TECHNICAL REPORT
70-69-PR

MODE OF ACTION OF RODENT REPELLENTS AND ATTRACTANTS

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and
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Northeastern University
Boston, Massachusetts

Contract No. DAAG 17-67-C-0070

September 1969

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

U.S. ARMY MATERIEL COMMAND

Pioneering Research Laboratory

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Foreword

The contract research reported here was conducted under DA Project 1J062110A585, Biological Research on the Protection of Materiel from Insects, Rodents and other Animals, Task 01, Factors Involved in Preventing Deterioration. It is a part of continuing studies on the sensory physiology of insects and rodents.

The research was concerned with the roles of taste and odor in the discrimination of repellent and attractive substances by the rat. A related purpose was to develop methodology for evaluating repellent effectiveness based on the effects of chemicals on the taste and odor senses.

Although this report represents the final report of Contract DAA6-17-67-C-0070, it includes data and discussions from two previous contracts: DA-19-129-AMC-386 (N) with the University of Massachusetts, and DA-19-129-AMC-691 (N) with Tufts University. It is logical, therefore, that this is an integrated report based on the results of the 3 contracts.

Mr. Theodore Nalwalk designed and constructed the flow systems and most of the apparatus used in this research. Miss Jacqueline Walthers and Mr. Frank Gordon assisted in the surgical and histological experiments.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care as Established by the National Society for Medical Research".

JOHN J. PRATT, JR., Head
Applied Entomology Group
Pioneering Research Laboratory
Project Officer

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ABSTRACT

The mode of action of rat repellents was investigated in a series of experiments concerned with food consumption, olfactory sensitivity and discrimination, adaptation and habituation, and performance. A variety of chemical repellents were used both in the animal's food and in the air. Comparisons were also made among laboratory strains and the Norway rat (both wild and tame), with and without lesions in the olfactory bulb. It was concluded that the odor of a chemical makes little or no contribution to the repellency of a chemical or to its value as a deterrent except when it has a signalling value from association with a painful taste or when it is a novel stimulus. Additional conclusions relate to methodology for testing the effectiveness of repellents, theory, and needs for future research.

Introduction

Rodent control takes the form of repellents or of toxicants. The logic is different for these two approaches since repellency requires an insult to the senses of the animal whereas poisoning requires either neutrality or an appeal to the senses in order to lure the animal to accept the bait. Even though the logics are different, both approaches have certain common problems associated with their evaluation and their effectiveness. Among these are questions of learning, physiological adaptations, and psychological habituations.

The usual purpose of a rat repellent is that of keeping rats at a distance from a to-be-protected material or place. For the repellent to function this way, it must have a volatility sufficient for it to be detected and responded to as an aversive at the desired distance. Unfortunately, the greater the volatility, the shorter the life of the chemical. Optimal volatility, then, is a function of olfactory sensitivity and the aversiveness of the odor to the animal. The problem is not really as simple as that statement makes it appear, however, since it can be expected that olfactory sensitivity will decrease with continued exposure to the odor and aversiveness may decrease with both continuous and intermittent exposures.

The problem is further complicated by the fact that volatile substances probably act upon more than olfactory receptors. They act upon pain fibers in proximity to the olfactory receptors (Tucker, 1963), upon taste receptors via transmission of the substance to the mouth, and upon receptors in the skin and eyes serving the senses of touch, temperature, and pain. Available repellents take advantage of this by attacking pain fibers in the skin, eyes, and mucosa. However, the effectiveness of these repellents still depends upon phenomena of adaptation and habituation which determine the sensitivity and the aversiveness of the chemical. In addition, it is reasonable to suppose that there are degrees of

acceptance of pain by the rat which depend upon such conditions as state of hunger and the availability of other food sources.

A repellent of low volatility has a long life, but it permits the animal a closer approach to the protected substance. Since it depends for its effectiveness on the production of pain in the mouth or later in the digestive system, this kind of repellent requires that the animal bite or taste it; the result is damage to the repellent, itself, and a subsequent loss of effectiveness as successive animals make the same kind of attack. Another weakness of low volatility chemicals is that other animals, or people, may also make contact with it. The purpose of volatility, therefore, appears to be twofold, i.e., that of repelling via inspiration or skin contact and that of warning. For these reasons, the distance or "odor" effects of the chemical appear to be more important to study than those effects associated with actual tasting or consumption. Our research was oriented largely in this direction, therefore, although attention was given to problems associated with ingestion as well.

All repellents in use appear to be acute toxicants used at less than lethal concentrations. An important control question was the possible lethality produced by continued consumption of these chemicals at levels which were repellent, but not toxic. Since no information was available about continued consumption, Experiment I was carried out (Teichner, Wagner, & Rountree, 1966). The experimental conditions of greatest relevance are shown in Table 1. As may be seen 11 groups of rats were put on a feeding regime in which the indicated chemicals were mixed into their diet at the concentrations shown. All were albino rats except three groups which were a hooded strain. There were five animals per group, all about four months old at the start, all male. The food used (Purina Chow) was their normal diet prepared in the form of a wet mash. The animals were fed in

TABLE 1

Experimental Conditions of Phases II and IV, Experiment I**

Group	Repellent		Test concentration (ppm) Phase II	Retest concentration (ppm) Phase IV
1	None (control group)			
2	Acti-dione:	B-2-(3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl glutarimide	2.5	2.5
			5.0	5.0
3	Acti-dione		20.0	20.0
			20.0	1000.0
4	Acti-dione		40.0	1000.0
5	Car-Ban T.A.:	Tributyltin acetate		
6	Car-Ban T.A.			
7	TNBAC	Trinitrobenzene aniline complex	500.0	500.0
8	TNBAC		1000.0	1000.0
9*	Car-Ban T.A.		20.0	1000.0
			5.0	5.0
10*	Acti-dione		20.0	20.0
11*	Acti-dione			

*Groups 9, 10, and 11 were hooded rats. All others were albinos.

**Adapted from Teichner, Wagner, and Rowntree (1966)

individual chambers once a day and allowed no other food. This regime was maintained for 26 days prior to Phase II of Table 1 at which time the food was contaminated. This was followed by a 9-day period of uncontaminated food and then by a second period of contaminated food (Phase IV).

Some of the concentrations presented were at the LD-50 for single consumption; it was expected, therefore, that some animals would not survive. Figure 1 presents the number per 5-animal group which did not survive and the portion of the experiment in which they died. As may be seen there were considerable differences between strains and between concentrations.

Figure 2 shows the consumption of contaminated food compared to that of uncontaminated food when the chemical used was acti-dione. Only those animals which survived the entire experiment were included. The figure shows a marked initial reduction of food intake followed by a systematic recovery. This is true in both phases although there is some suggestion that the initial reduction in Phase IV may have been the lesser one. In both cases, the amount of food eaten increased systematically within each phase until at the end of the phase food consumption was at least 60 per cent of that of the control group or of the prior uncontaminated level. These data are clear in showing that those rats which survive do so by regulating their food intake systematically to the point where they can accomodate levels of a contaminant which are otherwise lethal.

For present purposes, the greatest interest in the results of Experiment I is that: (1) a chemical may be defined as a repellent if, when mixed with a normal diet, it produces a reduction of normal food consumption; presumably, the greater the reduction, the more repellent the chemical may be said to be; (2) even those chemicals which are strong repellents lose their repellency as a result of changes in the tolerance of the animal to them. Whether the changes are physiochemical or behavioral or both cannot be concluded from the results of this experiment, but the question is, clearly, of great importance.

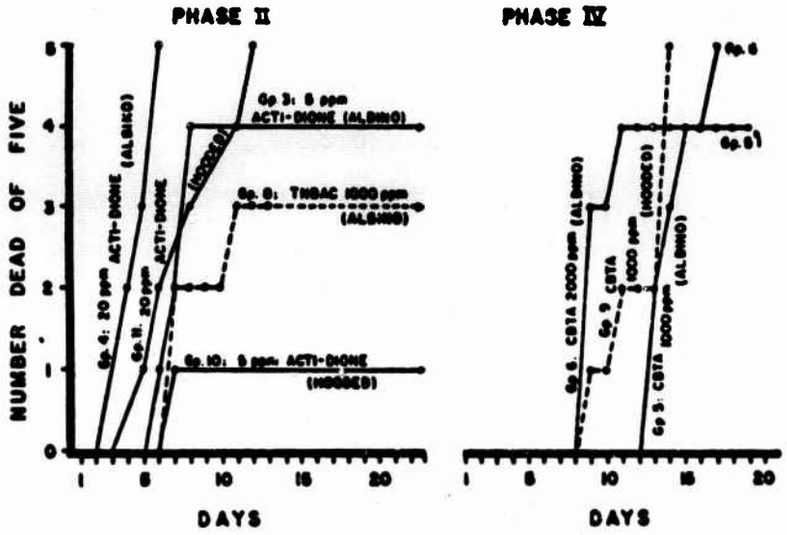


Figure 1. Number of Animals that Died and Survival Time for the Conditions of the Experiment I. (From Teichner, Wagner, and Rowntree, 1966).

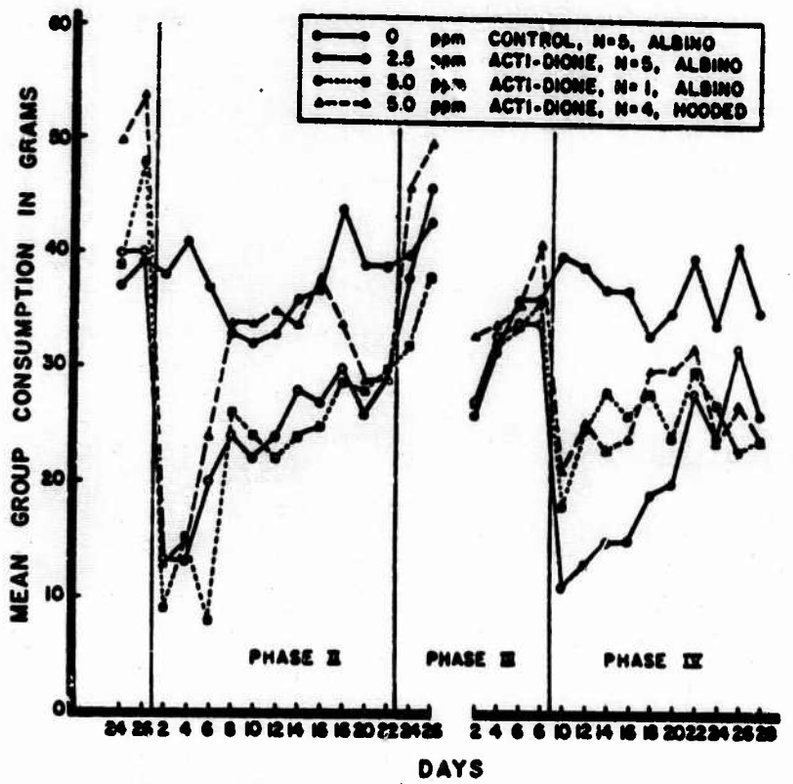


Figure 2. Food Consumption of Final Surviving Rats on Acti-Dione. (From Teichner, Wagner, and Rowntree, 1966).

Repellency is generally tested in term of a reduced intake of contaminated food or a reduction in attack on a treated material. Most such tests are for short duration (from perhaps one hour to 1-2 days). An implication of Experiment I concerns the need for testing for even much longer periods and under conditions when no other food is available. Another implication, in terms of our statement, made above, about low-volatility repellents, is that not only may the protecting repellent be damaged by the attack of successive animals tasting or biting it, but it may be damaged by successive attacks from the same animals.

It is likely that contaminated food consumption would not have recovered if other uncontaminated food had been available either at the same time or at a different time. From this it follows that the rated repellency of a chemical agent depends not only on the exposure conditions of the animal to the chemical, but also on the hunger level, and the availability of consumables other than that protected by the agent. Thus, a chemical may be highly repellent in one set of circumstances and much less effective in another. The degree of repellency must be stated in terms of the environmental conditions in which it is used and the state of the animal. All of the food consumptions to be reported were obtained under conditions in which no other food was available and in which the food presented was available only one time per day. All of the repellents used in the studies to be reported were selected in terms of the results of Experiment I or similar preliminary experiments. It should be noted that our interest was not in any particular chemical, but rather in using known chemical aversives as a tool with which to study the processes on which repellency depends.

Although we recognize that volatile substances act upon more than olfactory receptors, including the taste buds, it is convenient to refer to the effects of inspiring such substances as "odor" effects and we shall do this. Similarly, we shall call those immediate effects associated with ingestion, "taste", effects. The problem of determining the relative contributions of these two kinds of effect to repellency is made difficult by their

confounding during ingestion. Ideally, it would be desirable to compare the consumption of contaminated food in the presence of uncontaminated air with the consumption of uncontaminated food in the presence of uncontaminated air. Difficulties arise because even in the presence of trace quantities, it must be assumed, until demonstrated otherwise, that the animal has both the taste and the odor available during both conditions. The most favorable assumption that can be made, given present knowledge, is that in the presence of low air concentrations, the taste information is so small as to approach being negligible; the comparable assumption that the odor effect is negligible in the presence of contaminated food is not reasonable. Thus, logically, the best comparison that can be made is of the difference between taste plus odor associated with ingestion and odor alone associated with inspiration.

Even that comparison, were it made, suffers from logical difficulties since it cannot be assumed that the odor intensity associated with air contamination can be equated to that associated with ingestion. One or the other could be a stronger effect depending upon the concentrations selected and the psychophysical relationship involved. Finally, a difficulty arises as we have shown, in that the animal does not approach the consumption of contaminated food in the same way as for uncontaminated food. This gives a special advantage to comparison groups which have only the air contaminated.

The solutions to these problems, as we have approached them, are as follows:

1. The problem of a different approach to eating contaminated food was attacked by developing measures of repellency which are very highly correlated with the basic measure of food consumption, but which do not involve the actual consumption of contaminated food and which can be applied to both kinds of comparison groups. The fundamental premise was based upon well-established behavioral relationships which state that the greater the de-

privation of food (1) the greater will be the strength of a learned response which leads to food, and (2) the greater will be the amount of food consumed.

2. The problem of unequal odor effects associated with concentrations of repellent in food and in air was approached by developing a measure of aversion for use with inspired compounds so that air effects can be calibrated. With such a measure, comparisons can be made of the odor aversion of varying concentrations in food with the odor aversion of air concentrations.

3. Given the above two methods, a factorial experimental design carried out over a reasonable range of air contamination and of food contaminations will indicate the equivalences and differences between different air and food concentrations on dependent measures of repellency not used in establishing the independent repellency of each, that is to say the Ingestion X Inspiration interaction can be estimated.

Experiment II (Teichner, 1966) was performed as an approach to the development of measures of repellency in addition to that of the amount of contaminated food consumed. The situation was one in which the rat on a 23.5 hour deprivation schedule was fed wet mash (Purina Chow) for 25 minutes in an individual feeding chamber. Immediately following it was placed in the starting box of a relatively long straight runway the center portion of which was tilted upwards at 45 degrees. The goal box of this runway contained another portion of wet mash to which the animal was allowed access for five minutes. The measures taken were 25-minute food consumption, running time through the center portion of the runway, and 5-minute food consumption. The animals were trained to stable food consumption and running times before the 25-minute food was contaminated. The repellent used was TNBAC (see Table 1) mixed into the 25-minute portion in concentrations which were varied experimentally between 100 ppm and 400 ppm by weight. The overall results are shown in Figure 3. From the figure it is clear that both running time and the 5-minute food consumption may serve as measures of repellency.

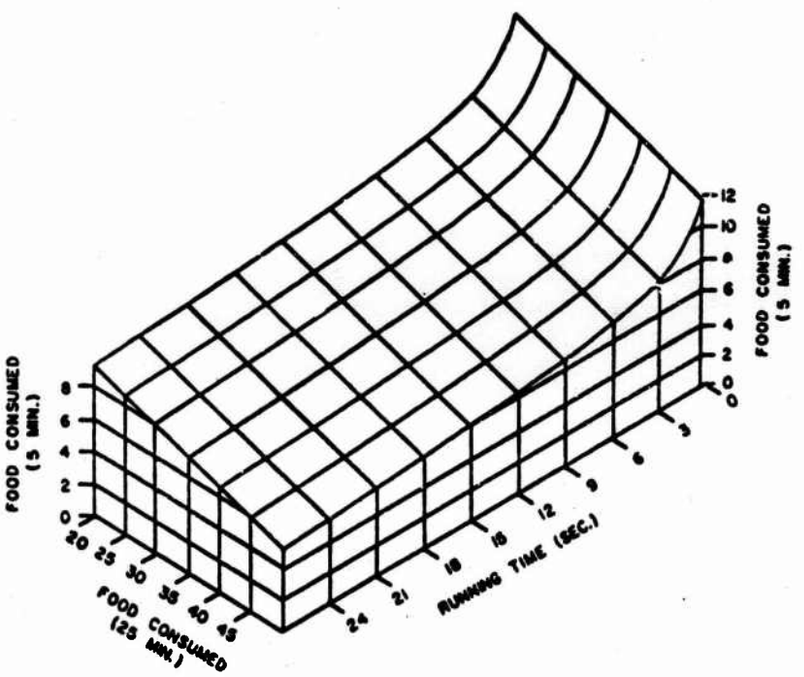


Figure 3. Goal Box Food Consumption as a Function of 25-Minute Food Consumption and Running Time. (From Teichner, 1966).

The results of Experiment II represent a large step toward a legitimate comparison between the two kinds of conditions described above. That is, a fair comparison may be obtained in terms of either or both running time and the 5-minute food consumption if a factorial design is used which provides various levels of contaminated air and food in combination during the 25-min. period. However, although the comparison can now be made fairly, the interpretation of the results will still not be completely clear. The problem remaining is that of choosing the air and food contamination levels. Since somewhat different sensory processes are involved, at least different in degree of stimulation, different sensory intensity curves are involved, and since such curves are known not to be linear, any comparison in terms of a specific concentration could be loaded one way or the other. That is, a given concentration in food might be an intense aversive experience via taste (or a weak one) and a weak (or intense) odor experience. Furthermore, as the concentration is varied within some limit, it might or might not exceed a detectable difference in aversion for either sense. Thus, the kind-of comparison needed must involve the independent scaling of both of the aversive reactions. Since it was shown that taste aversion can be measured by contaminated food consumption, a great deal of this research program was aimed at the question of how to evaluate the odor reaction. Besides its use in the manner indicated, such a measure has the additional practical value of also being a measure with which to evaluate the distance repellency of a volatile substance.

The technique developed relies on the fact that one is dealing with a respiratory agent and that the most logical selection of phenomena to be measured should be some aspect of the respiratory system. Respiration, itself, as a basic defining operation offers some difficult problems since it can vary with a variety of stimuli other than odors. Sniffing, however, may be regarded as a special kind of respiratory behavior used by the rat (and some other animals) as a means for investigating and sampling its environment. Sniffing is an air sampling mechanism which can be relied upon as

a reaction associated with odorants. Drawing upon knowledge of the reactions of the sense organs and to some degree upon intuition, a model or set of working hypotheses were generated about sniffing as a reaction to chemicals in air. The model is illustrated in Figure 4.

The ordinate of Figure 4 presents the three possible ways in which sniffing may be measured, i. e., the amount of time in a period of time during which the animal is sniffing as opposed to breathing without sniffing or breath holding, the number of sniffs in the period of time, and the amplitude (in arbitrary units) of sniffs which defines a big or small sniff. The situation assumed starts with a pre-exposure period in which the animal is presented only with clean or normal air. At time zero, the animal receives the chemical. At time x, the chemical is removed and a post-exposure period follows. The curves drawn indicate the hypothesized effects on all measures of sniffing when the odor is an aversive and when it is an attractant. They illustrate the following hypotheses as listed previously (Teichner, 1966).

1. The rate of sniffing and the amplitude or intensity per sniff should decrease with stimulation by repellents and increase with stimulation by attractants. The amount of change should be a function of the degree of aversion or attractiveness of the odorant.

2. With continued constant stimulation, sniffing should adapt; that is, the rate and amplitude of sniffing should return to the base-line level. The rate of adaptation should be a function of the attractiveness or aversion of the odorant.

3. Removal of the odorant provides a new stimulus condition and, therefore, should affect sniffing. Assuming complete adaptation, removal should be followed by an increase in sniffing regardless of the nature of the previously presented odorant. However, following removal of an aversive stimulus, sniffing should be greater and adaptation should be slower than following removal of an attractant.

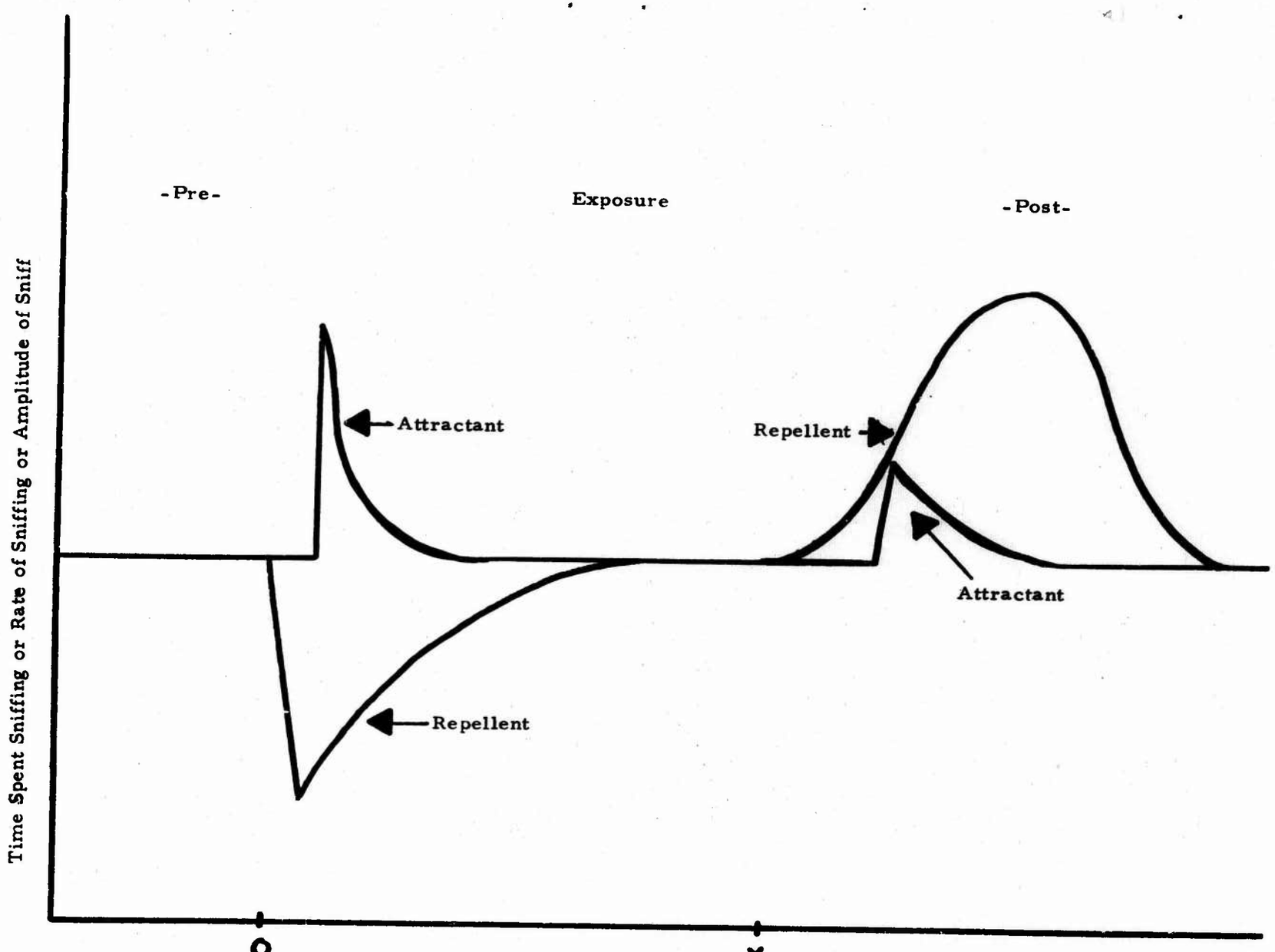


Figure 4. Hypothesized Effects on Sniffing of the Presentation (o) and Removal (x) of a Repellent and of an Attractant Odor.

4. Regardless of whether the stimulus is an attractant or repellent, sniff rate, time, and amplitude should be inversely related to the concentration of the odorant in the air. This is based on the assumption that, for an air sampling mechanism, the weaker the concentration, the greater must be the air sample required for a decision.

5. The speed of occurrence of the first sniff following presentation or removal of an odorant should be greater for aversives than for attractants.

6. The speed of response of the first sniff following presentation or removal of the odorant should be related directly to the previous concentration of the odorant in the air.

