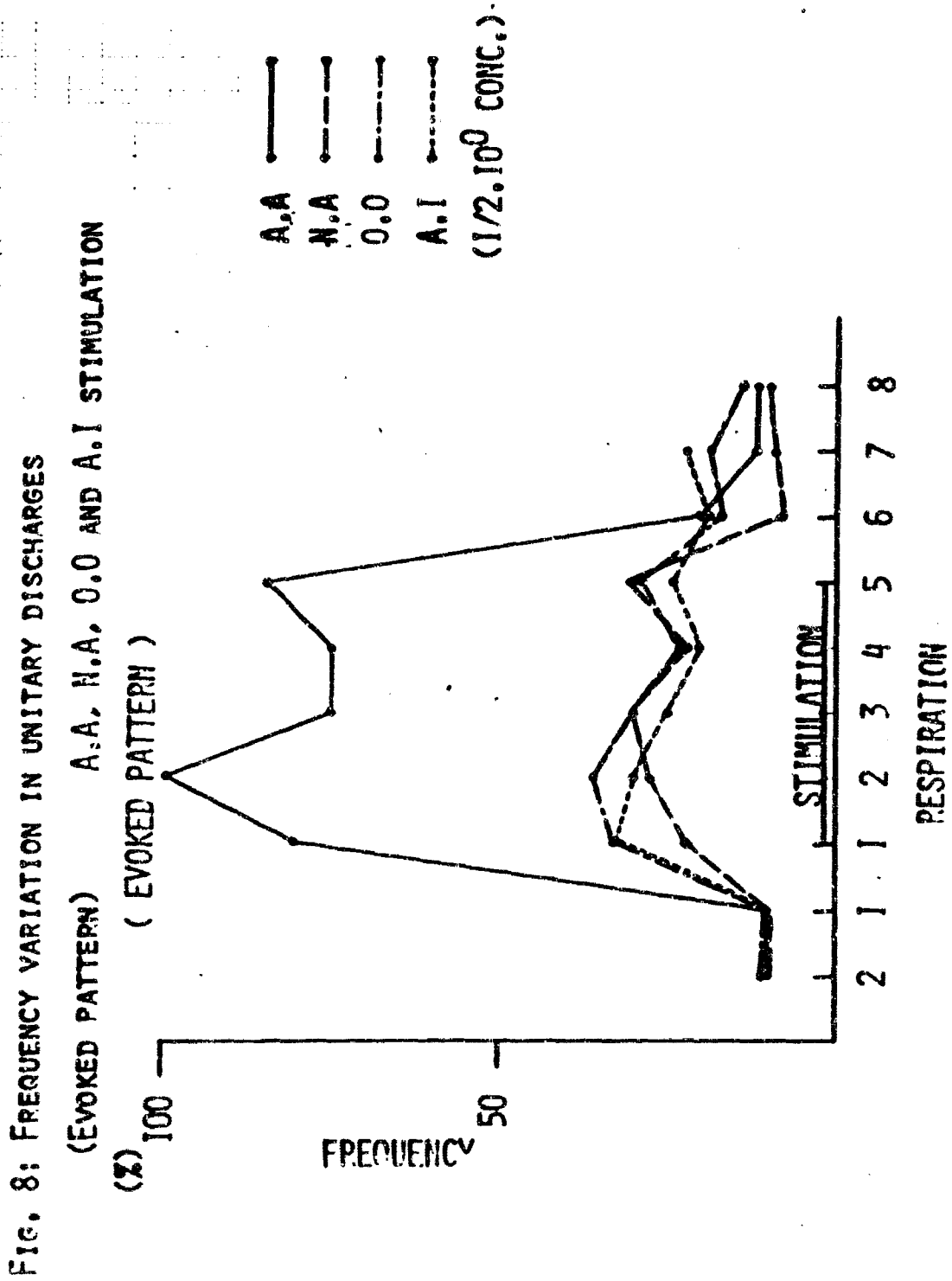


FIG. 2. EFFECT OF STIMULATION ON RESPIRATORY FREQUENCY
A.A., N.A., O.O. AND A.I. STIMULATION
(1/2.10⁸ CONC.)