Using repellents such as beta-nitrostyrene, tributyltin acetate and others and a highly attractive liquid food as an attractant source, it was possible to test some of these hypotheses and to confirm them. Details are presented elsewhere (Teichner, 1966; Teichner, Price & Nalwalk, 1967). The general procedure was one in which the animal was placed in a small chamber, unrestrained, and exposed successively to a flow of clean air, contaminated air, and then clear air again. Sniffing was picked up by microphones and recorded as a dc output. For example, Figure 5 (Experiment III) presents the effects on the per cent change of two sniffing measures during the contaminated period relative to the original baseline and of the second clear air period relative to the original for 2-Nitro-1 Phenyl-1 Propene (PNP) as impregnated on burlap at three different concentrations. The effect of beta-nitrostyrene on the change in rate and amplitude of sniffing during exposure to the contaminated air (Period 3) and following removal of the contamination (Period 5) is shown in Figure 6 (Experiment IV). Comparable data for the effects of the liquid diet odor are shown in Figure 7 (Experiment V). It may be seen that these data are not as clear as those for the aversive, but of considerable importance is the demonstration, at least with sniff rate, that the effect of an aversive odor (decreased sniff rate) is opposite to that of an attractant (increased sniff rate).

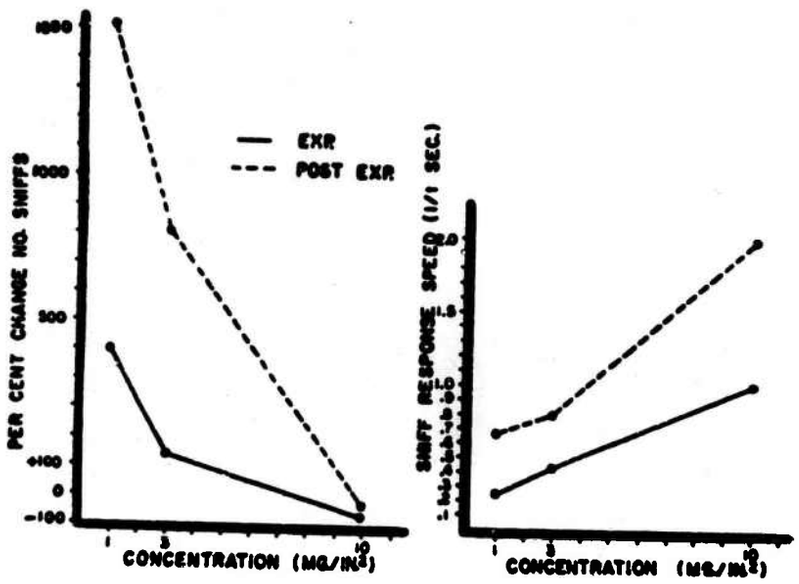


Figure 5. Mean Per Cent of Change in Number of Sniffs During the First Five Seconds Relative to the End of the Pre-Exposure Period (Left) and Mean Reciprocal Sniff Response Time (Right). (From Teichner, 1966 a).

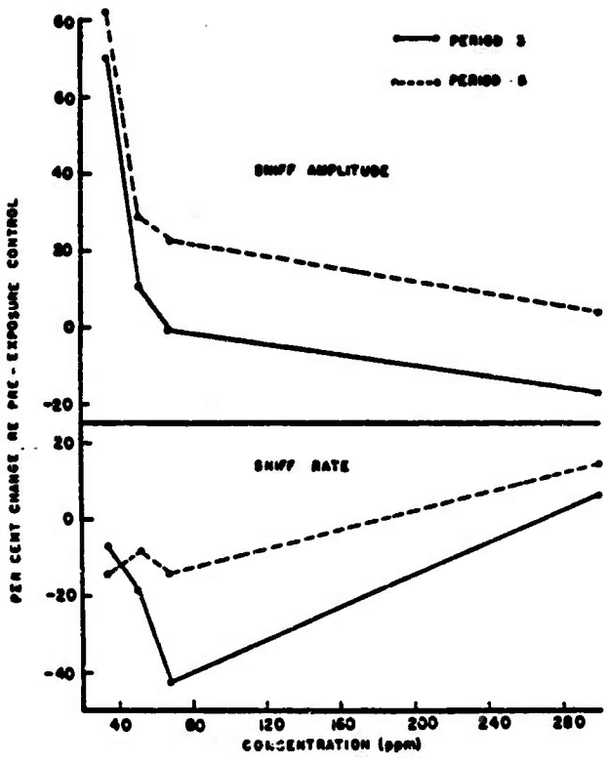


Figure 6. Relative Amplitude and Rate of Sniffing in Response to Beta-Nitrostyrene. (From Teichner, Price, and Nalwalk, 1967).

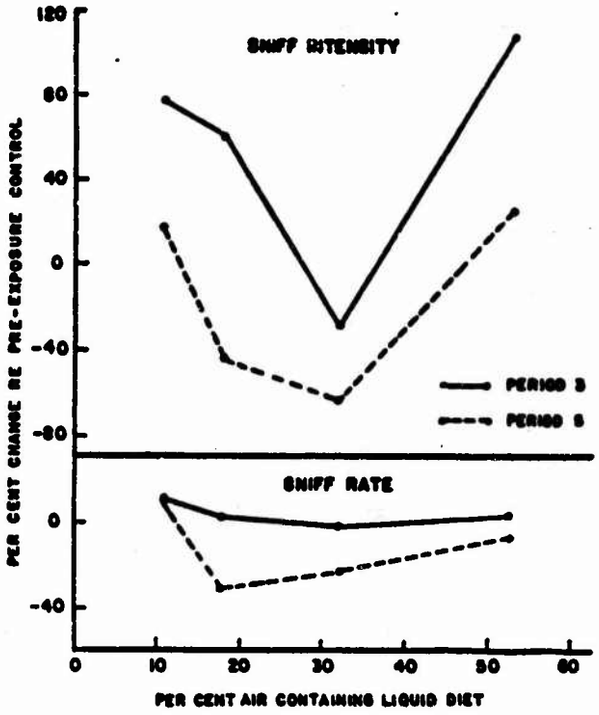


Figure 7. Relative Amplitude and Rate of Sniffing in Response to Food Odor. (From Teichner, Price, and Nalwalk, 1967).

The sniffing results are reasonably clear support for the model presented in Figure 4. Along with the results obtained in the feeding chambers and runway (Experiment II), they indicate the feasibility of a fair test of the relative contributions of odor and taste (i.e., the Ingestion x Inspiration interaction) to repellency and to attractiveness. That is:

1. In an individual feeding chamber allow 25 min. for the animal to eat its daily ration of wet mash.

2. Vary the concentrations of the chemical in the food and in the air in a factorial experimental design. The air concentrations should be pre-calibrated or pre-rated in terms of differences in sniff reactions.

3. Immediately upon completion of the 25-min. period, place the animal in the starting box of the runway. Five seconds later open the starting box door. Allow five minutes for consumption of wet mash in the goal box. Determine both food consumption and running time.

The details of food preparation, training and sniff measurement may be derived from the previously reported studies. The results of the experiment will provide the interaction between taste and odor. As part of this, it will indicate the aversion due to odor for given concentrations in food and the aversion due to the chemical in food at given levels of sniff-calibrated, odorous aversion. The same logic applies to attractants.

A large-scale, demonstration experiment of the sort described was carried out, but due to suspected unreliabilities in the data-collection, the results will not be reported. It can be said, however, that the experiment is perfectly feasible, although enormously time-consuming. It suffers also from the administrative necessity for the use of a team of data-gathers working on a highly coordinated schedule. These disadvantages can be tolerated as experimental necessities; they may

provide serious handicaps to routine testing. Nevertheless, we feel that they provide a methodology, to be improved upon, for testing and for research. In view of this, and of the greater need in the long run to deal with questions concerning odor repellency and attractiveness, primary emphasis was turned to studies involving sniff reactions. An additional important reason for doing this concerned the problem of adaptation and of habituation of the animal to the odor. It was felt that these phenomena would be unavoidable in the test described as well as in the application of the chemical in the real world.

In our previously-reported research we have noted that sniffing tends to decrease as a response to a novel stimulus with repeated exposure to the stimulus. Supporting findings have also been reported by Bindra and Spinner (1958). In our case, this phenomenon was especially marked as a day to day decrease in sniffing in the apparatus even in uncontaminated air; thus, the baseline against which a repellent effect was to be evaluated was being reduced, and since the effect of the repellent itself is to reduce sniffing, the possibility of even getting a measure was being threatened by the very process of getting it. This difficulty was overcome considerably by using hungry rats even in situations in which food consumption was not involved since it had been observed that such animals tend to have a higher basal sniffing rate. In addition, as expected, air containing a familiar food odor augmented the sniffing response. Even so, a between-day decrement was generally observed although not of as large a magnitude. The question arises whether this day-to-day decrement is increased when the air contains an aversive chemical. If so, the chemical may be considered continuously effective as an aversive. Experiment VI was designed to investigate day-to-day habituation with this question in mind.

It is conceivable that a chemical odor may be aversive and yet not act as a repellent if the substance being protected is itself uncontaminated. This would be indicated if the consumption of uncontaminated food were unaffected when food was presented in contaminated air. Experiment VI was set up to consider this problem as well. The basic experimental approach was also designed to have relevance to the general testing technique described above.

Experiment VI

Experimental Methods

A flow system was constructed which permitted the mixing of air channels (see Appendix I for details). Channel 1 contained chemically pure air. Channel 2 consisted of chemically pure air passed over food. The food was the animal's normal diet in the form of a wet mash. The mixture of these two flows will be called food odor. A third channel which duplicated Channel 2 contained in addition a predetermined quantity of tributyltin chloride (TBC) mixed into the wet mash. A mixture of Channels 1 and 3 made up the TBC or aversive air conditions. Thus, both air conditions contained the food odor. One contained an additional, known aversive. The concentration of TBC presented to the animal could be varied by varying its proportion to the wet mash, or for a constant proportion, by varying the ratio of Channels 1 and 3 in a mixture of constant volume, or by varying the temperature of the air holding the chemical.

The air mixture was presented to the animal in a small chamber. The flow rate through the chamber was 500 cc/min. The chamber, housed in a sound-dampened enclosure, was instrumented for an audio pickup which permitted the recording and monitoring of sniffing by an experimenter in an adjacent room.

Procedures

The animal was placed into the sniff chamber following 23 hours of food deprivation. During the first and third 60 seconds of this period the food odor was presented. The second 60 seconds was a TBC period. Sniffing was recorded from the 41st to the 120th second. Immediately following this, the animal was provided a 10-gram portion of uncontaminated wet mash and allowed 10 minutes to eat. The air during this 10-minute period was the same as the second 60 seconds of the previous 3-minute period. Following the 10-minute feeding period, the animal was returned to its individual living cage where it was held until all animals had completed this portion of the daily treatment. At that time, all animals were placed simultaneously into individual feeding chambers and given a 25-gram portion of wet mash for a 30-minute eating period. The air in these feeding chambers was always odorless; i.e., from a source comparable to Channel 1.

The wet mash was prepared 24 hr. in advance of use. For use in Channel 3, TBC was dissolved in methanol and then mixed with powdered food. This mixture was then placed into a fume hood for approximately 23 hours. Immediately before use, it was sorted into desired weighted portions; water was then added to form a thick paste. The same procedure was followed for all other food preparations except that TBC was not added except as noted below. After being presented to the animal, the food was re-dried and then re-weighed.

Prior to the experiment proper two groups of five hooded Long-Evans, male rats were placed on a two-week 23-hour food deprivation schedule, but with feeding in their home cages. Following this, they were put through all of the conditions described except that the air flow contained neither food nor TBC; i.e., training was with clean air. This training period was 15 days in duration. Food consumptions were determined, but sniffing was not measured during this period.

Experimental Design

A summary of the experimental conditions over the 20 test days is presented in Table 2. The intent of this design was to permit a comparison of habituation to TBC plus food odor and to food odor alone over a large number of days with intermittent changes in the odor condition introduced at different portions of the series. The latter was desired in order to determine the degree to which a recovery from habituation might occur with changes in the stimulus. As the table shows, the experiment was designed so that each group could serve as its own control as well as in comparison to the other group.

The first experimental day contained odorless air. Day 2 was the first day in which the animals had ever experienced any odor at all in the flow system. On this day both groups received the food odor alone. From Day 3-9 Group X received the food odor condition and Group Z received the TBC plus food in the concentration conditions noted. Thus, the first nine days provide the clearest basis for studying day-to-day habituation and for determining the aversive effect of TBC as an odor. The designations, 10/90 and 20/80 represent mixtures of 10 per cent and 20 per cent air from Channel 3 respectively.

Except for days 3-5 and 19 the TBC condition was always a 20/80 ratio. Except for Day 19 the wet mash in Channel 3 always contained 150 ppm of TBC by weight. On Day 19 the mash contained 1000 ppm. Except for Day 20, the food presented for consumption was never contaminated. On Day 20 the 10-minute portions contained 1000 ppm; the 30-minute portions were uncontaminated.

Results

As will be reported below, the same major trends are obtained regardless of whether the sniffing measure used is number of sniffs

TABLE 2

Experimental Conditions for Experiment VI

Days	Group X	Group Z	Form of TBC contamination
1	No odor	No odor	None
2	Food odor	Food odor	None
3-5	Food odor	TBC-Food odor	150 ppm in Channel 3; 10/90 mix
6-9	Food odor	TBC-Food odor	150 ppm in Channel 3; 20/80 mix
10-12	TBC-Food odor	Food odor	150 ppm in Channel 3; 20/80 mix
13-14	TBC-Food odor	TBC-Food odor	150 ppm in Channel 3; 20/80 mix
15-18	Food odor	Food odor	None
19	TBC-Food odor	TBC-Food odor	1000 ppm in Channel 3; 20/80 mix
20	Food odor	Food odor	1000 ppm in 10-minute food ration

per unit time, amplitude of those sniffs, or the amount or percentage of time per unit time spent in sniffing. Some experiments are presented, therefore, in one, and others in another of these measures. A complete analysis and justification will be presented in data to be presented later.

The sniffing results of this experiment are presented in terms of the mean number of sniffs per second per 20 seconds. The three-minute sniff measurement phase was divided into seven 20-sec. blocks as follows: the last 20 seconds of the first minute represents the food odor or baseline period; the next three 20-second periods represent successive portions of the test period whether the odor was changed or not; the last three 20-second blocks represent a final food odor or recovery period. We shall describe these in succession simply as the baseline period and Periods 1 and 2.

Figure 8 presents the mean number of sniffs per second for the baseline period of each day. In inspecting these data, it should be remembered that, except for Day 1, all points represent periods during which only the food odor was present. Thus, any effect of TBC on these measures is due to a persistence from previous days.

The data for Days 10, 11, 16, and 17 were lost in a laboratory accident. Since the primary questions were centered around the results of Days 1-9, the main purpose of the experiment was not affected. Even considering the missing data, however, and viewing the overall trends from Day 2 to Day 20, it is apparent that sniffing decreased more or less systematically regardless of the experimental treatments and in spite of temporary recoveries.

The effect of introducing the food odor on Day 2 was a very large increase in sniffing consistent with what would be expected for an attractant. The magnitude of the ordinate on this day is of some interest because of its very large value. To some degree, especially

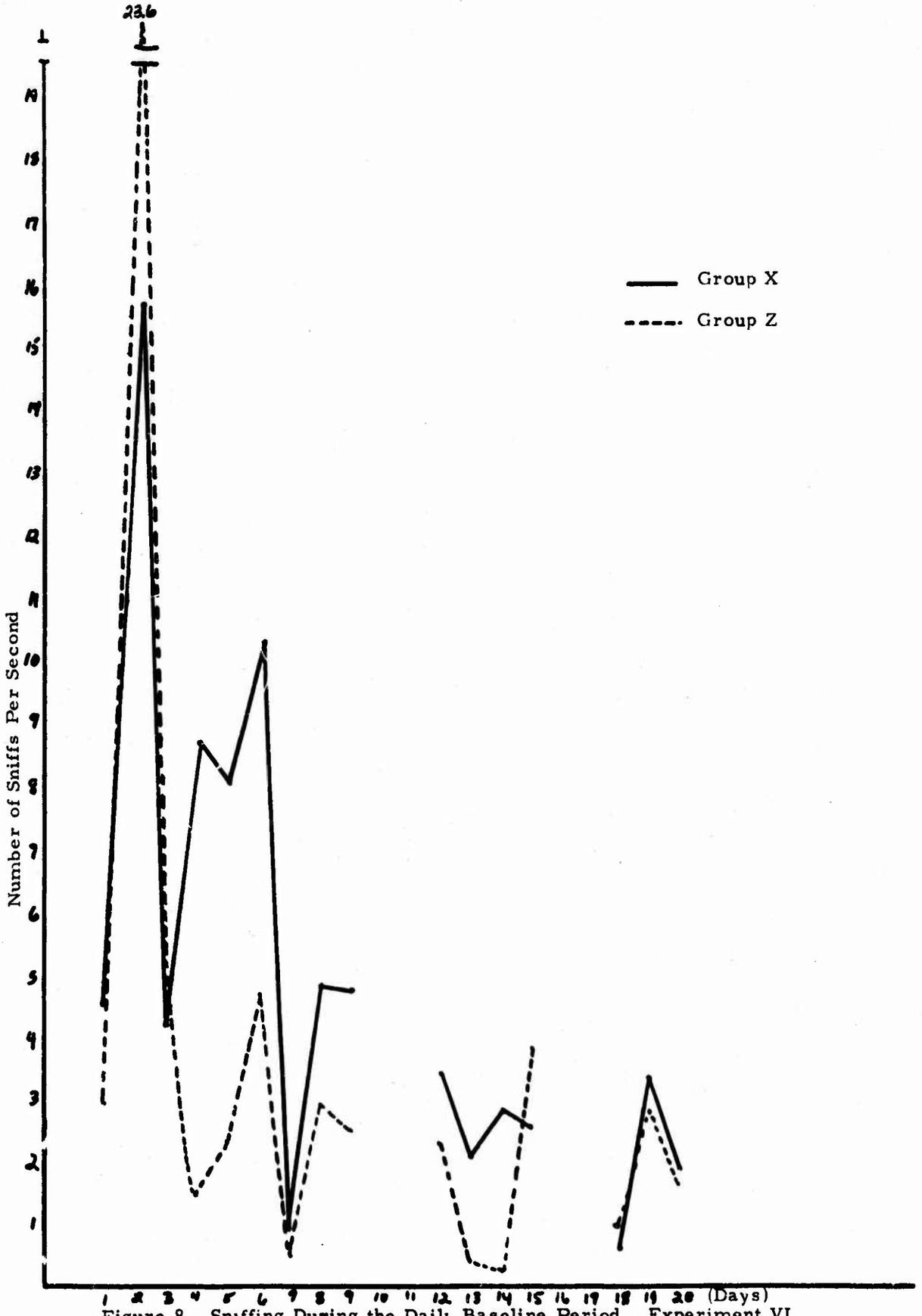


Figure 8. Sniffing During the Daily Baseline Period. Experiment VI.

for Group Z, this is probably an artifact of the technique of recording and scoring the data. The paper speed used was 2.5 cm/sec. for all measurements. For very high sniff rates it becomes very difficult at this chart speed to discriminate individual sniffs; thus, some degree of estimating is required which is not present at the more customary lower rates. In addition, extraneous noise associated with movement of the animal becomes more critical under these conditions. In spite of this, the results compare very faborably to those of Welker (1964) who reported sniff rates of up to 11 sniffs per second using cinematographic methods for rats under conditions which were less conducive to sniffing than those reported here. It may be noted that our data fall easily within that upper limit except for Day 2 and on that day a very high sniff rate is predictable from our earlier hypotheses.

The points of Day 3 still represent the same experiences for both groups since Group Z did not have the TBC until the baseline period of Day 3 ended. Day 4, therefore, shows the persisting effect of TBC from Day 3. The effect was clearly an aversive reaction, i.e., reduced sniffing rate. From this point on, Group Z recovered relative to Day 4, but not up to its Day 1 and Day 3 levels. At the same time, it remained consistently below Group X although it had the higher rate on Days 1, 2, and 3. Thus, while the data suggest some

sporadic partial recoveries on some days, it is reasonable to conclude that there was an incomplete habituation to the TBC odor. In a looser sense, even in the presence of food odor alone, these animals sampled the air very cautiously beginning with their first TBC experience and continuing throughout.

Unlike habituation to an aversive which is indicated by a recovery of sniffing, habituation to an attractant is indicated by a reduction in sniffing. It is hard to explain what happened to Group X on Day 7, but whether this day is considered or not, the pattern of behavior over Days 2-9 for this group strongly suggests an overall habituation. It is of considerable interest to note that the decreasing trend continues right through days in which TBC was presented. On these days, however, the rate of sniffing is already low enough so that demonstration of an aversive effect might be difficult.

Days 18 and 20 were food odor days for both groups. Day 19 represented an increase in the concentration of TBC in the food source of Channel 3 by a factor of four. The effect, as may be seen, was a slight increase in sniffing for both groups. This suggests a response to a novel, but not additionally aversive, easily identified stimulus.

Figure 9 presents a plot comparable to that of Figure 8 except that the data are for the first 20 seconds of Period 1. The figure shows the immediate effect of introducing TBC into the food odor. That effect for Group Z on Day 3 was not importantly different than the Day 3 response of the other group. The consistent downward trend on successive days, however, indicates that the TBC odor was aversive when it was present.

The response during this period depends upon both adaptation and habituation to the degree that they are involved. Both are expected to operate in the same direction so that their effects cannot be separated

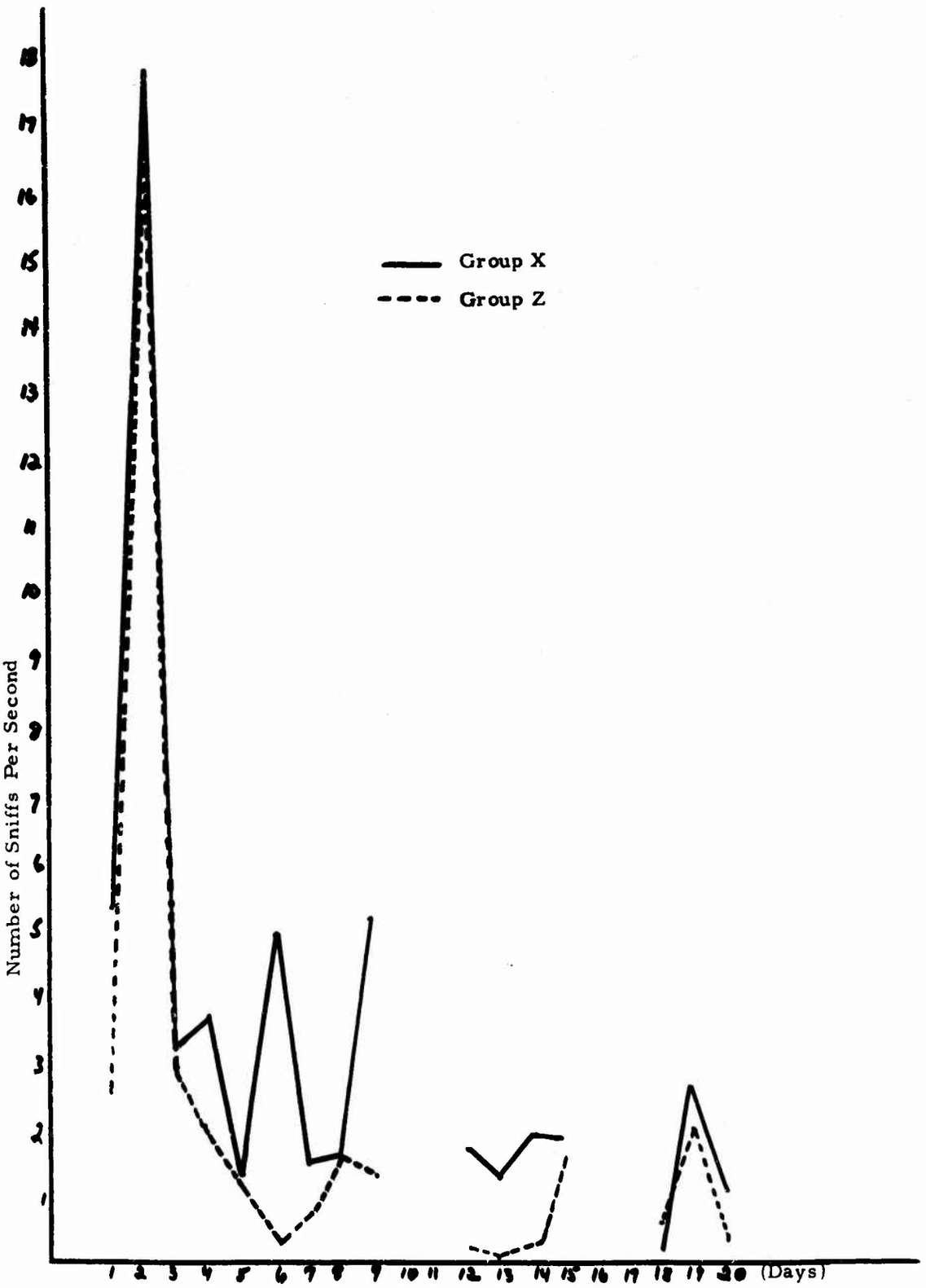


Figure 9. Sniffing During the First 20 Seconds of Period I. Experiment VI.

in this experimental period. Regardless, the figure shows again that there is a general day-to-day habituation which is independent of unique daily effects.