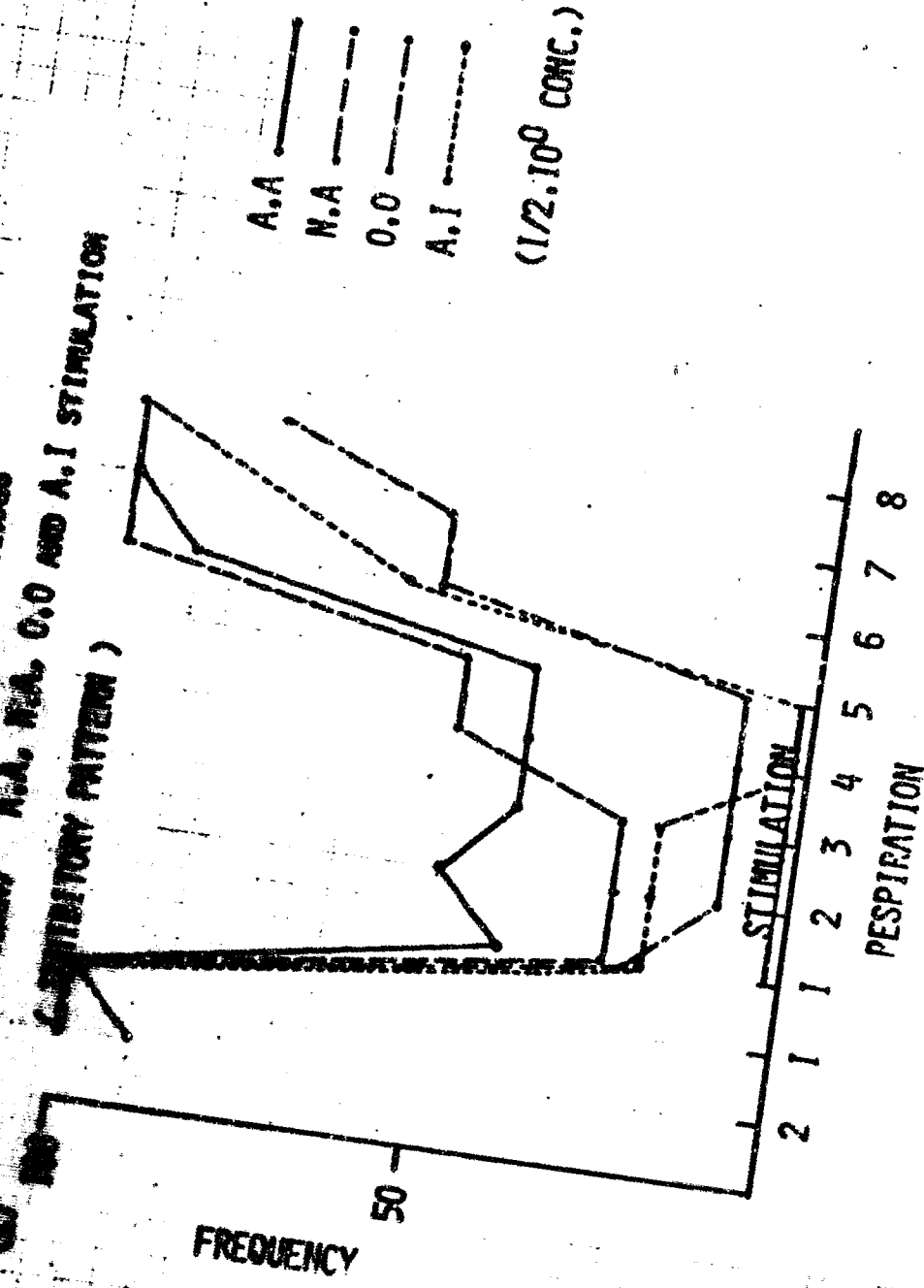
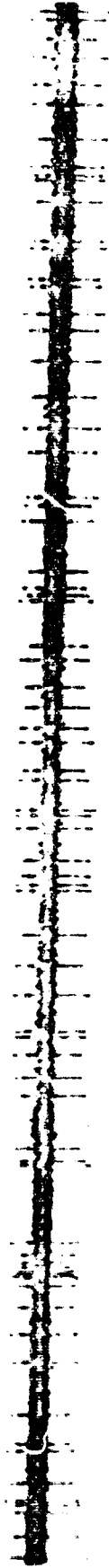


FIG. 10: UNITARY DISCHARGES IN OLFACTORY BULB
(NON-RESPONSE PATTERN)



LIST OF PUBLICATIONS RESULTING WHOLLY OR IN PART FROM
GRANT SUPPORTED ACTIVITIES

1. Moulton, D.G. (1967). Interrelations of the Chemical Senses. In: Kare, M. and Maller, O., (editors). The Chemical Senses and Nutrition. Johns Hopkins University Press, Baltimore . 249-261.
2. Moulton, D.G. (1967). Olfaction in Mammals. Am. Zoologist, 7: 421-429.
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4. Moulton, D.G. (1968). Telemetering of Spike Activity during Behavioral Response to Odorants. In: Tanyolac, N. (editor): Theories of Odors and Odor measurement. Technivision: London. 483-491.
5. Moulton, D.G. (1968). Electrophysiological and Behavioral Responses to Odor Stimulation and their Correlation. Olfactologia, 1: 69-75.
6. Moulton, D.G. (1969). Sensory Perception. J. Dairy Sci. 52, 811-815.

In Preparation

7. Moulton, D.G. Olfaction: Current Concepts. Proc. IX. International Congress Otorhinolaryngol.. (Mexico City, 1969).
8. Moulton, D.G., Fink, R. and Celebi, G. Olfaction in Mammals - Two Aspects: Proliferation of Cells in the Olfactory Epithelium and Sensitivity to Odours. (Ciba Foundation Symposium, London 1969). Little Brown & Co. Boston.
9. Ai, H., and Moulton, D.G. Single Unit Activity in the Olfactory Epithelium and Bulb of the Rat.
10. Marshall, D.A. and Celebi, G. A Variable Frequency Subminiature Transmitter for Telemetry of Neural Potentials.
11. Moulton, D.G. Odor Quality Discrimination (International Symposium on Gustation and Olfaction, Geneva, 1970).

12. Moulton, D.G. Correlation of Behavior and Electrophysiology in Olfaction. (N.A.T.O. Advanced Study Institute, Utrecht, Holland 1970). In: Johnson, W., Moulton, D.G. and Turk, A. (editors). Advances in Chemoreception II. Techniques in the Chemical Senses. Appleton-Century Crofts: New York.
13. Marshall, D.A. Olfactory Discrimination and Stimulus Coding in the Virginia Opossum.

(In addition to these publications, D.G. Moulton gave a brief account of work supported by this grant in a round table discussion, to be published in: Pfaffmann, C. (editor) 1970. Olfaction and Taste III. Pergamon Press).

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13. ABSTRACT

The research project concerned the mechanisms of odor discrimination. The major activities of this project are as follows:

(A) Analysis of single unit activity in the olfactory epithelium and bulb of the rat and goldfish.

(B) Development of telemetry techniques for recording neural impulses from freely moving animals.

(C) Development of behavioral and olfactometric techniques to be used in conjunction with (B). ←

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