Figure 10 presents the first 20-seconds of Period 3 during which both groups always received the food odor alone (except on Day 1). It is clear that there are no importantly consistent differences between the groups and, again, that there is an habituation over the experimental days. Of further interest in comparing this with the last figure is that from Day 4 on, the sniff rates of this figure are generally a little greater than in Figure 9. This suggests the recovery effect hypothesized in Figure 4. It is not a strong effect, however.

The effects of increasing the TBC concentration in the source on Day 19 is of particular interest and is not well-detailed in the previous figures. Figure 11 was prepared to look at this more closely. The figure shows the sniff rate per 20-second block for Days 18-20. Both groups had identical treatments on those days, i.e., food odor on Days 18 and 20; TBC plus food odor on Day 19. The data for Day 18 suggest no differences between the groups. The immediate effect of TBC on Day 19 was a reduction in the sniff rate for both groups. Group X recovered for a time during Period 1, but Group Z did not. Both groups show an increase in sniffing with removal of the TBC. The effects are small, but generally consistent with expectations. In fact, these expectations are also seen for Days 18 and 20 so that they cannot be considered significant for Day 19. What is unique about Day 19 compared to the other two days is that only on this day were the two groups separated. We conclude from these figures that the animals had, by this time, developed a time-bound, conditioned anticipation of TBC. The only possible effect of the increased concentration was to make the response of Group Z slightly stronger. This is not unreasonable since this group had had the greater number of TBC exposures over the course of the experiment.

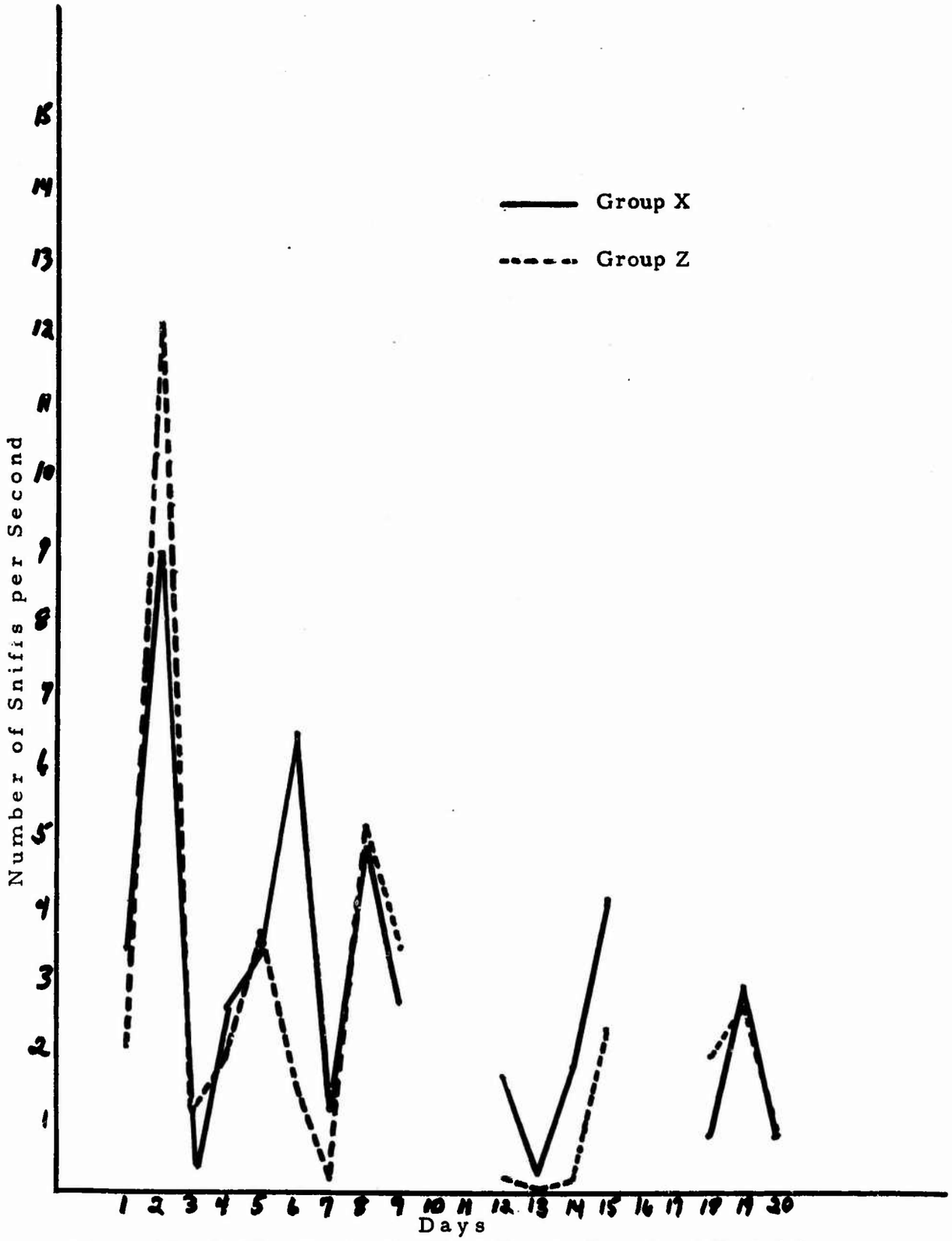


Figure 10. Sniffing During the First Twenty Seconds of Period 3. Experiment VI.

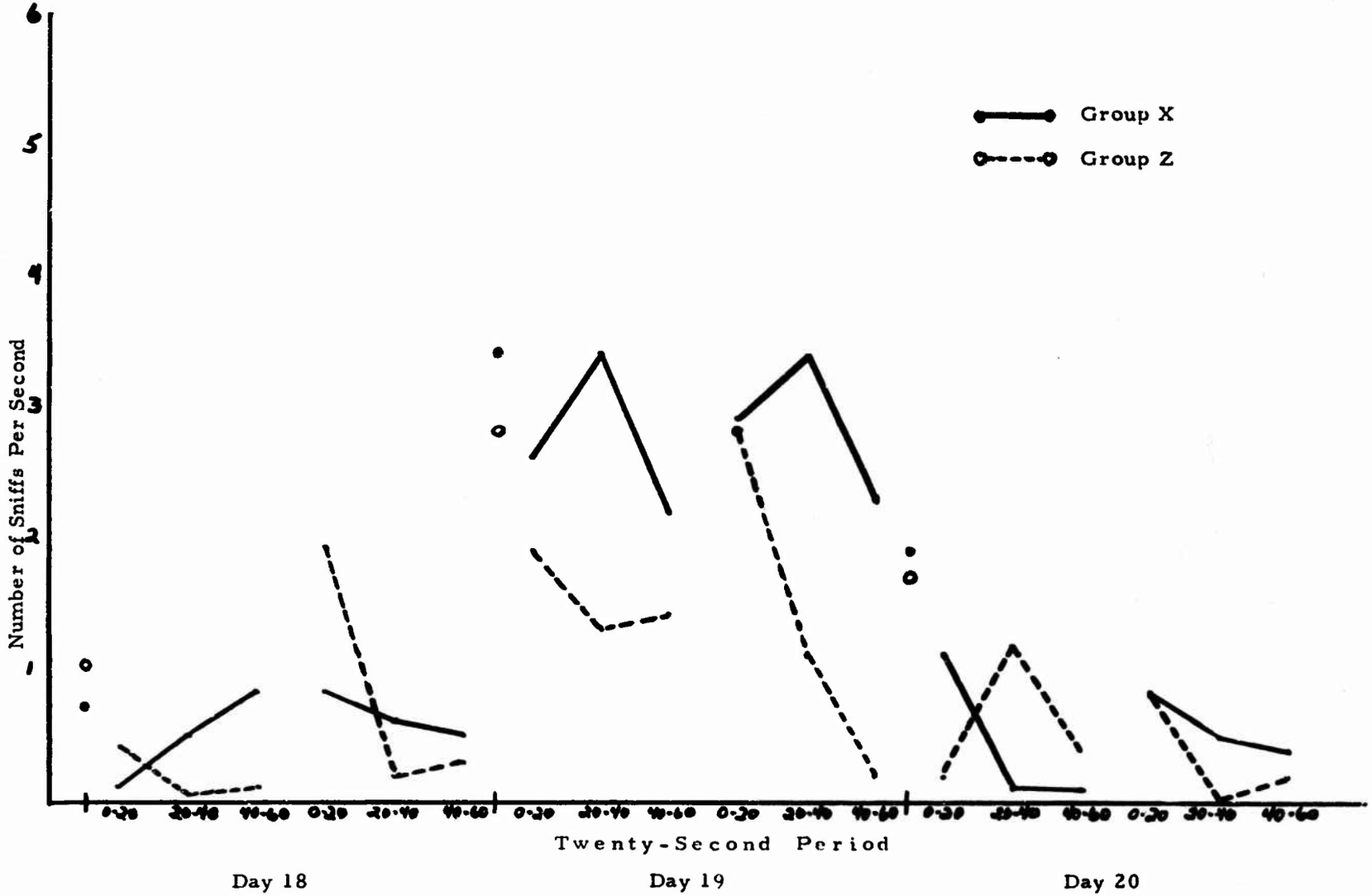


Figure 11. Sniffing During the First 20 Seconds of Each Period of Days 18-20. Experiment VI.

All things considered, the results obtained from analysis of the sniffing data suggest: (1) an habituation to food odor from day to day (Group X using days 1-3 as a reference); (2) a partial habituation to TBC odor (Group Z compared to Group X and to its own initial reactions to food odor and to TBC); and (3) an overall habituation to the stimulus situation regardless of intermittent changes in conditions and temporary reactions to them.

Figure 12 presents the food consumption data. The first t. days are the days just before the experiment. The 10-minute feeding period was conducted with either the food odor or TBC while the animal was eating. There is no evidence at all that eating was influenced by either. Nor is there any worthwhile suggestion in the data of a relationship between the previous sniffing and either of the food consumptions. The only positive aspect of these data that we can interpret in a relevant fashion is that when the 10-minute portion was contaminated on Day 20, food consumption in that period was reduced and that this effect persisted into the 30-minute portion. The relation between the two on this day is consistent with our earlier results showing that the less eaten in the contaminated period, the more that is eaten of the uncontaminated portion.

The reduction in food consumption on Day 20 during the shorter period is clear, but compared to comparable data reported above, it is not very large. The concentration used was considered high for direct food consumption. This result raises interesting questions. That is, either 1000 ppm is not a large dosage for this compound or the presence of this compound in the air while eating was so familiar to the animals by this time that they did not discriminate it as a highly aversive substance even in food. Some support for the latter is given by the fact that Group X ate less since this group was less frequently exposed to the TBC and, therefore, would be more likely to treat it as an aversive.

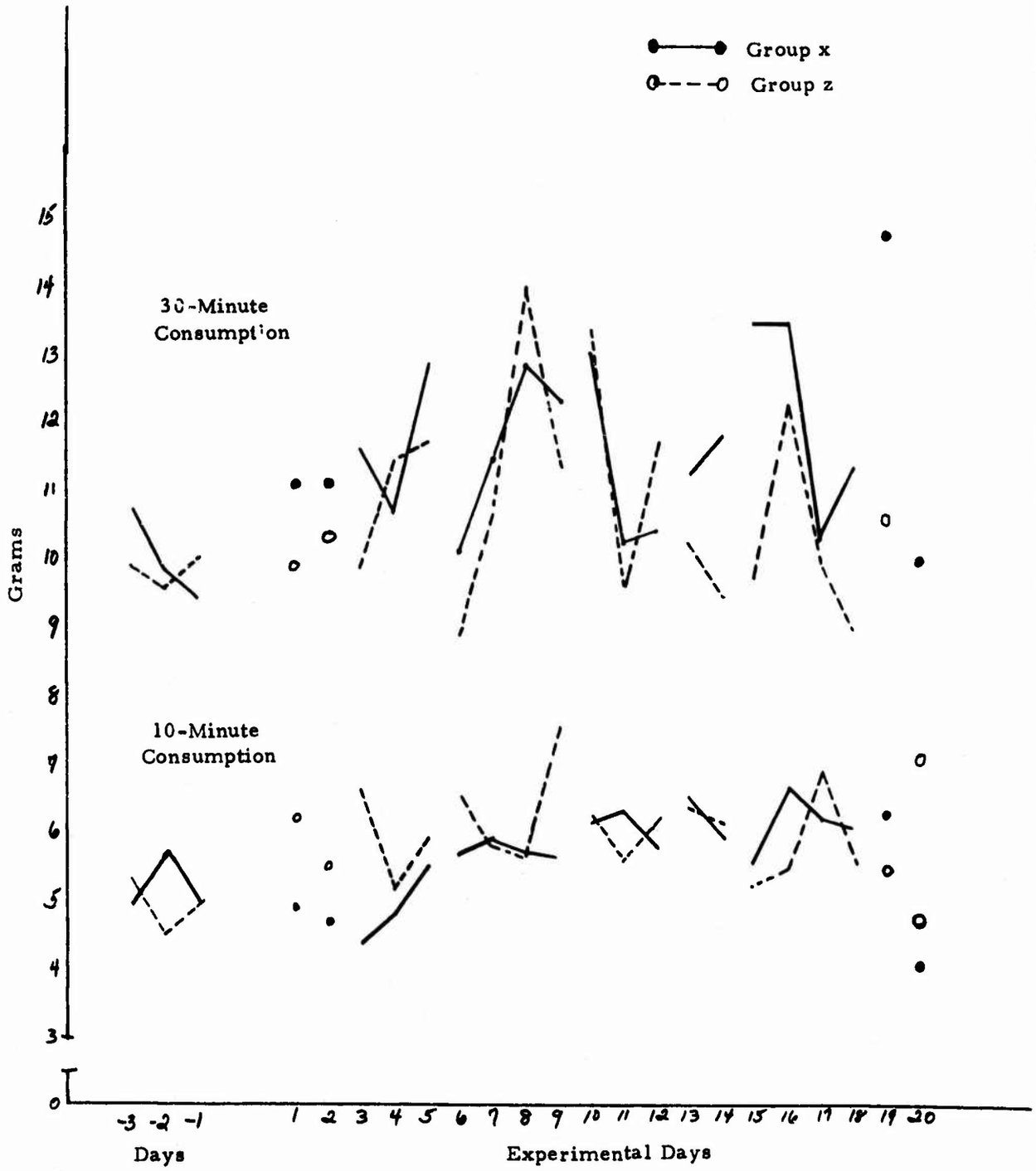


Figure 12. Food consumption. Experiment VI.

In any case, in terms of our original experimental question the data are clear in showing that a substance which can be shown to have aversive properties as an odor does not necessarily act as a repellent to food consumption. Comparing this finding with those of Experiment I suggests that the critical conditions for an aversive odor to act as an important repellent to eating is that it be associated with an aversive taste during the initial exposures.

Experiment VII

This experiment was intended to obtain a variety of kinds of preliminary information for guiding further research. Some of the results have general value and, therefore, are reported.

One concern of the experiment was with the problem of adaptation to odor. The basic question was whether animals forced to remain in an air-contaminated environment adapt to a repellent odor sufficiently to reduce the effectiveness of that odor as an aversive barrier. This was studied with the use of a short runway (Appendix I) in which the odor was presented to the animal in the starting box for prescribed periods before the animal was released to the runway proper. Two air streams directed upward from the floor to exhausts in the ceiling of the runway just before the goal box contained the same compound in the same concentration. The goal box contained a small, dry, food pellet (.01 gram). Animals maintained on a 23-hour food deprivation schedule were pretrained to run to this reward.

A problem associated with this kind of experiment is the effect of delaying the animal in the starting box on running performance. The animals were pretrained on a variety of starting box delays, therefore, prior to the test phase.

A second purpose of the experiment was to evaluate the utility of a more precise specification of the concentration of repellent in the air inspired by the rat. An approach to this is by calculation using the ideal gas law equation. This estimate assumes an equilibrium state and that, of course, was not the case. Nevertheless, the law provides a useful approximation and is often used for this purpose.

There are a number of problems associated with this use of the gas law equation. For one, the vapor pressure must be known and it has not been specified for most compounds used as repellents. Another difficulty is that the actual experimental variable to be manipulated becomes the air temperature since, for constant pressure, it is the temperature which will determine the concentration. It is possible that the animal will react to temperature differences, however, and since the concentration increases as the temperature increases, the two variables are perfectly confounded. This approach, then, can be useful only when the temperature differences are so small that differential responding to them does not occur or when the experimental design provides controls which permit the evaluation of the chemical effect over and above the temperature effect. This experiment was designed with such controls in mind.

The compound used for this experiment was dibutyltin diacetate (DBDA)*. Three air temperatures, 24°C., 30°C., and 34°C. were used to vary the concentration. Calculated values of the concentration are expressed in moles/liter as a function of temperature in Figure 13. The experimental concentrations, read from the figure, were 6.8×10^{-12},

* We are indepted to Mr. Robert Ringwood of the M&T Chemical Co. for the constants used in the calculations: Molecular weight = 351.02, Freezing Point = 10°C., 2mm, Boiling Point = 139°C., 5mm. On this basis the constants, a and B can be determined from: $\ln P = a/T + B$ and then used to calculate the values in Figure 13.

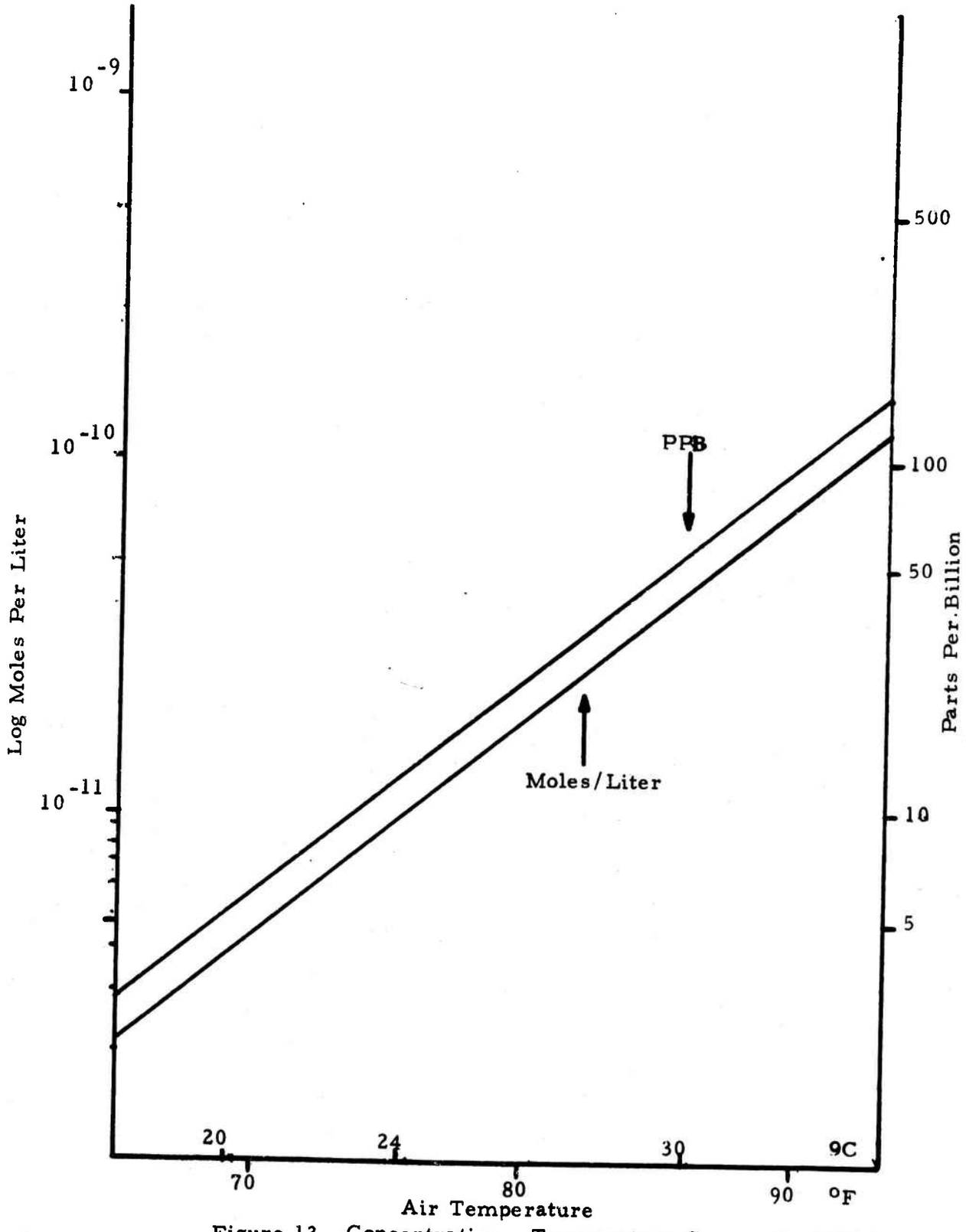


Figure 13. Concentration - Temperature Curves for DBDA.

2.8×10^{-11}, and 3.0×10^{-11} moles/liter. The equivalent values in parts per billion are 9.4, 30.1, and 50.2.

Another purpose of the experiment was to investigate the relationships among the three basic measures of sniffing, rate or number of sniffs per unit time, time or duration of sniffing, (or percent time spent sniffing), and average peak amplitude in a behaviorally-performing situation. That is, we already had data from rats enclosed in a small glass chamber (Experiment IV) in which sniffing was unrelated to a subsequent behavior. Those data suggested that the functions are different. In runways, the animal is confined (in a larger space) for a delay period and then permitted free running. The relationships among the measures might differ from those obtained in a more restrained situation. If all three measures were to show the same trends, as suggested by Fig. 4, a considerable economy in data analysis could be achieved by using dependent measures of convenience.

Finally, this experiment was intended as very preliminary to an exploration of the effects on sniffing and performance of surgical interference in the olfactory bulbs. A summary of the anatomy of the rat's olfactory system and of our experience in attempting to interfere with it is presented later. It may be noted here that attempts to produce a variety of kinds of lesions in the bulbs of animals prepared for this experiment yielded seven animals with lesions that could be reasonably confirmed by later histology. The lesions in all cases were very small. These animals constituted the experimental group data of this experiment. Four animals with sham operations made up the control group. The experiment was performed three months after surgery when the animals were approximately seven months old. We emphasize that the experiment was set up to be exploratory in several ways rather than definitive in any way.

Procedures

For approximately one month prior to the experimental phase, all animals were maintained on a 23-hour food deprivation schedule. Each animal was run five trials per day in the runway with a .01 gram food reward. Each of the five trials was for a different starting box delay period, viz: 10, 25, 40, 65, and 80 seconds. The animals were run one trial at a time and then returned to their home cages until all other animals had been run through that trial. They were run in the same sequence of subjects every day; however, the order of the delay period was balanced so that each animal started with a different delay period each day and was followed through a different delay sequence. The actual order of the delays was randomized initially.

The experimental phase was identical to the training phase except that the starting box air and the air barrier before the goal box were contaminated with DBDA at flow rates of 500 cc/min. Clean air from a compressed source was passed over a pure sample of the compound at temperatures of 24°C., 30°C., or 34°C. to provide calculated concentrations of 9.4, 30.1, and 50.2ppb respectively.

Experimental Design

The experimental design over the 9-day test period following training is shown in Table 3. The design consisted of three similar three-day sets. Each set consisted of a fresh air or uncontaminated day followed by two contaminated air days. Each set represented a different concentration. Over the 9 days the first set of days represented 30°C.; the second set was at 24°C. which was the smallest concentration; and the third set was at 34°C. which was the largest concentration. The experimental design was completed factorially by a comparison at all conditions of concentration and delay periods between lesioned and unlesioned animals. Thus, the design was a

TABLE 3

Experimental Conditions of Experiment VII

Day	Condition
1	Clean Air; 30° C
2	DBDA; 30°C, 2.8×10^{-11} m/l; 30.1 ppb
3	DBDA; 30°C, 2.8×10^{-11} m/l; 30.1 ppb
4	Clean Air; 24° C
5	DBDA; 24° C; 6.8×10^{-12} m/l; 9.4 ppb
6	DBDA; 24° C; 6.8×10^{-12} m/l; 9.4 ppb
7	Clean Air; 34° C
8	DBDA; 34° C; 3.0×10^{-11} m/l; 50.1 ppb

2 x 3 x 5 factorial of the repeated (correlated) measurements type. The order of the three-day temperature sets was selected in the hope of minimizing biases that might be associated with an overall habituation or with an overwhelming persistence in response to the highest concentration if it had come first.

Results

The mean time spent sniffing per second in the starting box for the various experimental conditions is shown in Figure 14. Day 1 represents the pure effect of delay time unaffected by any experience with varying chemicals or temperatures. The figure is clear in showing that after the chemical was introduced on the later days it eliminated the trend associated with the delay variable on Day 1. Both groups suggest an increasing and then decreasing function on Day 1. All other curves are essentially flat.

A comparison of Days 1, 4, and 7, all of which were fresh air days, does not suggest that sniffing depended importantly upon the air temperatures involved. On the other hand, all of the data obtained from Days 4-9 were clearly affected by the experience had on the first three days. That the effect is at least largely due to the chemical is suggested by the fact that the temperature on Days 4-6 was the same as the normal temperature of the starting box in which the animals had been trained. Yet, the curves are depressed. In any case, the data do not suggest any adaptation to the conditions due to length of time of exposure as far as sniffing is concerned, since sniffing did not recover at the longer delays. The data suggest that DBDA is very aversive since all concentrations were very small.

A clearer picture of the general effect may be seen in Figure 15 which shows the same data pooled over delay periods. Here it may be seen that the control group showed a systematic reduction in time

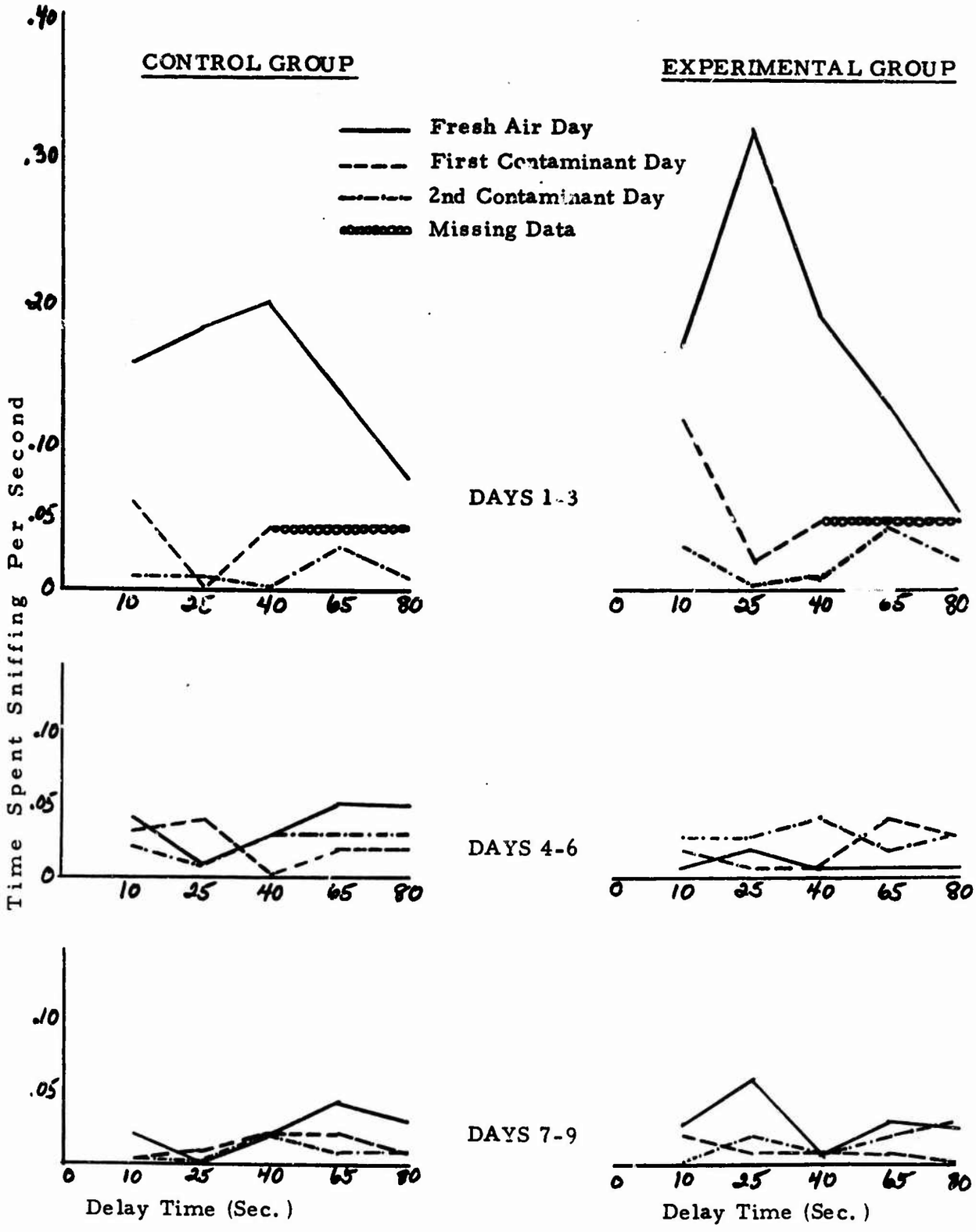


Figure 14. Sniffing Time for the Conditions of Experiment VII.

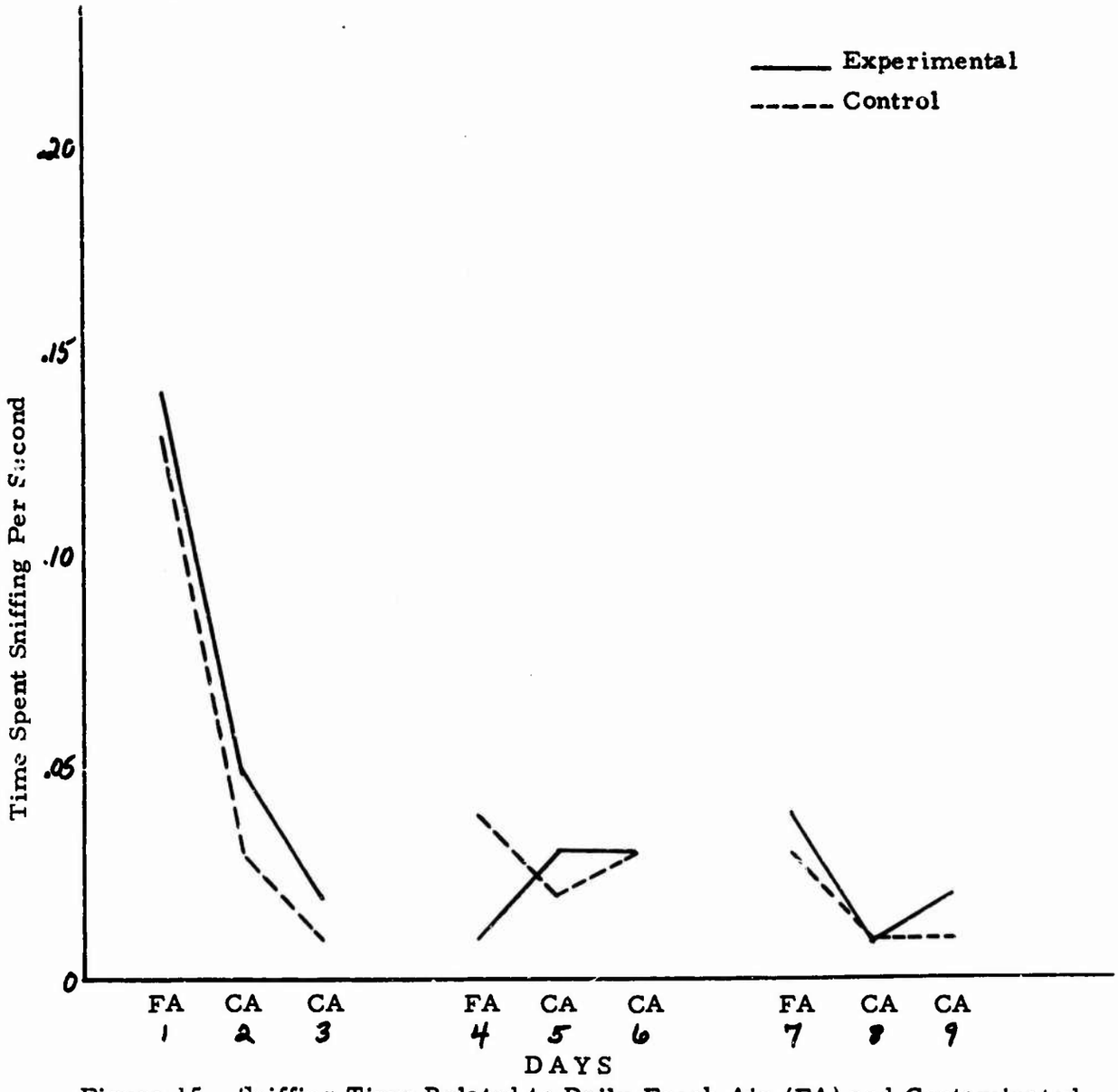


Figure 15. Sniffing Time Related to Daily Fresh Air (FA) and Contaminated Air (CA) Exposures. Experiment VII.

spent sniffing over days. Day 6 is a possible exception. Thus, the data suggest neither a sensory adaptation, i.e., delay effect, nor an habituation, i.e., between-days effect. If anything, there is the suggestion of an increasing, learned aversion over days.

The results obtained with the experimental group are similar except for Days 4-6. There it may be seen that these animals tended to spend more time sniffing on contaminated days than on the fresh air day. This is consistent with our theoretical expectations if it is assumed that the effect of the lesions was to reduce the sensitivity of the animals so that what was a relatively strong stimulus for the control group was a relatively weak one for the experimental animals. That expectation is supported on the other two sets of days where it may be seen that the experimental animals tended to spend more time sniffing throughout. The differences, however, are very small.

Figures 16 and 17 present the same kind of plots for the mean number of sniffs per second. There are some differences between those two figures and the previous two as far as details are concerned, but the overall conclusions about the effects of the experimental conditions are similar. The data of Days 4-6 are clear also in suggesting a loss of sensitivity of the experimental animals. The results are much less clear than those obtained with the time measure.

Figures 18 and 19 present similar plots for the mean amplitude of the sniffs. Although we consider this the least reliable of the three measures due to problems associated with recording, there is no major difference in the trends. It is reasonably clear that the two groups did not differ in any basic tendency toward sniff amplitudes. Again, the Day 4-6 data suggest a different effect of the weakest concentration on the two groups; again, they suggest a reduction in ability of the experimental groups to evaluate the stimulus.

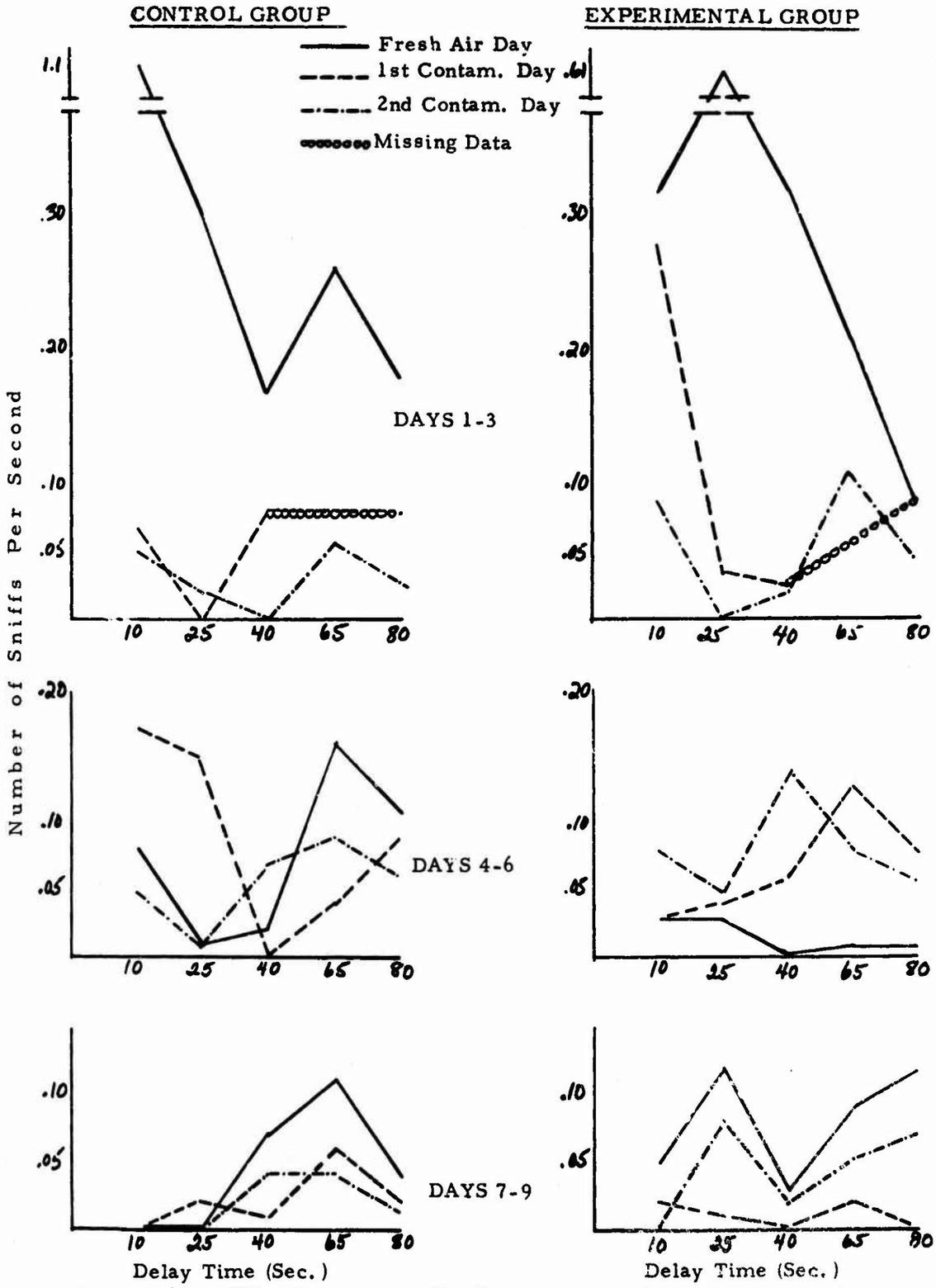


Figure 16. Sniffing Rate for the Conditions of Experiment VII.

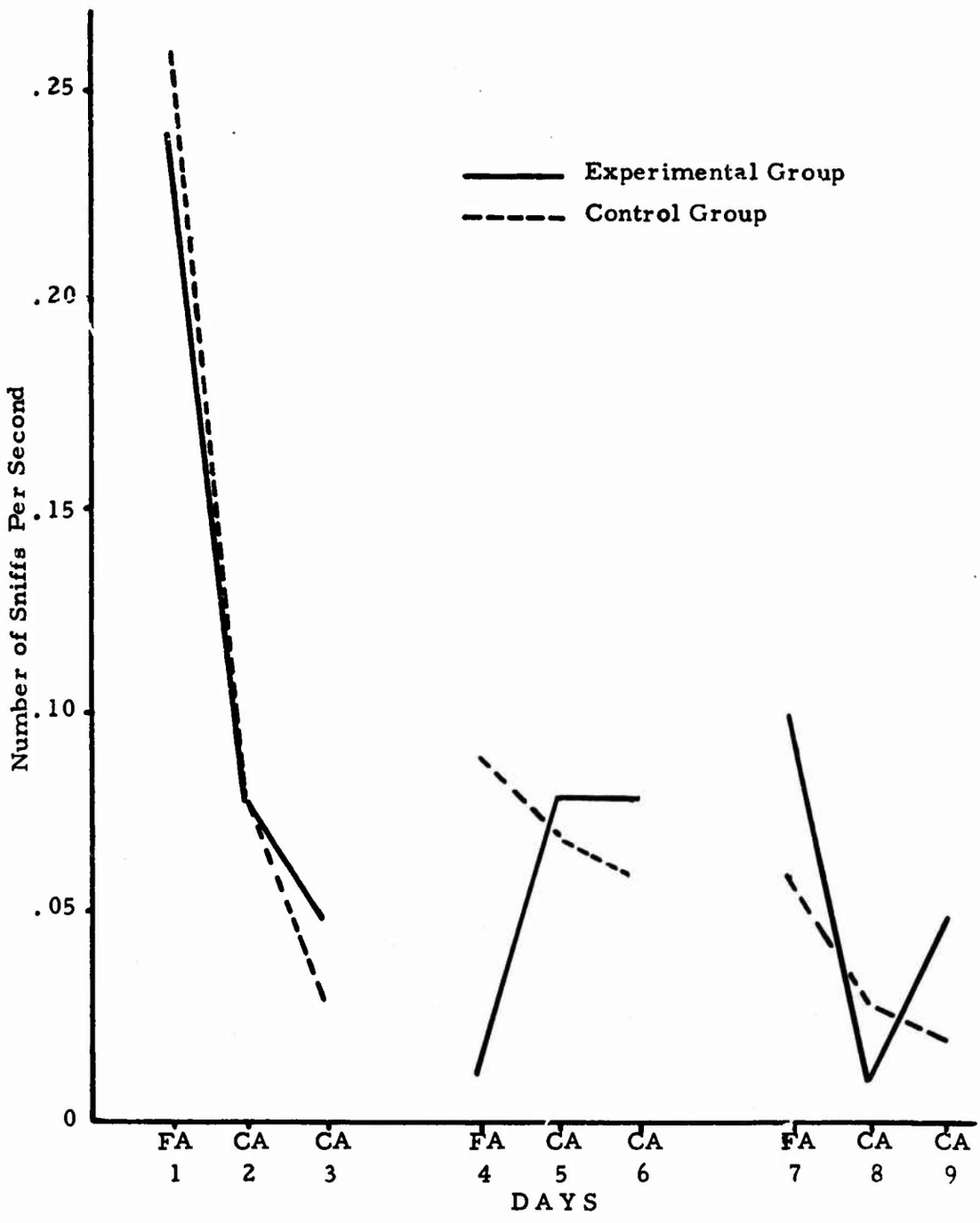


Figure 17. Sniffing Rate Related to Daily Fresh Air (FA) and Contaminant Air (CA) Exposures. Experiment VII.

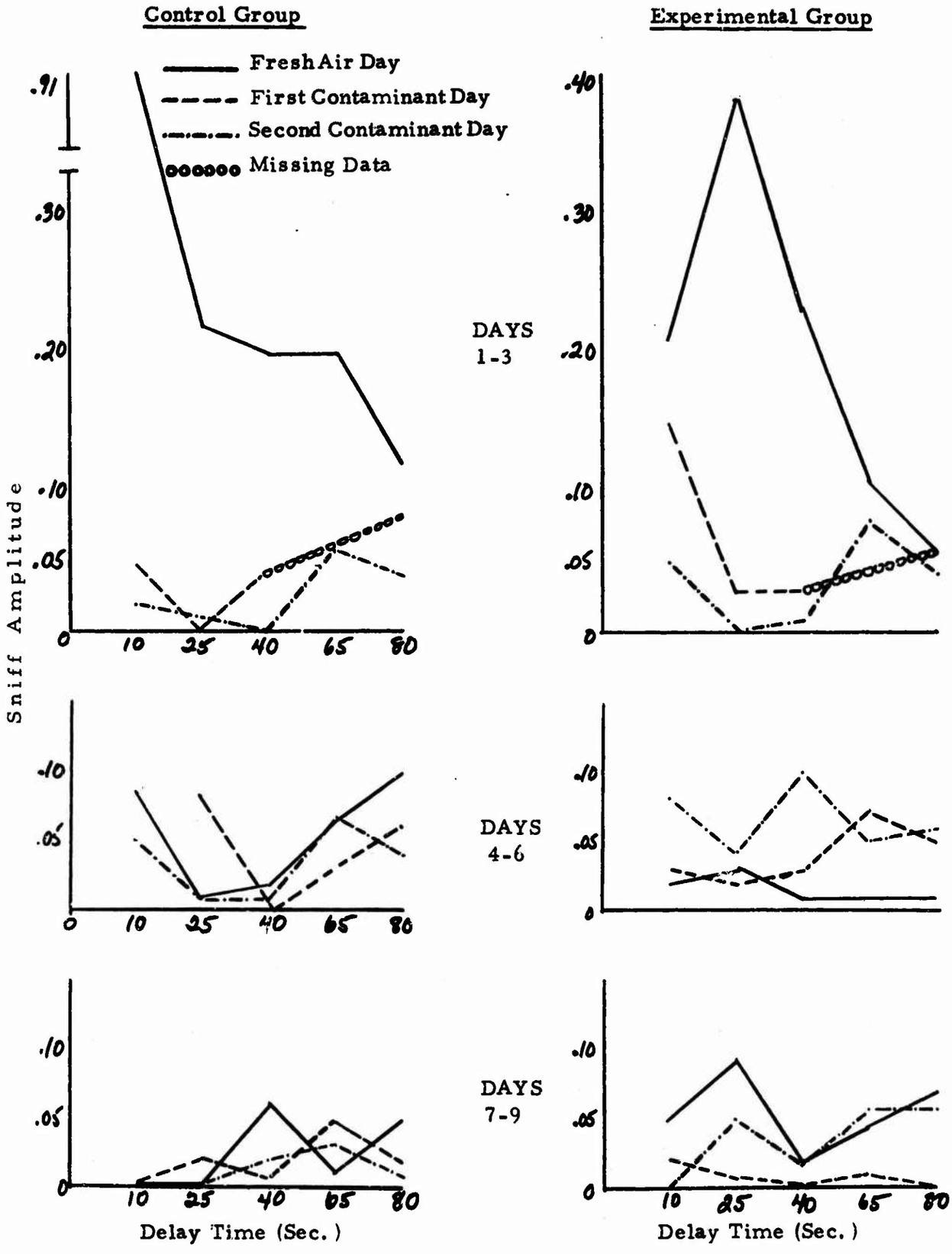


Figure 18. Sniff Amplitude for the Conditions of Experiment VII.

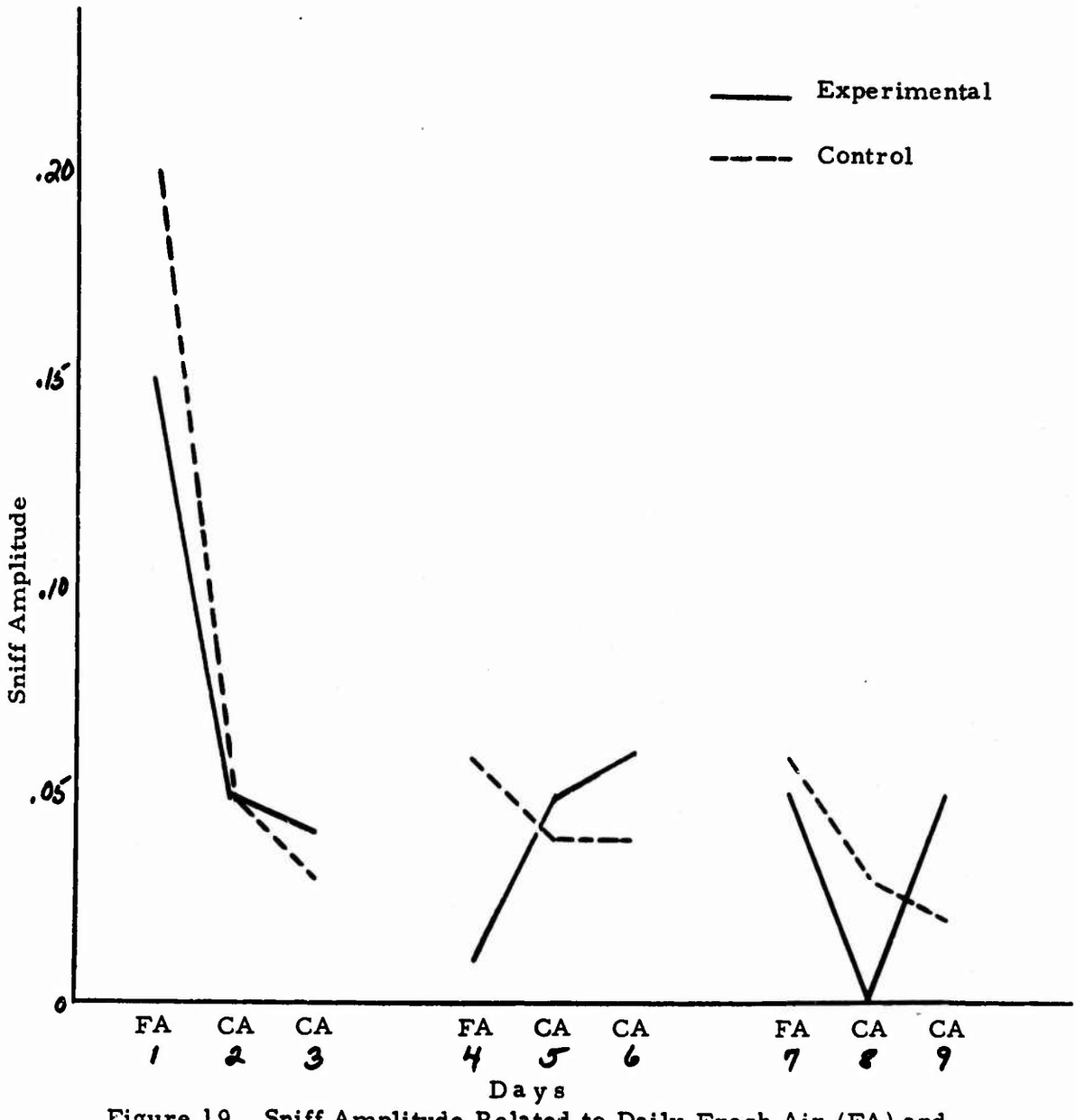


Figure 19. Sniff Amplitude Related to Daily Fresh Air (FA) and Contaminated Air (CA) Exposures. Experiment VII.

Looking across Figure 14-19, the safest hypothesis concerning the two animal groups appears to be that the experimental animals may have spent slightly more time sniffing, but, perhaps, with smaller amplitude sniffs except in the presence of weak odorants. This conclusion is extremely tentative, of course. Much less tentative is the suggestion that, as measured by sniffing variables, for DBDA over the range of concentrations used, there does not appear to be either an adaptation or a habituation for exposures of at least 80 seconds.

The mean running time is presented in Figure 20 and 21. In Figure 20, on Day 1, it can be seen that the effect of starting box delay as a variable tended to be faster running as the delay period increased. The last point of the control group is an inversion of this trend. In view of the small number of animals, this inversion should be viewed as error. The data also suggest an interference to running on Days 2 and 3 since the Day 1 curves are consistently lower. Since the sniffing curves do not suggest a day-to-day habituation in this period, it would appear that the barrier did operate as a deterrent. This is suggested again on Days 7-9, but not on Days 4-6. Since Days 4-6 represent the training temperature, we cannot conclude that this failure of the animals to be deterred was due to the low concentration. It is as easy to conclude that they were deterred on other days by the higher temperatures of the air barrier.

The clearest comparison of the two animal groups is provided by the fresh air days. The general conclusion suggested across all of the data is that the lesioned animals tended to spend slightly more time sniffing in the starting box and to run more quickly when released. The number and peak amplitude of sniffs was essentially the same for the two groups, a result in agreement with Welker (1964).

Table 4 provides Spearman Rho correlation coefficients between each pairing of sniff measures and between the time spent sniffing and running time separately for each group of animals and for each three-day set. Although the number of animals is very small, all sniff inter-correlations for the experimental group are significant at the

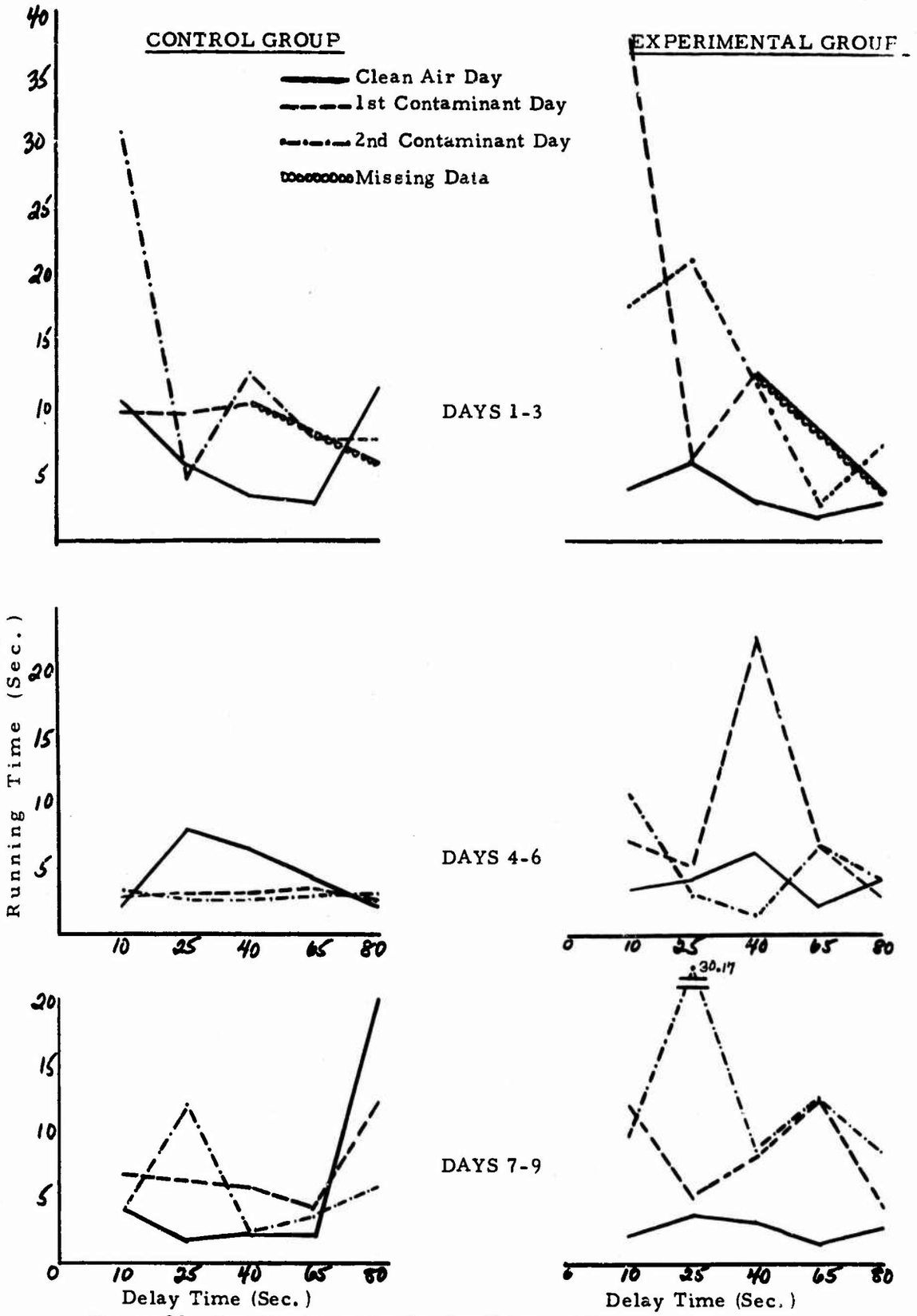


Figure 20. Running Time for the Conditions of Experiment VII.

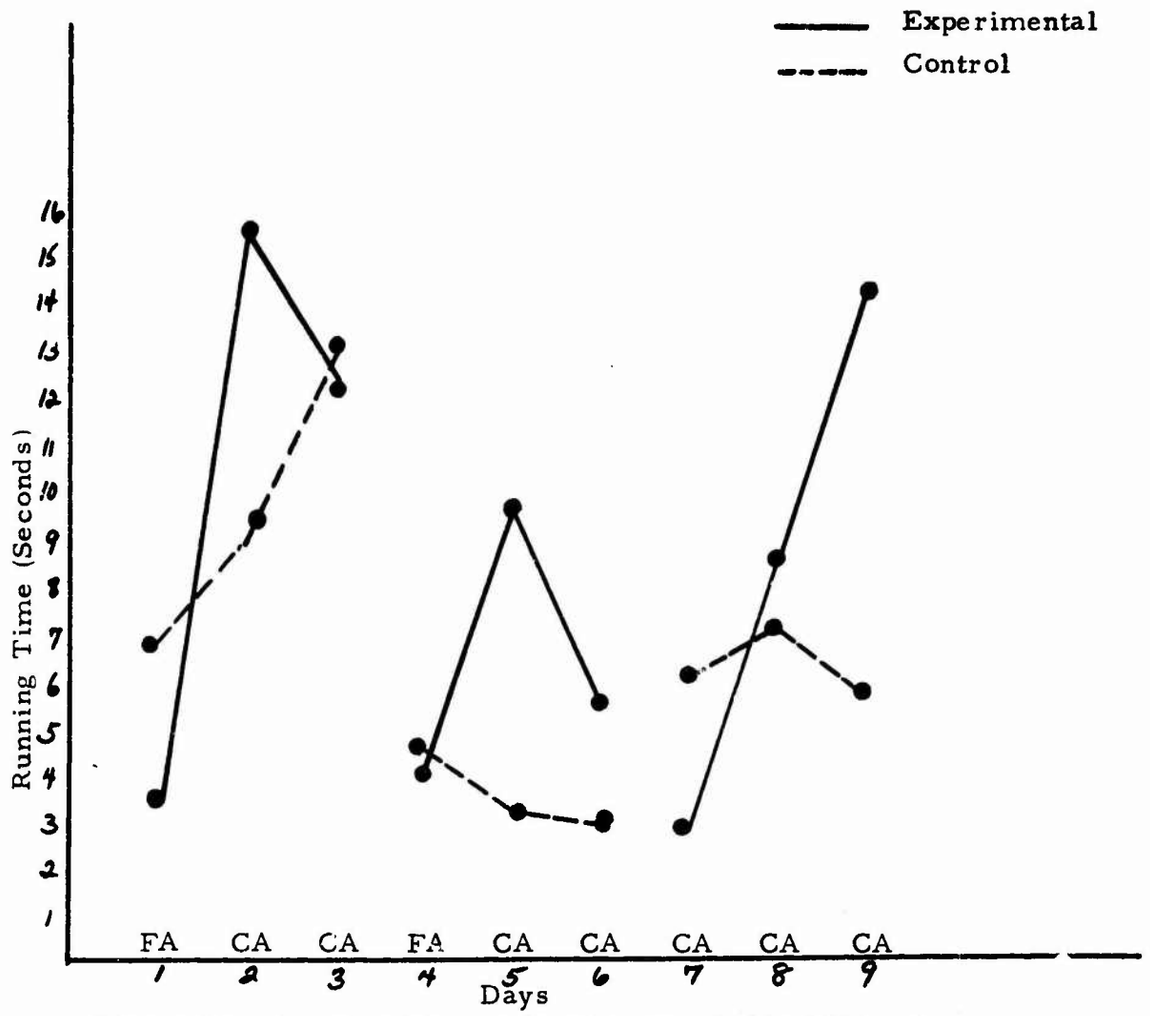


Figure 21. Running Time Related to Fresh Air (FA) and Contaminated Air (CA) Exposures. Experiment VII.

TABLE 4

RHO Correlations for Experiment VII+

Group	Time spent sniffing vs. no. of sniffs/sec.	Time spent sniffing/sec. vs. amplitude of sniffs	Time spent sniffing/sec. vs. running time	No. of sniffs per sec. vs. amplitude of sniffs
		Day 1		
Experimental	.94*	.99*	-.26	.93*
Control	1.00**	.99	.66	.99
		Day 2		
Experimental	.86*	.97*	-.57	.82**
Control	.99	1.00**	.47	.99
		Day 9		
Experimental	.97*	.99*	-.18	.99*
Control	.94	.90	.43	.96

* $p < .01$

** $p < .05$

+ For N=4, the coefficient must be equal to 1.00 for significance at $p < .05$.

.05 or .01 level. For the control group, which contained only four animals, a coefficient of 1.00 is required at the .05 level. This was actually attained for two of the comparisons, and the rest were very high. All in all, the table suggests that the more time an animal spends sniffing, the greater the number of sniffs, and the greater the amplitude of the sniffs. For many purposes, then, it appears that the three measures are interchangeable. On the other hand, to answer special questions, or for specific situations as described previously, there is probably useful information to be gained from an analysis of all three.

The correlations with running time were not significant, which suggests a greater variability among the running time measures since the sniffing time measure was highly correlated with the other sniffing measures. At least, this probably accounts for some of the low inter-correlations. An important trend is suggested, nevertheless, for further consideration, i.e., an inverse relationship for the experimental animals and a positive one for the controls. If supported, this suggests that the experimental animals which spent more time sniffing in the starting box ran faster when released whereas the opposite was true for the control animals. Also interesting is the suggestion of important individual differences in sensitivity which have a bearing on the behavioral measure.

The data do not permit any conclusion about the effects of air temperature vs. concentration on the sniffing and running responses. It was hoped that the fresh air days would have provided a baseline against which the contaminated days could be compared for chemical effect. However, although the first repellent condition used was not the most severe, it did have a persistent effect which overwhelmed the later fresh air days. At least for strong aversives, problems of this sort are so severe, apparently, that it must be concluded that temperature manipulations should always be avoided as a means for varying

comparison concentrations. At the same time the results indicate how important it is to maintain the temperature of the air at the same level for fresh air and contaminant concentrations.

A major suggestion from this preliminary experiment is the possibility that lesioned animals have a different relationship between their sniff reactions to the chemical and their response to an air barrier containing the chemical. Related to this, these results do appear to provide a clear support for use of an apparatus situation in which a contaminated air barrier is used to evaluate repellency.

Experiment VIII

Based upon Experiment VII, a second experiment was set up to study the effect of exposure to a repellent chemical in air on the repellent effectiveness of the chemical. The working hypothesis employed was that if an animal is exposed to a chemical continuously over a short time those receptor processes and/or behavioral processes which respond to the chemical will adapt and/or habituate. The effect will be a reduced aversion to the chemical.

The general methodology was the same as that of the previous experiment. The same short runway was used. The chemical was DBDA at an air temperature of 30°C. (2.8×10^{-11} m/l) presented at a flow rate of 500 cc/min. to the animal while the animal was in the starting box. The same conditions were presented as the air barrier before the goal box.

Subjects

Thirty, experimentally naive, male hooded rats, 112 days old at the start of training were used as subjects. Loss of one animal during training reduced the subjects to 29.

Training

The animals were put on a 23-hour food deprivation schedule beginning three weeks prior to the experiment. The day before the experiment each animal was allowed five minutes of exploration of the apparatus with the starting and goal box doors open and two .01 gm. pellets in the goal box. After this day and for the next 35 days, they were trained to run for one .01 gram pellet. During training they were given three trials per day, about 20 minutes apart. Each of the three trials represented a different delay time in the starting box. The times used were 10, 40, and 80 seconds. Air flow was always uncontaminated, but otherwise simulated the experimental conditions. Running times, but not sniffing, were recorded.

Experimental Procedures

The animals were matched by rank order to form two groups of approximately equal mean running time and variance based upon all data of the last three training days. One group of 14 animals was then always run one trial per day with a 30-second delay in the starting box; the other of 15 animals was run one trial with a 120-second delay. This procedure of one trial per day at a new, constant delay was initiated three days before the test series. The experimental series which followed was eight days long. On the first day and the last two days the animals were presented with fresh air exactly as before. On the intervening five days the air was a mixture containing DBDA as noted above. Sniffing was recorded in the starting box on the first, third, and fifth DBDA days and on all three fresh air days.

Results

The sniffing data were analyzed in terms of time spent sniffing per second, number of sniffs per second, and amplitude of sniffs. Figure 22

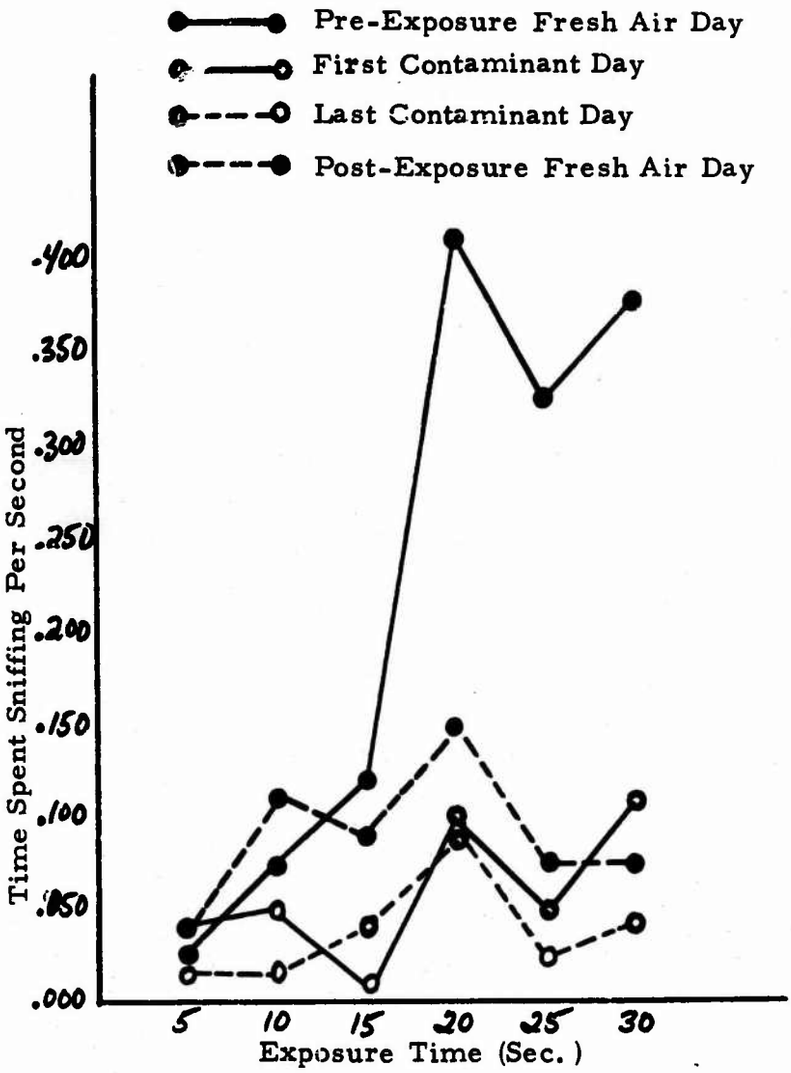


Figure 22. Mean Sniffing Time Per Second of the 30-Second Group as a Function of Exposure Duration. Experiment VIII.

presents the mean time spent sniffing per second for each five seconds for the 30-second group on the first and last DBDA days and on the fresh air days just before and just after those days. Again there may be seen a strong depression of sniffing associated with DBDA. The curves do suggest an increase in sniffing as a function of time, with DBDA, but the rise is not nearly so great as with the pre-exposure fresh air day. Thus, these data cannot be used to support the idea of an adaptation or habituation as a function of exposure time. The same kind of data are presented in Figure 23 for the 120-second group. The conclusions permitted are the same. In fact, the data for the first 30 seconds of this figure are reasonably comparable to those of the previous figure.

The running times of the animals are presented in Figure 24. Also shown are the mean sniffing times per second per day. The figure shows an immediate effect associated with presentation of DBDA in the air stream just before the goal box. That is, both groups show a decreased running time on the first contaminant air day; the response of the 30-second group is marked which suggests that the 120-second group may have developed a tolerance for the chemical. Since there was no evidence of adaptation, such a tolerance would have to have some other basis. On the days following, there is a trend suggesting an increasing recovery so that by the last day recovery is complete for both groups. Removal of the DBDA from the air stream on Day 8 appears to have produced a second slowing effect and tendency to recover.

Figure 24 also shows the sniffing times obtained in the starting box. It is clear that the chemical had a marked effect on both groups. There is no indication of a recovery (habituation) of sniffing during the contaminant period; some recovery is shown on Day 7, but it did not continue on to the next day. On this basis it would seem that over the course of this experiment, the chemical retained its properties as an aversive odorant, that it did serve as a deterrent, but that its effect as a deterrent, as shown by the running time measures, was only temporary.

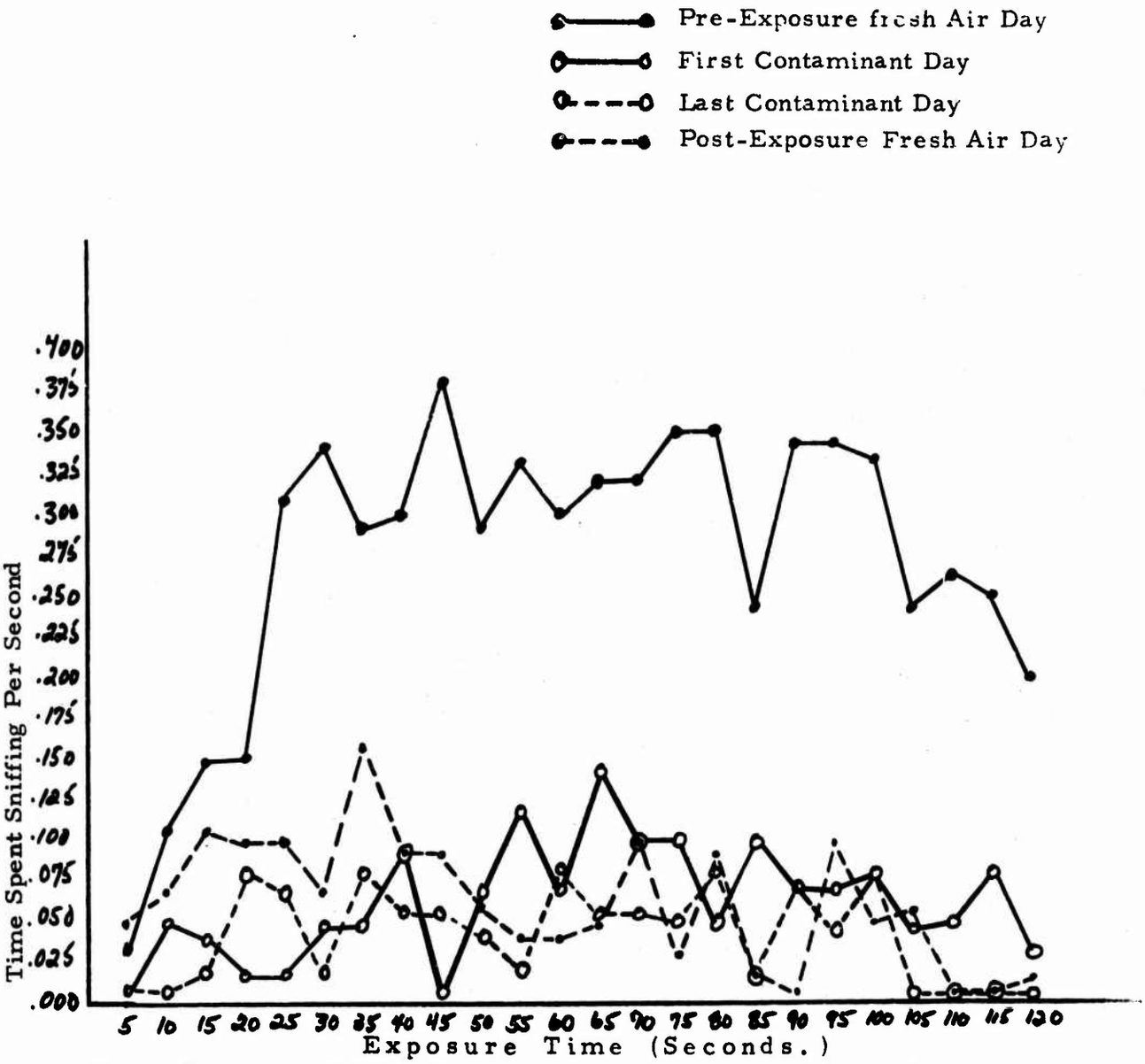


Figure 23. Mean Sniffing Time Per Second of the 120-Second Group as a Function of Exposure Duration. Experiment VIII.

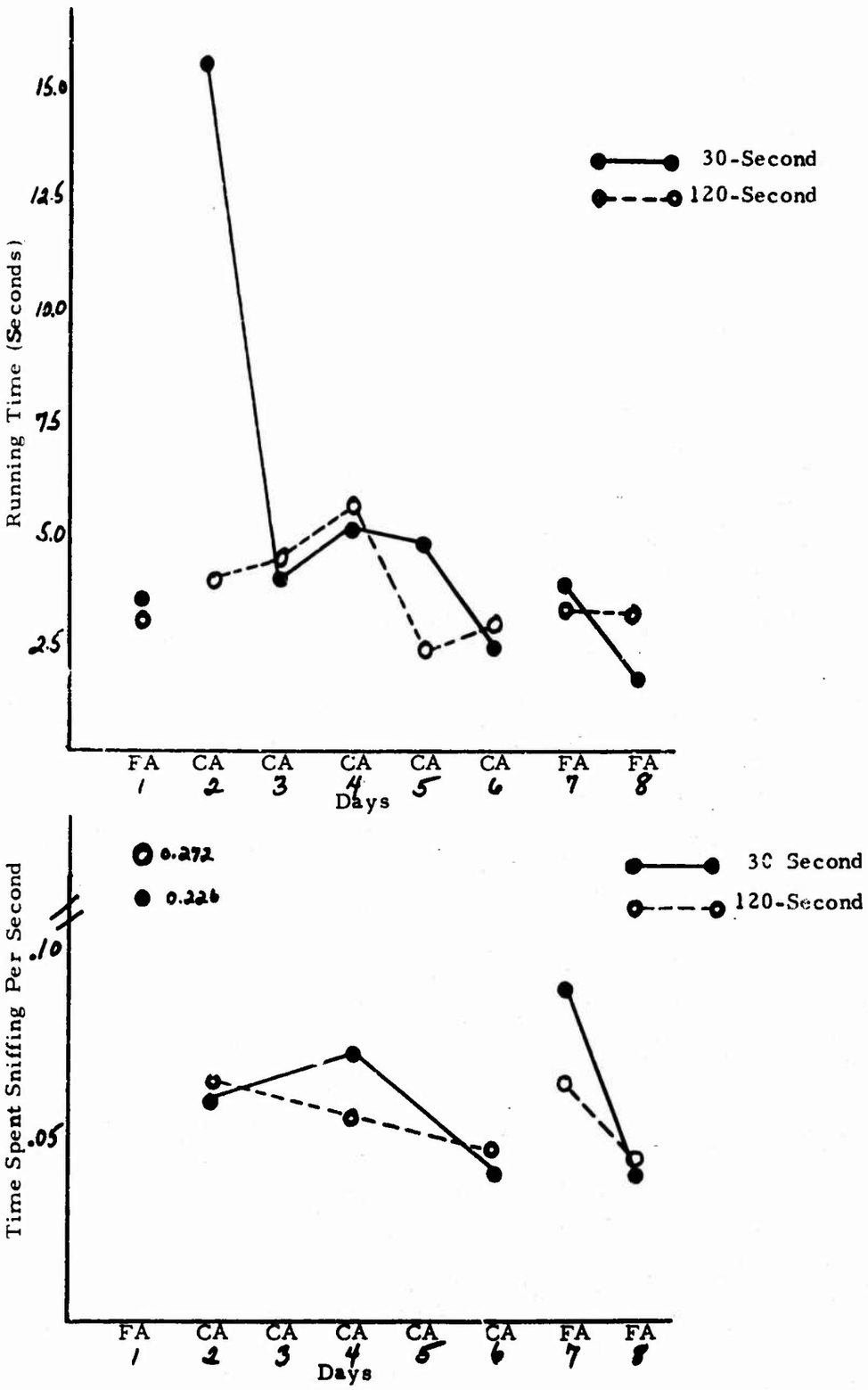


Figure 24. Daily Running Times and Sniffing Times. Experiment VIII.

To study the inter-relationships among the dependent measures, rank order (rho) correlations were obtained separately for each group between each pair of sniffing measures and also between time spent sniffing and running time. This was done separately for Day 2 (day before DBDA exposure), Days 3 and 7 (first and last DBDA days) and Day 8 (first post-exposure fresh air day). The results are shown in Table 5.

As shown in Table 5, of 24 correlations among the three measures of sniffing, all were positive and all but two were statistically significant Those two were found for the 120-second group on the last DBDA day; both involved time spent sniffing. Plots of the results comparable to those in Figures 22-24 but with number of sniffs and amplitude as the dependent measures, did not suggest any conclusions different from those presented. This result, along with the correlations, agrees with the preliminary findings of Experiment VII.

Of the eight correlations between time spent sniffing and running time, only three were significant. Six, including two non-significant ones, were negative. There is no consistency that seems useful about this. However, three of the four correlations involving the 30-second group were significant whereas none of the correlations within the 120-second group were significant. This does suggest that there may be some kind of factor operating during the delay period which affects individual differences or which affects the relationship between sniffing and running. An example of where such a factor could operate was in the finding in this experiment of a much greater running decrement for the 30-second group to DBDA.

Experiment IX

The purpose of this experiment was to replicate the previous one and, in addition, to explore again the possible effect of lesioning of the olfactory bulbs. The same animals as used in the previous experiment were re-used for this one. They were maintained on the

TABLE 5

RHO Correlations for Experiment VIII

Group	Time spent sniffing vs. running time	Time spent sniffing vs. amplitude	Time spent sniffing vs. number	Amplitude vs. number
		Day 1 Fresh Air		
30 sec.	-.5598**	.8479*	.8676*	.9762
120 sec	-.1018	.6071**	.7161*	.8391*
		Day 2 Contaminated Air		
30 sec.	.6986*	.7680*	.6578*	.8239*
120 sec.	-.1953	.6160**	.8527*	.8106*
		Day 6 Contaminated Air		
30 sec.	-.4848**	.4724**	.5259**	.9509*
120 sec.	-.1706	.2010	.3301	.5716**
		Day 7 Fresh Air		
30 sec.	-.1358	.7383*	.5533**	.8015*
120 sec.	..2064	.6687*	.8500*	.8067*

* $p < .01$

** $p < .05$

food deprivation schedule between experiments, but given no further apparatus experience until the beginning of the present series.

Four groups of animals were formed of the previous two groups by assigning nine animals of the 30-second group at random to an experimental group and retaining five for controls; similarly 10 animals of the 120-second group were assigned to an experimental group and five retained for controls. In the experiment, the animals were run with a 30-second or 120-second delay as before.

In the week before surgery each animal was run in the apparatus for two fresh air days and then for two contaminant air days as a means of retraining. Over the next several days monopolar electrodes were used to lesion the bulbs as close to the incoming afferent (afferent to the bulb) fibers as possible. Control animals received identical treatment except that no electrodes were introduced. Ten days following the last operation, the animals were re-introduced to the apparatus for three successive days, fresh air, one trial per day with a 30- or 120-second delay as appropriate. Running time, but not sniffing, was recorded on those three days. The fourth day was identical except that sniffing was recorded. Days 5, 6, and 7 were contaminated air days (DBDA) and Day 8 was a fresh air day. Sniffing and running were recorded every day from Day 4 to Day 8.

Later histology revealed that the lesions made were very small. Of the 19 lesioned animals, only 10 appeared to have reasonable evidence of lesioning. Five of these were in each group. Thus, the data available for analysis were from five control and five experimental animals in the 30-second group and from five control and four experimental animals in the 120-second group.

Results

Analysis of the data in terms of differences between delay periods did not suggest any important relevant differences. The two control and the two experimental groups were pooled, therefore, to increase the reliability of the comparison between lesioned and non-lesioned animals. The results are shown in Figure 25 for both running times and time spent sniffing per second.

The differences between the two groups in sniffing is very small, but of great interest since the experimental animals consistently sniffed less on the fresh air days and sniffed more on the contaminant air days. Looking at Days 4 and 8 as comparison days, it appears that the control animals reduced the amount of time spent sniffing over the contaminated days; there is the suggestion of a possible small recovery over these days. The Day 8 point for these animals helps make it clear that they were really responding to the chemical on the previous three days. The lesioned animals also showed a depressed sniffing time during the three contaminant days compared to Days 4 and 8, but the effect was much smaller. The fact that it was smaller accounts for the reversal of amount of sniffing between the two groups. Nevertheless, that the chemical was detected and treated as an aversive by both groups is shown in the running times on Day 5. The control animals exhibited both a greater reduction in sniffing and greater increase in running time on this day. The lesioned animals, however, also increased their running time. The difference in running time was maintained after Day 5; both groups recovered partially from Day 5, but only the lesioned animals suggest the possibility of an approaching complete recovery.

The data of this experiment, of course, are confounded by the previous experience of the animal with the chemical. The differences

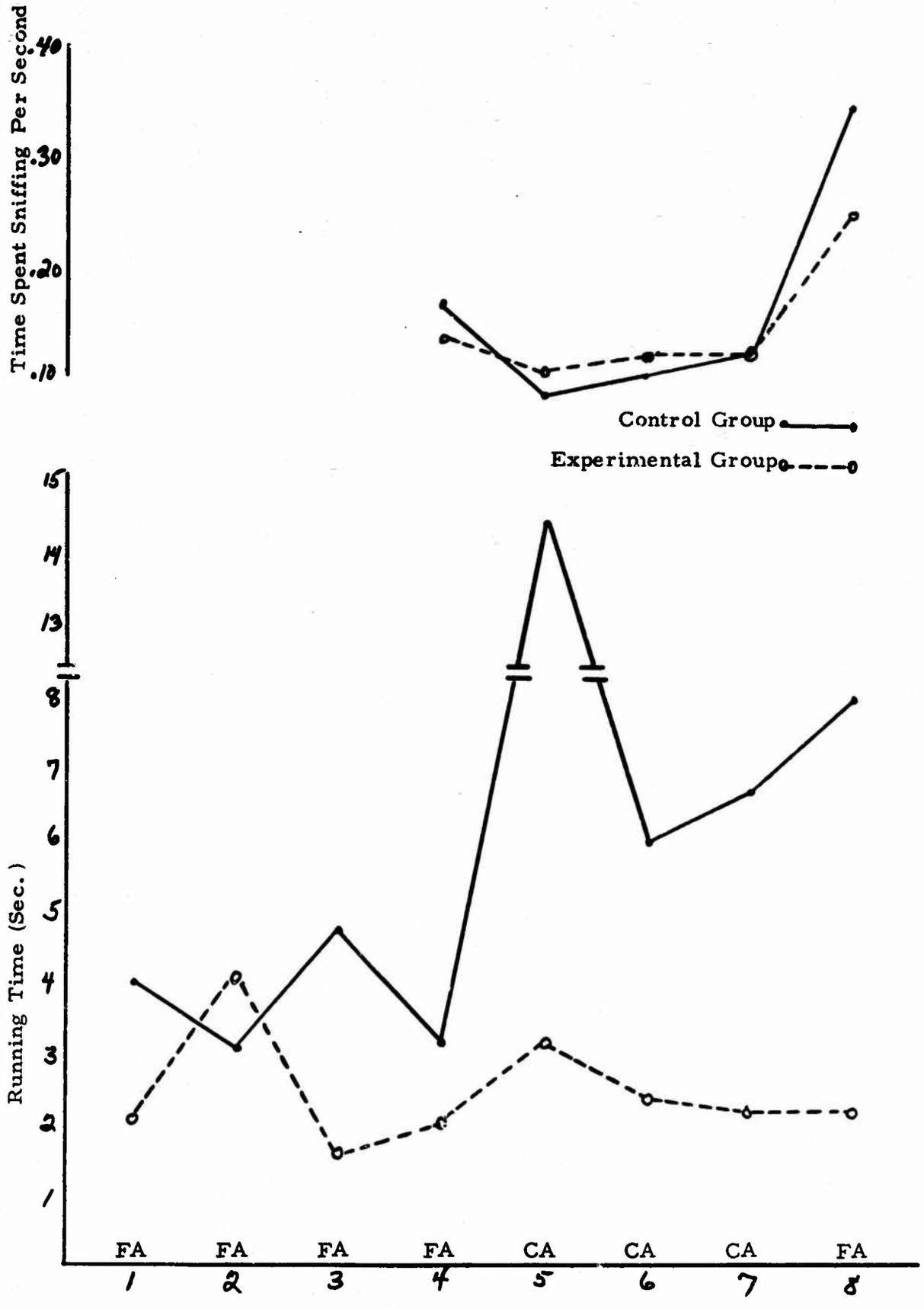


Figure 25. Daily Running Time and Sniffing Time. Experiment IX

between groups are also tentative because of the minimal tissue damage produced by the lesioning procedures. In spite of these problems, however, when both sniffing and running are considered together, the data suggest that the lesioned animals may have been less sensitive to the chemical and, therefore, that it was less aversive, and that for these reasons, it was less effective as a deterrent.

Experiment X

The purpose of this experiment was to explore the possible differences between wild rats and laboratory animals with regard to the conditions of the last experiment. Of interest was the question of possible differences not only between strains, but with regard to the added effect of differences in previous living conditions. Are there differences between gentled, laboratory-bred rats and ungentled rats bred in the wild?

Six adult male, Norway rats, estimated to be between four and six months old at the time of capture were placed on a 23-hour food deprivation within a few days after admission to the laboratory. The animals were captured in Scarboro, Maine. These animals were never handled directly. Rather they were transferred from individual living cages to plexiglass carrying cages designed to accomodate easy transfer. They were then transferred to plexiglass inserts placed in the starting box of the apparatus. A second insert in the goal box permitted removal of the animal and transfer back to the carrying cage.

During the first three weeks after starting the deprivation schedule, the animals were accustomed to the transfer procedures and allowed to explored the apparatus. Following this they were trained in the apparatus for seven days, four trials per day using a .01 gm. food reward. Three of the animals were delayed in the starting box

for 30-seconds and three for 120-seconds. Fresh air was passed through the starting box and the pre-goal air barrier at 500 cc/min. From Day 8 through the rest of the experiment only one trial per day was given.

The pre-operational series is considered as beginning on Day 8. This was a fresh air day followed by three contaminated air (DBDA) days and then by a fresh air day. On the next day all animals were lesioned. Eight days were permitted for recuperation. On the ninth day following surgery (Day 13 in the experimental series) and for the next two days, the animals were retrained, one trial per day with fresh air. Following this (Day 16) they were given another fresh air day, three contaminated air days and a final fresh air day (Day 20). Sniffing was measured on all DBDA days and on the fresh air days just preceding and following.

Results

One animal died during the recuperation period. Subsequent histology indicated that all of the five remaining animals had small lesions comparable to those of the previous experiment. Plots of sniffing did not suggest any adaptation to the chemical within the starting box. For these reasons, as with the previous experiment, the data of the two delay groups were pooled to provide a larger sample. The results are shown in Figure 26 in terms of the median running time and median sniffing time per second for the five animals.

As a result of an apparatus malfunction, the running times of Day 6 were not recorded. The point shown is interpolated. Regardless, the training period can be seen to have resulted in a rapidly improving learning curve so that Day 8 serves as a reasonable pre-contaminant baseline. The effect of the chemical on Days 9, 10, and 11 was an increased speed of running so that, at least on those three

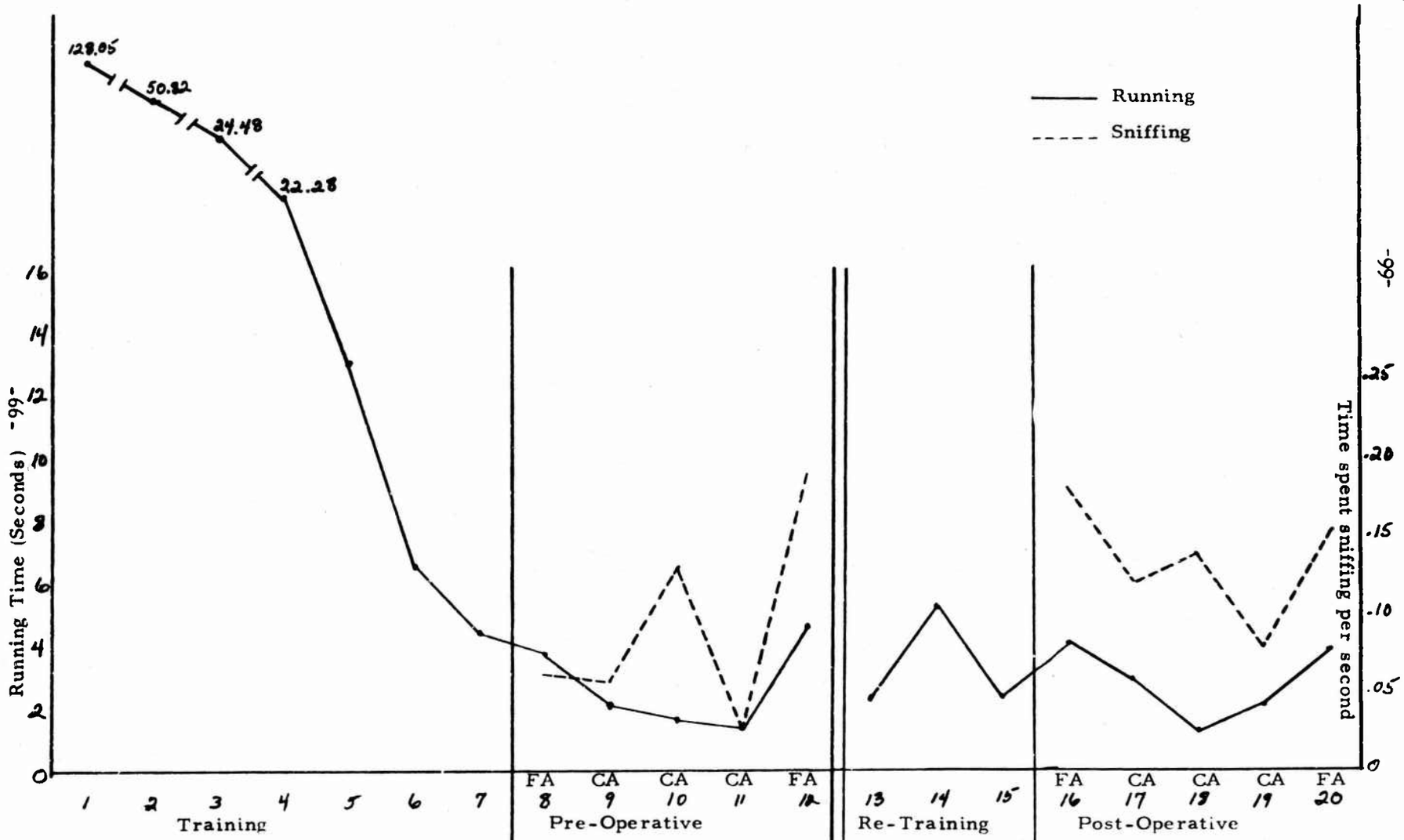


Figure 26. Running Time and Sniffing Time of the Captured Norway Rat during the Conditions of Experiment X.

days, the chemical acted as to enhance running to the goal box. The sniffing data for those three days do not really suggest that the chemical was aversive. On the other hand, the sniffing observed on Day 12 is a typical recovery phenomenon characteristic of a first post-exposure fresh air day and suggests that the previous days were depressed.

The post-operative sniffing data are more characteristic of previous data and reasonably clear in showing differences between fresh air and contaminated days. Thus, sniffing decreased with the contaminant and tended to recover with fresh air. Of considerable interest also is that the general level of sniffing was greater than before, an observation which suggests a reduced sensitivity, i.e., a need for a larger air sample. In any case, these data are clear in showing that the chemical acted as an aversive, but contrary to the previous results with this chemical, it not only did not act as a deterrent; running speed increased over the level represented by Days 13-16.

These results need to be made clearer. In particular, an important difference from Experiment VII is suggested by the data, especially those obtained post-operatively. That is, unlike the laboratory strain in which the chemical acted to slow down running to food, in these animals it speeded it up if it did anything. The hypothesis is very appealing that for these animals the response was to the chemical in the starting box and to the apparatus in general, i.e., that they were motivated very importantly in the first place to learn to go to the goal to escape from the apparatus and the chemical whereas the other animals, at least at the end of training, had learned to go to the goal box with food as the primary incentive. The issue is not clear both because of the exploratory nature of the experiment and because the level to which

the wild rats had been trained was less. It is possible that the effect of the repellent might be different at one stage of practice than at another. These questions deserve serious investigation.

Otherwise, this experiment does not suggest that the responses of the wild rats to the chemical were different from those of the laboratory rats. No evidence of adaptation during the delay period was seen in either; the sniffing level tended to increase after surgery as compared to before, and regardless of whether running time decreased or not, in both strains instances of aversion indicated by sniffing have not been seen to accompany a deterrent effect of the chemical.

Experiment XI

The previous experiment was concerned largely with the effects of an aversive odor on the behavior of wild rats. The present experiment was intended to inspect the effect of a chemical repellent on food consumption, the basic definition of a repellent as we have used it.

The animals used were the offspring of two female, Norway rats captured along with those males used in the previous experiment. Two of the males were used as studs. Fifteen male rats, 120-130 days old were used. These animals had received some handling after weaning, but they were not handled at all for at least the last two months prior to the experiment. Transfers were accomplished as in the previous experiment.

The animals were put on a 23-hour food deprivation regime for one month prior to the experiment. During this time, as was the case for all of the previously reported experiments, they were provided Purina Chow pellets in their individual living cages. Water was available ad libitum.

The experimental series was 24 days in duration. Each day the animals were allowed 30 minutes in individual feeding chambers in which the air flow could be regulated. The food was identical except that it was prepared from Purina meal and provided in the form of a wet mash. Food preparations were made as reported previously. When the food was to be contaminated, the chemical was dissolved in methanol and the solution mixed with the food. This preparation was then dried in a fume hood for 24 hours. Water was added prior to serving. Weighings to .01 gram were made on the dry food prior to serving and on the re-dried remains 24 hours later. The compound used was trinitrobenzene analine complex (TNBAC). The concentration in the food was 250 ppm by weight. Uncontaminated food was prepared the same way, including mixture with methanol, except that the chemical was not added. Air contamination was provided by passing air at 500 cc/min., 24°C over the pure chemical.

The first 19 days involved neither contaminated air nor contaminated food. On Day 20 the air to the feeding chambers was contaminated. On Day 21 the food was contaminated, but the air was not. On Days 22 and 23 the air was contaminated, but the food was not.

Of the fifteen original animals, six were discarded during the experimental series because of a refusal to eat the wet mash at all. These animals did accept small supplemental feedings of their normal food in their living cages. Without these feedings, they would have

starved. The remaining nine animals had no supplemental feedings. The mean food consumption per day of those nine animals is presented in Figure 27.

At least in the laboratory, when the feeding place of rats is changed and/or the nature of the food is altered, it is usually observed that there is a drop in food consumption. Whether the initial low level of consumption shown in Figure 27 is due to one or the other or both cannot be determined. It may be seen that from Day 1 on there was a systematic increase in food consumption until around Day 12. Beyond this, through Day 19, food consumption varied relatively little. The horizontal line drawn between Day 12 and Day 19 represents an estimated stable food consumption drawn by eye for comparison purposes. The mean deviation from this line of the eight days from Day 12 to Day 19 was 0.02 gram. It is reasonable to assume, therefore, that individual variations over this period represent error and daily variations for individual rats and that the line is useful as a baseline for comparison purposes. Using it this way, food consumption on Day 20 represents a decrease of 1.15 gram or 10.6 per cent. Whether this decrease is statistically significant or not, it seems to have little practical significance since as an effect it is very small and since the effect, if it is real, did not appear on Days 22 and 23 which were identical in treatment and which should probably have exhibited a greater decrease since they followed actual food contamination. On the other hand, on Days 21 TNBAC in food reduced the food consumption to approximately the level of the first day.

As reported earlier in Experiment II, TNBAC in food at 250 ppm produces a marked reduction in food consumption in a laboratory strain of rat. A gradual recovery follows, however, and, in fact, a large degree of recovery occurs even to concentrations in food of 1000 ppm (Exp. I). At the present level of comparison, 250 ppm, the

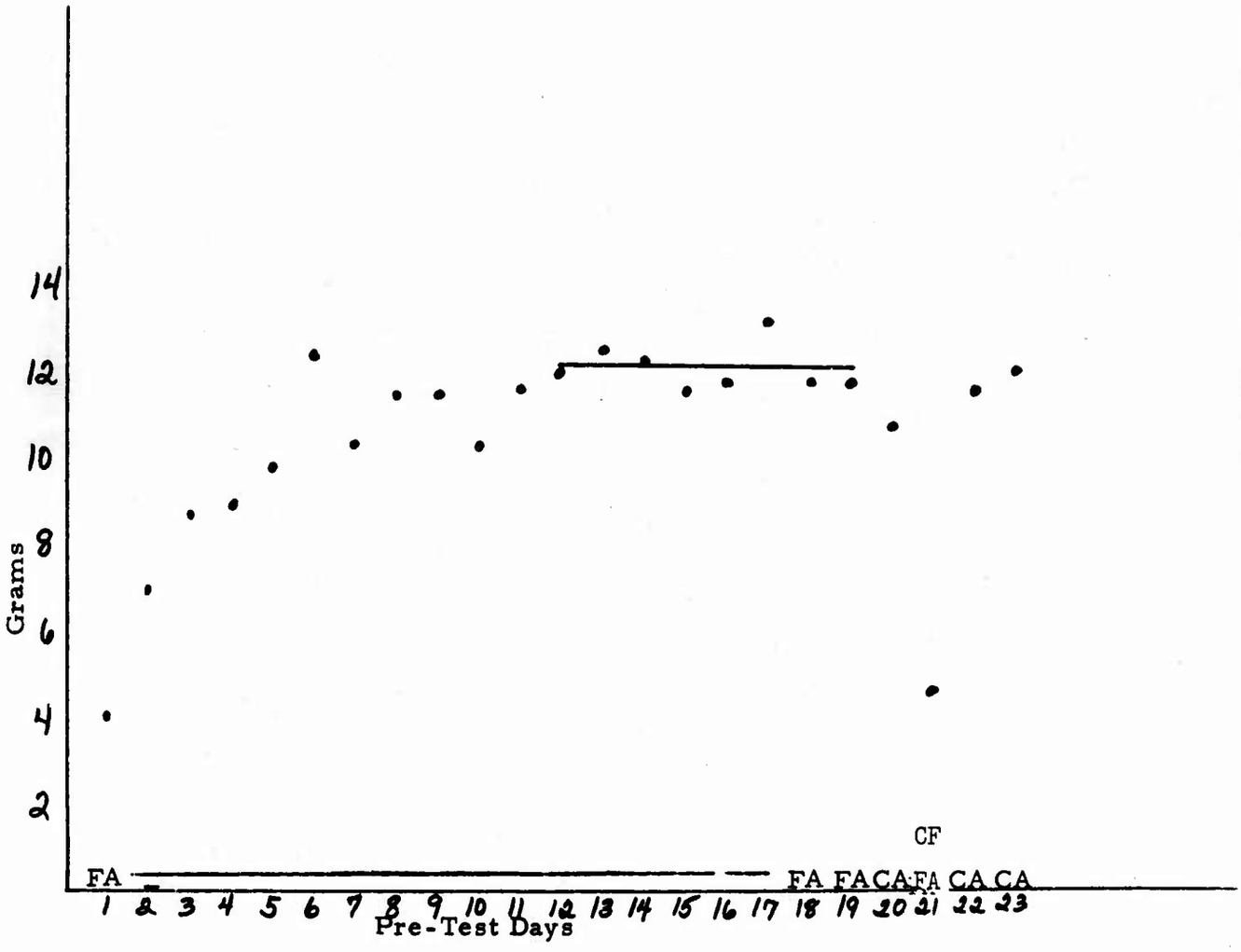


Figure 27. Food Consumption of the Norway Rat, First Laboratory-Born Generation, During Pre-Test, During Exposure to Fresh Air (FA), and Uncontaminated food Contaminated Air (CA), and Contaminated Food (CF). Experiment XI.

data do not suggest any different effect for the wild strain as far as the acceptance of contaminated food is concerned. We assume, but cannot be sure, that recovery would have been comparable.

Neither Experiments I or II involved air contamination so that no comparison can be made in this regard. Further, since sniffing was not measured in this experiment, we cannot say anything about the aversiveness of the odor measured in this way. It is clear, though, that if the odor were aversive, it had little or no effect as a repellent to food consumption. In the previous experiment, using wild rats, where we have reason to suppose that the odor was aversive, it did not act as a deterrent to locomotion. It seems reasonable to conclude, therefore, that although they may be aversive as odors, neither DBDA nor TNBAC have an important influence on either the behavior or the food consumption of <u>hungry</u> rats of this strain whether wild or born in the laboratory, but not gentled. These chemicals appear to be repellents only when taken into the mouth and then their effectiveness as repellents is reduced with repeated experience.

Experiment XII

Up to this point our study of sniffing in regard to odors had been concentrated on the use of that mechanism by the animal to evaluate odor sources in its environment. We were concerned with sniffing as a means for identifying odors as attractants or aversives and scaling them for intensity in each case. We were also concerned with habituation and adaptation to odors as might be revealed by changes in sniffing reactions. This experiment was aimed at the question of odor as a cue with which the animal could make a discriminative or selective response, and the role of sniffing in so doing.

The experiment was conducted in two parts each using the same animals but in a different set of conditions and with different basic questions in mind. Part I, conducted in a straight runway, was concerned with the relative effectiveness of an attractant (or at least a non-aversive odor) in the starting box as a cue indicating the presence of food in the goal box. This was compared to the use of the omission of the odor as the cue. Once learned, the more effective the cue as a signal, the faster the running of animal to the goal should be.

It was expected that the odor would provide a more effective cue than would non-odor since it provided a positive signal. The second part of the experiment employed a Y-maze in which the same odor indicated the proper choice of goals for the previous odor-cue animals and the non-odor arm of the apparatus provided the cue for the non-odor animals. The final treatment in this part of the experiment was a substitution of an aversive odor for the one that had been used.

Subjects

The subjects were 18 male, hooded rats of the Long-Evans strain. They were 120 days old at the start of the experiment. The animals lived in individual cages on a 23-hour food deprivation schedule starting two weeks before the beginning of training. They were gentled by handling for 10 minutes per day during this period.

Apparatus

The short runway used earlier was employed for the first part of the experiment. A Y-maze of which the short runway was a modifiable portion was used for the second part. See Appendix I for details.

The odor was presented in the runway only in the starting box. In the Y-maze it was presented as an air barrier just before one or the

other goal box. The goal not preceded by the odor had a fresh air flow. The air flow to these three places was always 1000 cc/min. at 30°C. When used, the repellent was DBDA at that temperature. Otherwise, the odor was obtained by passing the air flow over a standard liquid diet (# 116 E. C., General Biochemicals, Chagrin Falls, Ohio). In both cases the source was filtered, compressed air passed over five milliliters of the liquid.

In the straight runway sniffing was measured in the starting box. In the Y-maze sniffing was measured in the starting box and in the choice arm of the Y.

Part I

This portion of the experiment used the short straight runway. Odorant air was presented only in the starting box. Air flow was 1000 cc/minute at 30°C. The odor was obtained by passing the air over five milliliters of a liquid diet (# 116 E.C., General Biochemicals, Chagrin Falls, Ohio).

For two days just before training each animal was placed in the starting box for 30 seconds with the door closed and sniffing was recorded. The air flow system was not operated during this time. The animals were then matched into two groups of approximately equal mean based upon the average time spent sniffing during these two days. The initial training which followed these two days consisted of two trials per day for five days with five 45-milligram food pellets as reinforcements in the goal box. The Odor Group always experienced the liquid food odor in the starting box; the Air Group was always presented with a clean air flow. Animals were delayed for 30 seconds in the starting box before release to the runway.

Following the five initial training days, the animals were run for 10 more days with five trials per day. The five daily trials were divided into combinations of three and two trials of odor or air according to whether or not the odor was presented. The sequence was arranged into a Gellerman (1933) series in such a way that there were five odor and five non-odor trials over pairs of consecutive days. The Odor Group was reinforced only on trials in which the odor was present in the starting box; the Air Group was rewarded only on non-odor trials. The reinforcement schedule was 50 per cent, therefore. Throughout, all trials were spaced approximately five minutes apart.

Sniffing was recorded from the starting box for two days for pre-matching purposes as noted above. It was also recorded during the first four trials of training during which the Odor Group was always presented odor and the Air Group presented clean air. Finally, sniffing was recorded on all five trials of Days 1, 6, and 10 in the experimental series, i.e., the days in which both odor and non-odor trials were presented to both groups.

Results of Part I

The mean time spent sniffing per second on those days for which sniffing was recorded during the experiment is shown in Figure 28A. A general observation that can be made from the figure is that the Air Group sniffed slightly more at all but two points. Since this group also sniffed more on the last three pre-experimental days, no significance can be attached to the observation except that the initial matching criterion may not have been extensive enough.

Inspection of Figure 28 shows that the sniffing response of both groups fluctuated over the experiment somewhat,

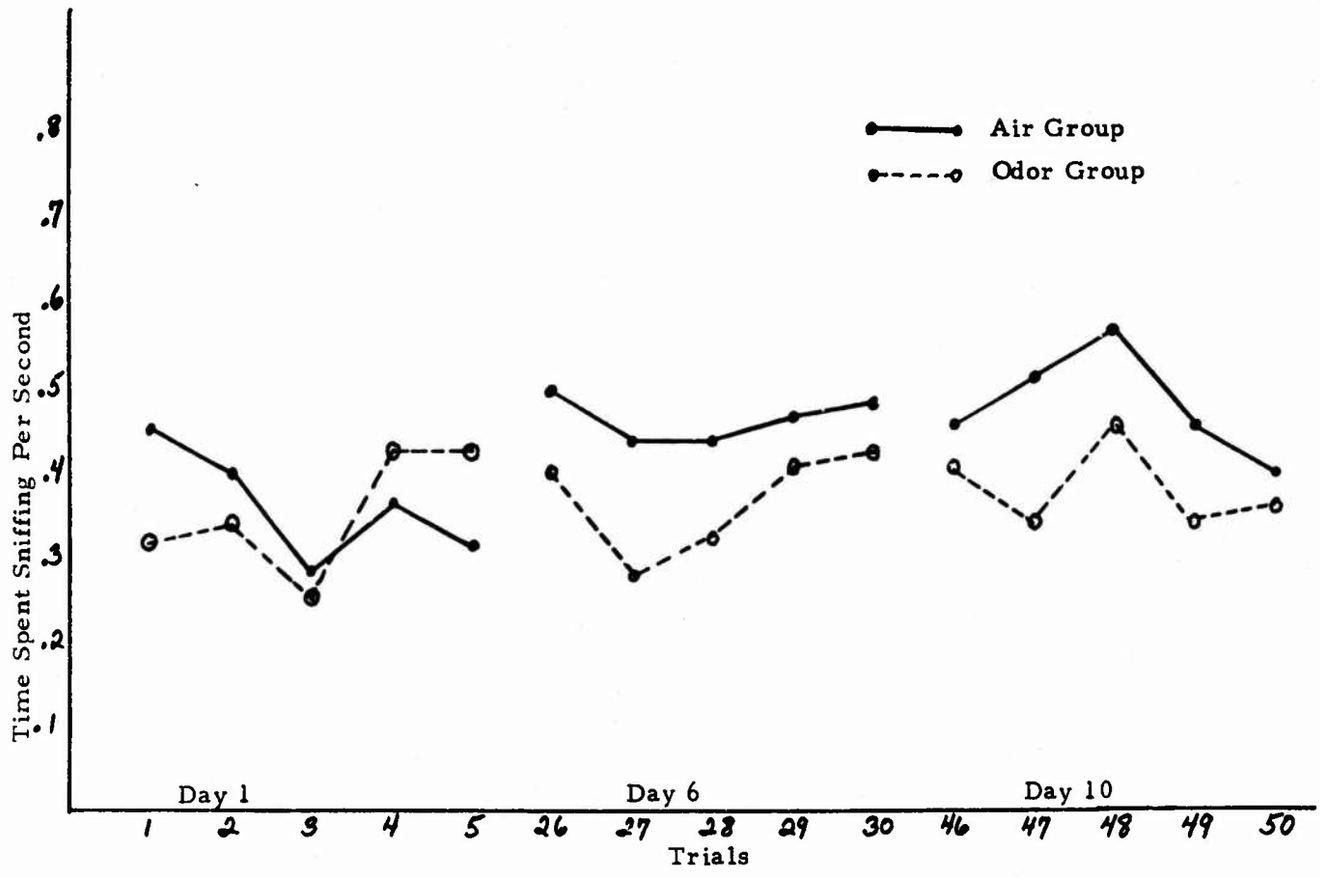


Figure 28. Sniffing Time in the Starting Box at the Straight Runway. Experiment XII, Part I.

though not greatly, and without any particular differential sensitivity to the presence of the odor or to its absence.

Figure 29 presents the running data in terms of the reciprocal of running time (i.e., speed). The data are means of five trials across pairs of days. It is clear that both groups increased their running speeds systematically over the course of the experiment and that the performance of the Odor Group increased more rapidly. The curves are typical learning curves for the reciprocal transformation.

The data of Figure 29 provides no evidence of a differential effect due to the presence or absence of the odor as a cue. The Odor Group, in fact, performed slightly better during the initial practice period and simply maintained this superiority later. Neither group exhibited an advantage on cued trials as opposed to non-cued trials whereas had the odor been an effective cue, its presence should have been associated with better performance for the Odor Group when present and for the Air Group when absent.

Taking both figures together, the results of Part I suggest that the odor did not provide cueing value within the length of time given to learn. Further, the sniffing data provide no evidence to indicate that the liquid food odor served as an attractant since sniffing to it did not increase. In fact, sniffing decreased at first and then tended to recover. Thus, if anything, the odor must be regarded as a mild aversive or as a novel stimulus. Since the reduction in sniffing found was small and recovered relatively quickly, it would appear to be classified best as a novel stimulus.

Part II

The purpose of this part of the experiment was to explore the role of an odorant as a cue for discrimination. The two groups of

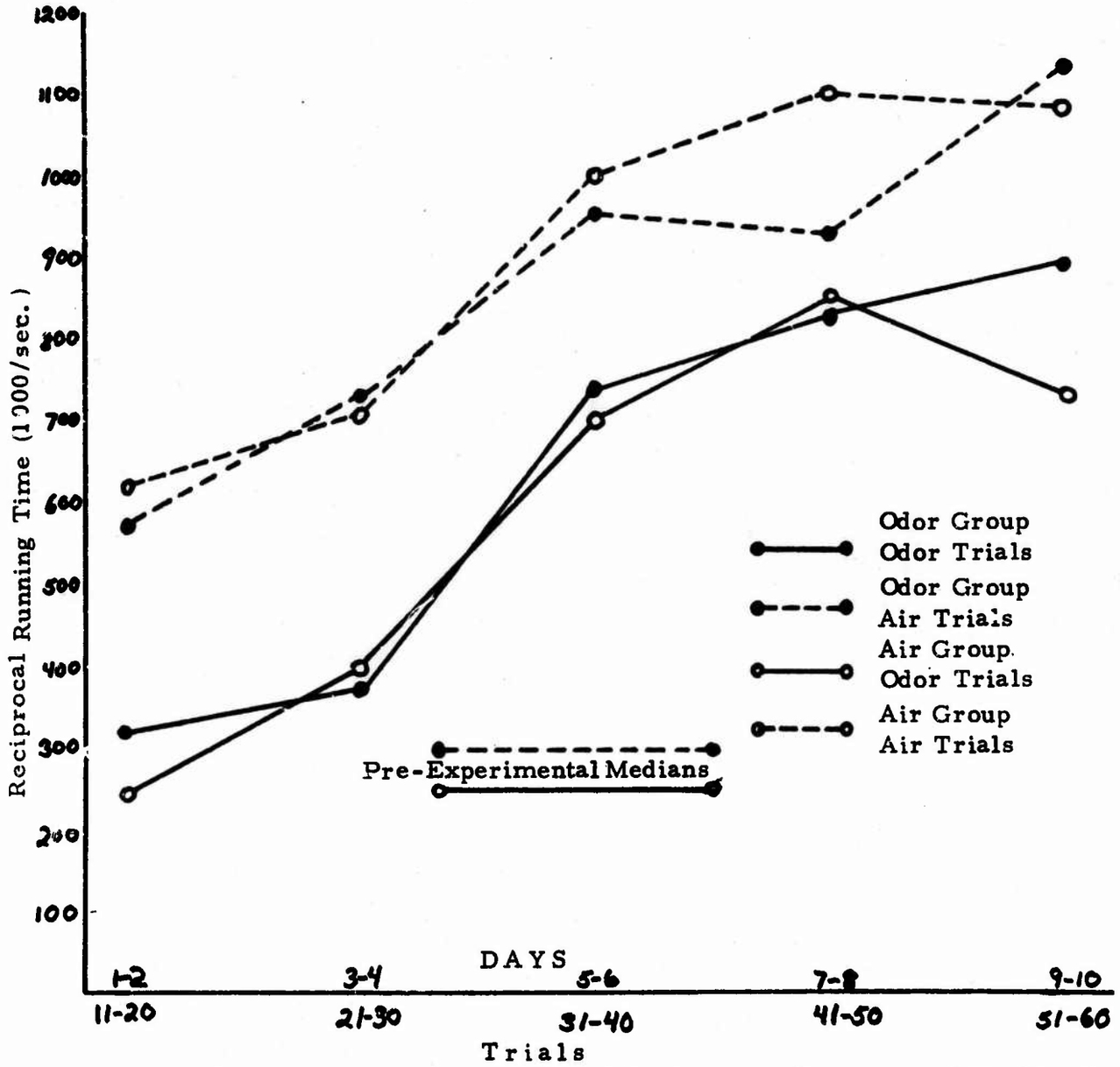


Figure 29. Running Speed in the Straight Runway. Experiment XII, Part I.

animals employed above were used immediately and without change to form two groups for use in the Y-maze. The presence of the liquid food odor in the right arm of the maze indicated for the Odor Group that it would be rewarded for choosing the goal in that arm. On the same trial, the correct goal for the Air Group was on the other side. The reinforcement for a correct choice was five 45-milligram pellets.

In this part of the experiment, as before, the animals were delayed for 30 seconds in the starting box, but without any air flow to that box. The only air flow was the air barrier presented just before each goal box. This flow was comparable in quality to that used in Part I except when DBDA was used. Since the air temperature was 30°C. the concentration was the same as used previously.

A non-corrective procedure was used throughout. That is, once the animal had entered the goal box, whether the right one or not, the goal box door was closed; the animal was retained in the box for about 2-3 seconds, and then removed. The Initial Training consisted of five trials per day for 11 days with a Gellerman series of left-correct and right-correct over pairs of days. (The results of one of these days were lost in the laboratory accident mentioned earlier.) Following this the animals were given a Final Training of 10 trials per day for 11 days with the sequence balanced over a similar quasi-random arrangement for pairs of days. In the starting box, sniffing was recorded on the first three trials of days 1, 2, 9, 14, 18, 19, and 22. In the running area of the Y-maze, sniffing was recorded on the first five trials of days 3, 5, 7, 11, 16, and 20. In all cases precautions were taken to clean the equipment and to space trials 15-20 minutes between trials for a single animal so as to minimize artifactual odors and olfactory adaptation. Food odor vs. non-odor was used throughout except that on the last four days, DBDA (30°C.) was substituted as the odor.

Although discriminative learning experiments with the rat usually require a great many trials to establish even a low level of learning, using choices as a dependent measure, it was felt early in the initial training that the animals were not responding to the odor at all.

This feeling was supported, of course, by the results of the previous experiment. Therefore, to enhance the possibility that the animals would attempt to use the odor as a discriminative cue, the doors of the goal boxes were kept closed during the running period. Thus, the animals were forced to remain in front of the door of their choice for two seconds prior to opening of the goal box door. In so doing, their heads were directly into the air stream. This did not affect the running time measures since the photoelectric pickup had already been triggered by that time.

Results of Part II

Figure 30 presents the performance data in terms of the percentage of correct choices and the speed of running (i.e., reciprocal of running time). Each value is the mean of 10 trials. As may be seen the development of correct choices was slow and reached a maximum mean value of about 70 per cent. There appears to be no difference between the two groups in this regard. On the other hand, although the speed of running did not show a steady increase over the course of the experiment, the Air Group developed a small but consistently greater speed during the final training period. It is not possible to conclude one way or the other about the effect of the aversive odor.

Figure 31 illustrates the sniffing results. In the starting box the Air Group spent more time sniffing. The reverse was true in the Y-maze itself where the Odor Group spent more time sniffing. No evidence of a repellent effect is indicated.

In order to evaluate the effect of odor cueing on learning, the animals were classified into two groups according to whether or not they were making at least 70 per cent correct choices over the last four days with no single day below that level. On this basis eight

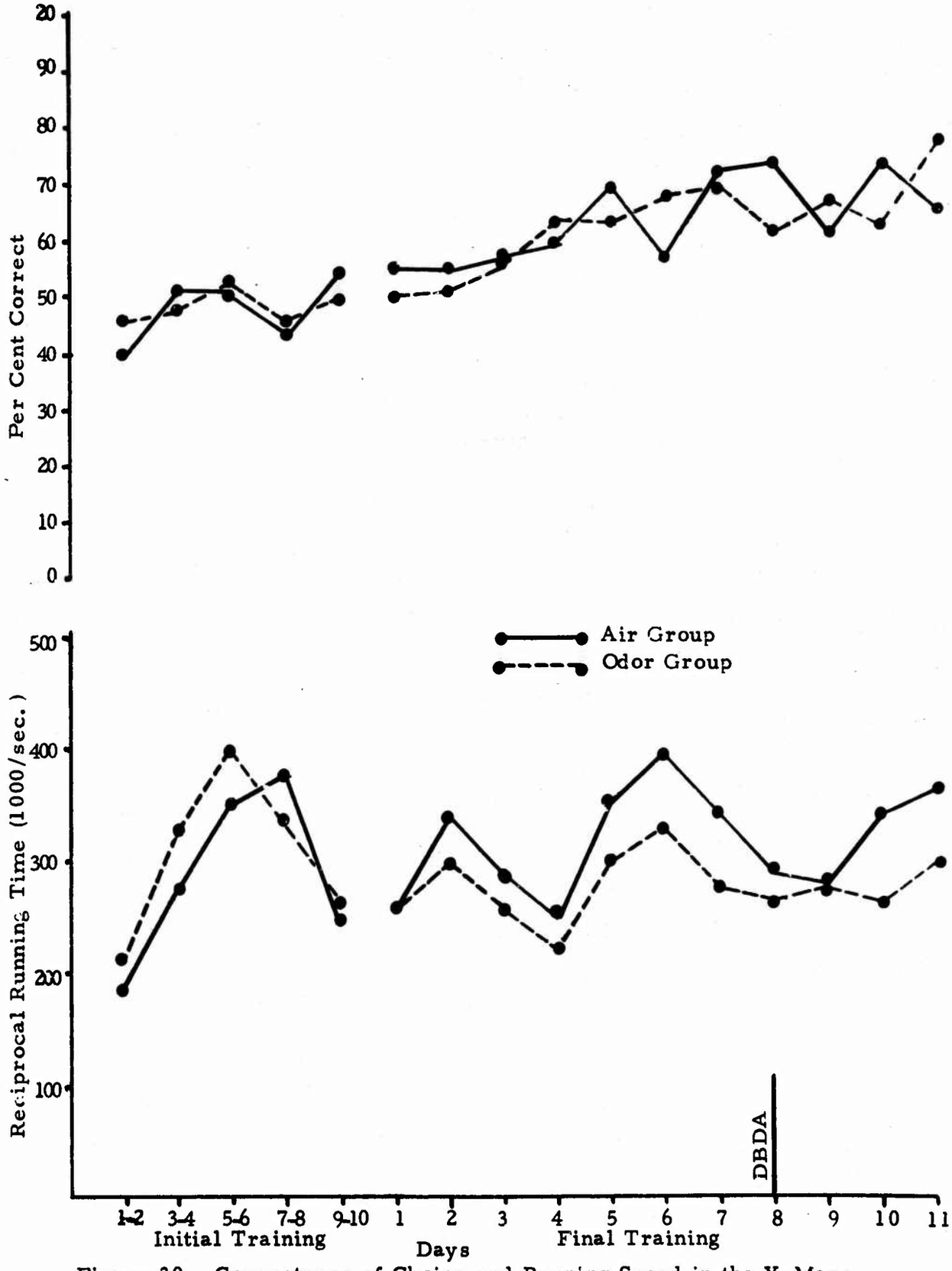


Figure 30. Correctness of Choice and Running Speed in the Y-Maze. Experiment XII, Part II.

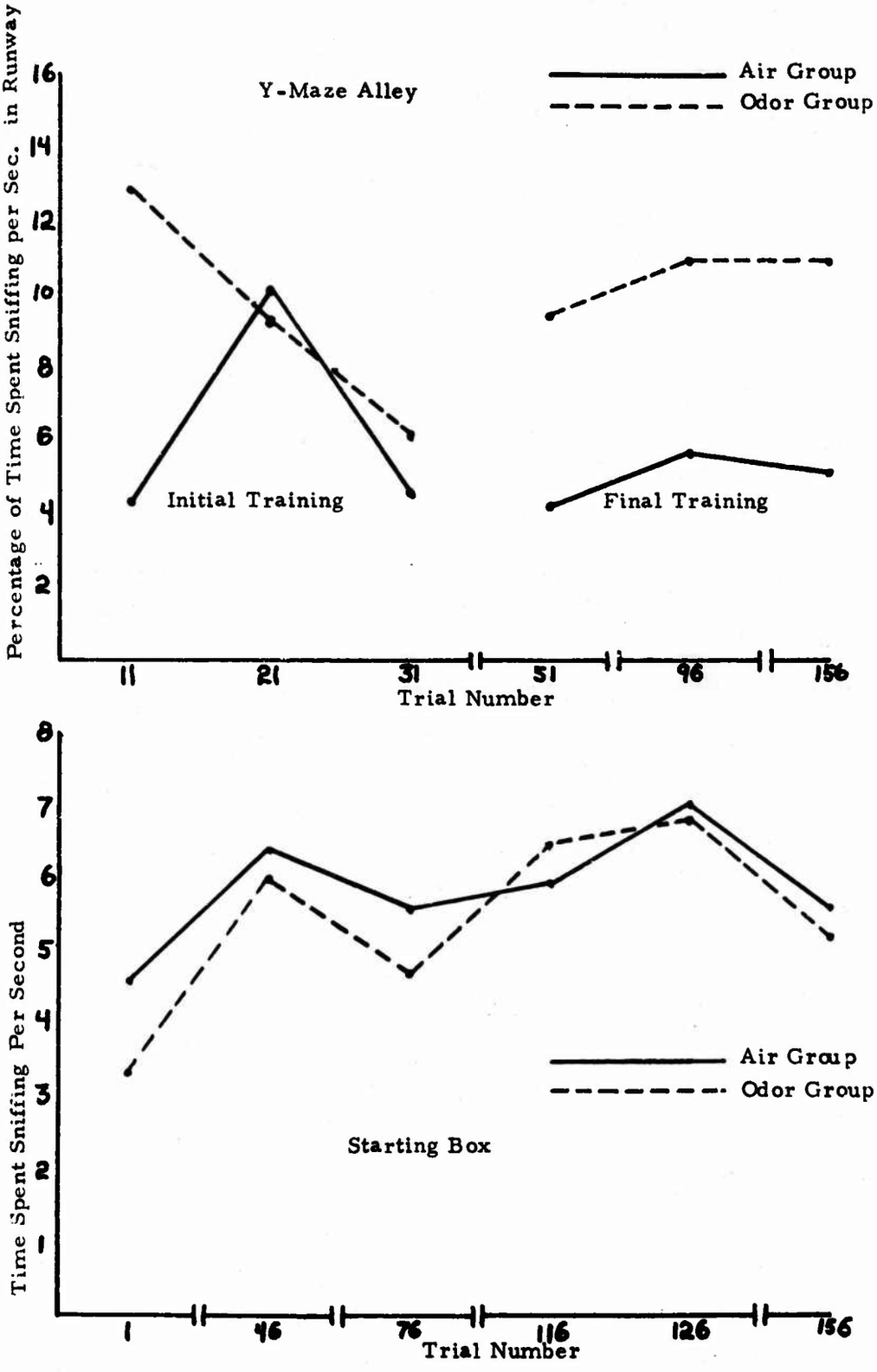


Figure 31. Sniffing in the Starting Box and Alley of the Y-Maze. Experiment XII, Part II

animals were found to have learned and 10 to have failed to learn. Since Figures 30 and 31 did not indicate a main effect due to the presence or absence of the odor, the animals were pooled across these categories to form a Learners and Non-learners group. The running speeds and choices of these two groups are shown in Figure 32.

These results appear to be very clear. The Non-learners performed at chance levels (50 per cent) until Day 5 of the Final Training. From this point on their performance improved slightly, though erratically, so that by the end of the experiment they were performing at an average of 64 per cent correct. On the other hand, the Learners were almost at that level by Day 2 of Final Training and improved more or less consistently so that by Day 8 of the Final Training they were performing at a mean of 90 per cent correct. Use of DBDA as the odor appears to have decreased performance in both groups although the Learners did not show this effect until the second day of it. Both groups appear to have been in some stage of recovery by the end of the experiment.

The running speeds in the figure show that the Learners ran more slowly throughout the experiment except for the first six days where their performance is essentially the same as that of the other group. Both groups also show a decrease in speed with the introduction of DBDA. This conclusion is weakened by the drop in the curves on the day before that. However, the maintenance of the reduction and the change in the behavior of the curves, from more or less cyclic to non-cyclic suggest strongly that something was actually affecting running.

Considering the figure as a whole, it seems clear that Learners did in fact use the odor discriminatively, but ran more slowly. The effect of the aversive odor as a substitute was to interfere with both choosing of the correct goal and running speed. The effect on the

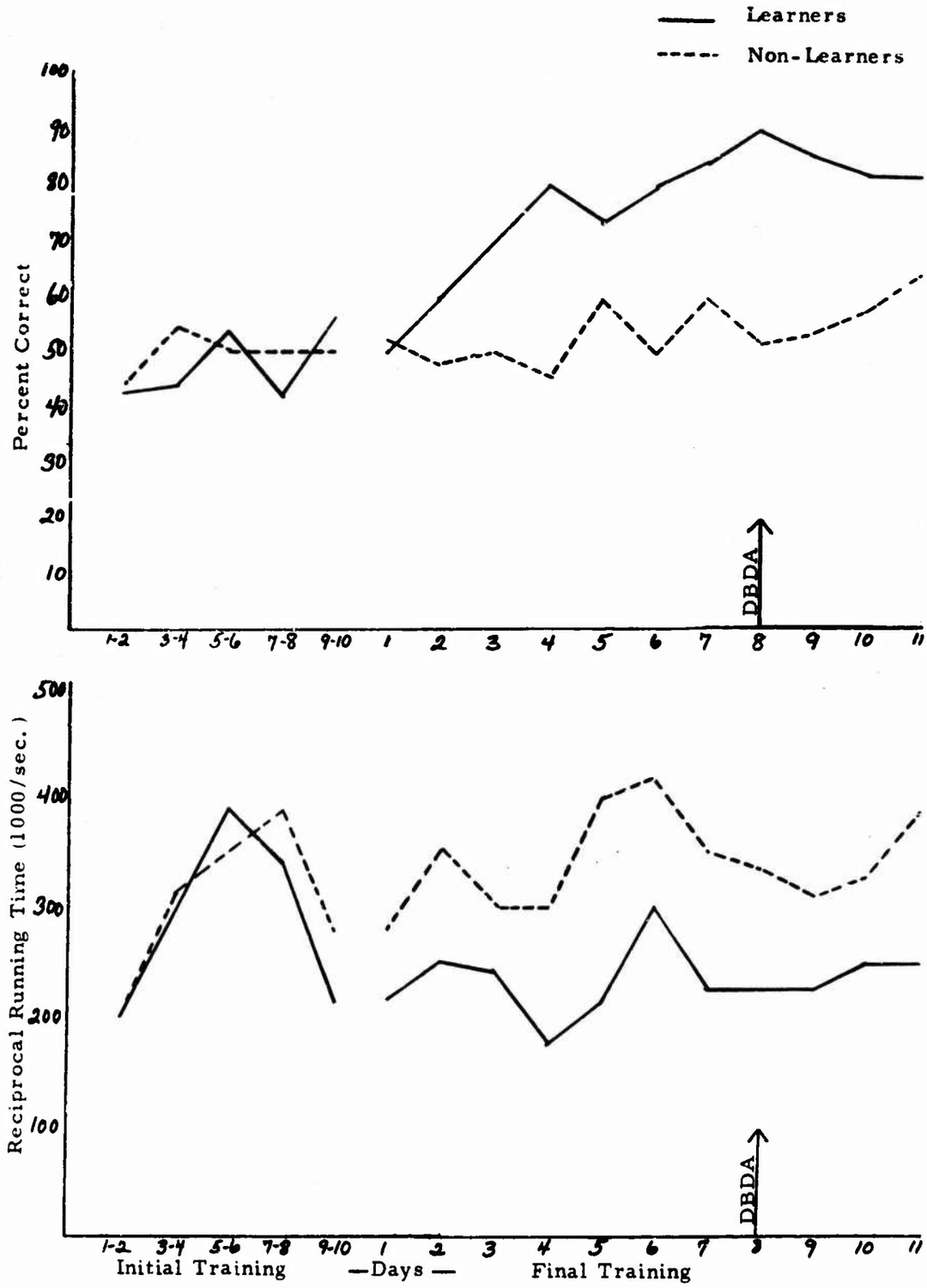


Figure 32. Correctness of Choice and Running Speed in the Y-Maze by Learners and Non-Learners. Experiment XII, Part II.

running speeds of the Non-learners was greater, presumably because they were running fast enough to show a large decrease. Similarly, the effect of DBDA on the percentage of choice was greater for the Learners.

The sniffing data are reported in Figure 33. These results also appear to be reasonably clear. The Learners sniffed consistently less in the starting box and, in general, consistently more in the choice section of the apparatus. The repellent did not reduce the sniffing of Learners in the Y-maze. It appears, then, that Learners were animals that investigated differential odor signals more carefully, and in so doing sacrificed running speed. Learners also habituated to a constant olfactory condition to a greater degree.

In order to evaluate the suggested interrelationships among sniffing and performance measures in this experiment, rank order correlations based upon all 18 rats were obtained as shown in Table 6. Although the correlations cannot be called high, the relationships suggested by those which are statistically significant are strongly supportive. They may be summarized as follows:

1. The greater the time spent sniffing in the choice section, the greater the percentage of correct choices.

2. The greater the speed of running in the Y-maze, the less the percentage of correct choices.

3. The greater the amount of time spent sniffing in the choice section, the slower the speed of running.

4. The greater the time spent sniffing in the starting box of the straight runway, the slower the speed of running.

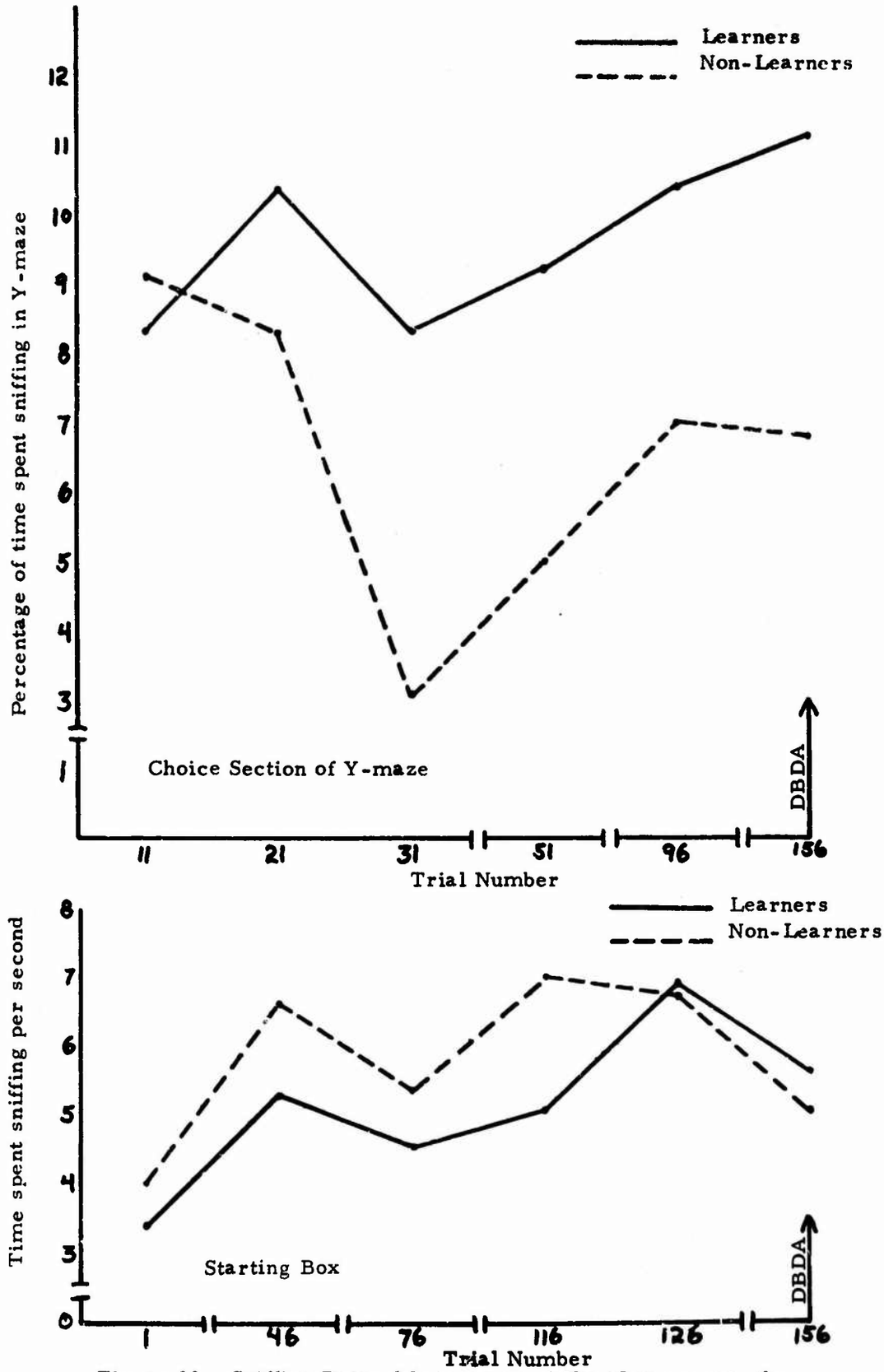


Figure 33. Sniffing Data of Learners and Non-Learners in the Y-Maze. Experiment XII, Part II.

TABLE 6

Rank Order Correlation, Experiment XII

Y-Maze	r	p
Choice vs. sniffing in choice section	.47	$<.025$
Choice vs. sniffing in starting box	.03	$<.05$
Choice vs. speed	-.42	$<.05$
Speed vs. sniffing in choice section	-.75	$<.005$
Speed vs. sniffing in starting box	.01	$<.05$
Sniffing in starting box vs. sniffing in choice section	.17	$<.05$
Straight Runway		
Speed vs. sniffing	-.51	$<.025$

Integration of Findings and Conclusions

The primary purpose of this investigation was to study the mode of action of rat repellents and attractants. The practical justification for such an investigation lies in the universality and the seriousness of problems of rat control and in the need for information on which to develop improved repellents. A related purpose of the study was to acquire information which might assist in the development of methods for evaluating the effectiveness of repellents. A fundamental issue to the whole study was the relative importance of taste vs. odor (i.e., ingestion vs. inspiration) in repellency. The entire approach to conventional chemical repellents depends upon this question.

In this section of the paper we shall attempt to integrate our diverse results via a set of questions and conclusions. Each will be accompanied by some discussion intended to show its basis in our thinking and, in some way, to evaluate our confidence in it. It is recognized in doing this that all empirical conclusions are probabilistic in nature; in science nothing is ever proven and from our point of view the work described is only a beginning.

1. Do wild rats differ from laboratory strains in their response to repellents? - This question must be considered before any other in order to evaluate the degree to which the results obtained from laboratory strains can be generalized to wild rats. It is an old question steeped with folk lore and personal bias. Neither the present investigation, nor so far as we know, any other study, has data which are directly relevant. To obtain directly relevant data, it would be

necessary to compare the behavior of a generation of a laboratory strain born wild with wild rats under conditions of no interference. To our knowledge no one has done anything which approximates this. To say that the wild rat in the laboratory is not like the laboratory rat in the laboratory is not the same thing as saying that the laboratory strain in the wild is not like the wild rat strain in the wild.

Our experiences with wild rats in the laboratory include both casual naturalistic observation and attempts to put one kind of wild rat through objectively measurable experiences with which it can be compared to laboratory rats. Our experience is not extensive in either case and we would want to extend our research considerably in order to increase our confidence in our feelings about this. Nevertheless, within the scope of what we have done, our experience suggests that the captured wild rat is impossible to handle, but that it breeds and eats like the others. We found that the first generation born in the laboratory was easy to handle when small. When adults they were difficult to handle, but they had not been handled for a long time between and this difficulty is also the case with long inbred laboratory strains. At present we have a second generation born in the laboratory, now about six months old, and they are very gentle. As far as this kind of observation is concerned, it would seem that the distinction between wild and tame is more meaningful than that between strains.

We have conducted two different experiments with wild rats, one with captured rats and one with a next generation. When the results are compared with other experiments using other kinds of rats, we find no basis for concluding that there is a difference in response to repellents whether ingested or inspired. Only one finding can be viewed as a possible difference and that was in the observation that captured rats tended in one comparison to increase the speed with which

they ran through a contaminated barrier to a goal, whereas Long-Evans rats tended to decrease their speed. We shall discuss a related problem in some detail later. At this point, it should be noted that this difference in response could also easily represent the difference between animals in tameness.

Although we do not have extensive sets of data to present, with regard to what we have done, and in terms of any scientific hypothesis, we cannot conclude that there is any native difference between the wild rat strain that we have used and a variety of laboratory strains in their response to atmospheric or consumed aversives. We are willing to hypothesize that the trapped wild rat is less easy to habituate either to handling or to apparatus. We have no reason, however, to suppose that it would never habituate. Certainly as far as taste and odor reactions are concerned, we know of no reason to restrict generalizations from the laboratory rat. Perhaps the generalization would be more comfortable if the laboratory rat were not gentled and not handled as we did with the wild rats. Note that nothing we have said questions the claim that some laboratory strains may be gentler, i.e., more easy to gentle, than others. We are saying only that the wild rat may be more difficult to gentle, but that it can probably be done and, once done, the differences of interest here would probably disappear.

2. What is a chemical repellent? - A chemical may be defined as a rat repellent if, when mixed with a normal diet, served in customary form, and in the usual eating place, it produces a reduction in normal food consumption. Our results, like many others, indicate that food consumption will be reduced if: (a) the place in which food is offered is novel, (b) the manner in which it is served is novel, (c) the time at which it is offered is novel, and (d) the animal has other food available. If these conditions are present, no reduction

in food consumption can be attributed to the presence of a chemical in or on the food. Their absence is a requirement of testing.

The degree of repellency of a chemical depends upon the length of time of food deprivation of the animal (within limits, of course). Animals on a novel food deprivation will not normally consume as much food as animals which have been conditioned to a food regime of the same length. How repellent a chemical in food can be said to be depends upon how well-established the hunger cycle is. In our opinion, consistent with essentially universal practice in behavioral studies, the most effective and convenient schedule is a 23-hour food deprivation or something close to it depending upon the time allowed for testing. A variety of data available in the older literature indicate that normal food consumption decreases at some longer deprivation period, but increases to about that one. On this basis we recommend a 23-hour cycle for general testing since it provides not only a constant level of hunger, but also a high level of normal food consumption. To demonstrate a loss in food consumption for only mildly hungry rats even by comparison with a control group is not a very powerful test.

3. When is a chemical repellent a deterrent? - In a sense this was just answered; yet, it bears repetition in this context. Given that it has been demonstrated that a chemical in food, under appropriate testing conditions, produces a large reduction in food consumption, or a larger one than some other, it will be an effective deterrent to the degree that the conditions required for testing are met. Thus, under field conditions, it will operate as a deterrent to the degree that the animal is not hungry, other food is available, it is novel, etc. The more that the field conditions differ from the required testing conditions, the less effective the repellent will be as a deterrent. Thus, its maximal value as a deterrent depends upon more than its value as a repellent.

4. How long will a chemical remain repellent? - Aside from loss of the chemical as a physical event, the repellency of a chemical at a constant concentration decreases with increased exposure to it. What we mean by this is that the animal develops some kind of tolerance or willingness to accept a given level of repellency. Our early studies of prolonged food consumption indicated vast pathological changes in those animals which survived and increased their consumption of contaminated food. The slow nature of the increase suggests either a loss of pain sensitivity or an increased pain tolerance, or possibly both. Regardless, conclusions about the repellency of a chemical should be based upon repeated testing under the standard test conditions. We would suggest that standard testing should be based upon:

a. a 23-hour hunger cycle established for not less than 21 days. This is based upon a well-established literature;

b. feeding conditions as described above;

c. repeated daily testing for not less than 10 days. Lethal doses are not assumed.

5. To what degree do ingestion (taste) and inspiration (odor) determine repellency and attraction? - Excluding the possibility of sex-related odors, our research leads us to conclude that odors which attack the eyes and pain fibers in the mucosa can be called aversive in that the animal shows an important reduction in sniffing in their presence. Continued exposure does not seem to produce a sensory adaptation in the sense that sniffing is resumed or recovers. Nor do repeated exposures produce an habituation, i.e., a loss of the initial sniffing reduction when exposed. Of the various chemicals that we have used, DBDA is very effective in this manner. Other chemicals that we have used appear to allow exposure-to-exposure habituation,

even though (within the limits of our testing) little recovery occurred during continuous exposure.

It is very important to note that any stimulus may be aversive when novel. Many chemicals used as repellents are probably not much more effective as aversives than neutral, but novel stimuli (e.g., lights, sounds). This is a question too that needs experimental study. In any case, across all of our experimental work, it appears that no chemical that we used when presented in air alone was a deterrent to either locomotion or food consumption except on a very short temporary basis even though it could be shown to be highly aversive as an odorant.

Considering the problem of attractant odors such as food odors, we have used the odor of the animal's usual diet and the odor of a novel liquid diet. In this regard, first, a comparison of Experiment XII with Experiment V is very important. In Experiment V the odor of the liquid diet was found to be highly attractive; in Experiment XII the odor was either neutral or slightly aversive. The difference between the two experiments is that the animals in Experiment V were provided with a daily supplemental diet of the liquid food before the experiment, and thus, had the opportunity for a taste and odor association. In Experiment XII the animals never had the food available for consumption. Their only experience with it was with its odor. A related finding concerns the findings of Experiment XII which indicate that use of the liquid food odor (highly attractive in Experiment V, as noted) did not lead to any different rate of learning than did the absence of odor as a cue. This was true both for simple, straight locomotion to a close goal and for a left-right discrimination. Furthermore, substitution of an aversive had a slight disrupting effect on performance, but not of a sort to indicate that the animals were really confused, repelled,

or misled by it. It would seem that the animals learned the concept of odor or of non-odor as a signal and may have benefited when the odor was a positive cue, but the nature of the odor was not important for this.

Experiment VI is also relevant to this context. The result of interest is the finding that animals which were repeatedly exposed to a chemical (TBC) in air while eating can lose their normal reaction to the chemical when placed in food. That is, when the chemical was mixed into food at a high concentration, the food consumption was affected much less for those animals which had experienced the greater number of exposures to it in air while eating uncontaminated food.

Considering the results as a whole, it seems reasonable to conclude that:

1. A non-sex odor does not serve as an attractant unless it has first been associated with a desirable taste.

2. An odor may be aversive if it has been associated with an aversive taste or if it produces pain. If it is aversive for the latter reason only, it is unlikely to be a deterrent for a hungry rat. In fact, it can acquire the properties of a safe-to-eat signal.

On this basis we conclude that the odor of a chemical makes little or no contribution to the repellency of a chemical or to its value as a deterrent except when it has a signalling value from association with a painful taste or when it has value as a novel stimulus. In terms of the development of chemical repellents of the sort generally in use, we recommend that emphasis be placed on the gustatory, ingestional, or taste properties of the compound. An exception could be for situations where it may be possible to provide the animal with both the taste and odor at some distance before he reaches the area to be protected. If

the animal can be conditioned in this way to use the odor as a warning signal, there may be value in developing the odorant attack. Finally, in this regard, the odor does temporarily slow the animal down in that it serves as a novel stimulus. But this effect is only temporary. We shall speculate below about possibilities for maintaining the effect.

Finally, we conclude that the methods developed in this investigation all can serve as the basis for the development of refined, testing techniques. Other questions in this context which appear to be very important concern the distinction that we have made between aversiveness, repellency, and deterrency. The present results suggest very strongly that these properties be related now to the problem of taste or ingestion independent of odor. In addition, however, they should be studied in regard to odor alone to determine what it is necessary to do to make an aversive odor a deterrent.

Speculations on Control of the Rat

There is sometimes a gain to be had by questioning what appears to be a well-established premise. This may be done by making another premise and comparing the two in terms of available information. If the result of doing this leads to an ambiguity or even to the possibility that the original premise must be supplemented by a new premise, the effort will have been worthwhile. In this section we wish to challenge the well-accepted premise that the exploring rat is primarily in search of food. As will be seen, we shall not reject that premise, but we shall suggest that it is applicable only to limited circumstances. If our speculations have any merit and if they were supported experimentally, an altered approach to the problem of repellency would be suggested.

Consider the behavior of a foraging rat. It is commonly assumed that it searches for food using olfactory signals as a guide to direction

or location. Once food has been found and consumed, it is assumed that the animal learns to return to that source on the basis of visual, tactual, auditory, and olfactory stimuli which are associated with the path to the food. There is no question that the actual behavior of the rat appears to be consistent with these assumptions. Rats find food and they learn to return along a single path to the source if the place of the source remains constant.

Methods for keeping the rat away from food are based on the assumption that the rat will seek and will find the food. Chemical or other barriers are used in the hope that the animal will not penetrate to the food. This has been a forlorn hope since even the most effective barriers lose their ability to deter very quickly. Such a consistent failure alone is sufficient to make worth while any questioning of the basic concepts underlying the concept of chemical or other repellents.

A rat in the real world in the process of learning the path to a desired place must learn to make discriminating responses to a large number of stimuli. The path from nest to food may be very complex and require a variety of associations of the sort, e.g., turn left at A, then right at B, go to C, climb over D, etc. Some of the stimuli may be visual, some tactual, some may be odors. A generally accepted theory postulates that the associations formed are established on the basis of an ultimate food reward; those nearer in time to the actual reward are developed more quickly. We are not challenging this basic general principle; rather we shall question whether in the world of the rat it operates in terms of food or some other reward.

In the first place, the assumption that rats learn to use food location cues effectively is not consistent with a long history of psychological research on the topic of discrimination learning. Experiments of this sort are of two general kinds. In the first kind the

animal is provided with a positive and negative stimulus (visual, auditory, olfactory). A common procedure is to use a bright light as one stimulus and a weak light as the other. The response is often simply turning in the direction of, or going to, the positive stimulus. In a Y- or T-maze this often means going to the right if the (say) bright light is on the right, and going to the left if the bright light is to the left. The correct response leads to a food reward; the incorrect response does not. The second class of discrimination experiment is similar except that instead of receiving food as a reward, the animal avoids punishment, usually an electric shock if it makes the correct run. Both situations are characterized by one simple sensory discrimination and the requirement for correctly associating one simple response, such as turning, to it. A careful look at the multitude of such experiments performed since at least 1900, and still being done, will show that to learn this simple discrimination, the rat requires hundreds of rewarded trials and that, after all of those trials, 8 out of ten, or 80 per cent, correct choices is considered a very high level of performance. Surely, a rat in the real world would not survive if it could do no better than that!

Another commonly used apparatus places the rat on a grid in front of a low hurdle. A light or tone is presented 5-10 sec. before the onset of current to the grid. No discrimination is required. The rat is expected to use the light or tone as a simple signal to avoid the shock by jumping over the hurdle. The expectation is reasonable in one sense, but in fact few, if any, experimenters using this apparatus achieve 80 per cent avoidance reactions after hundreds of trials. Theories have been proposed to account for this; some researchers have run animals as many as 50-100 trials per day for months with no greater success than described. It is true for this situation as well as for the discrimination-learning one that some small percentage of animals will improve at a greater rate and to a higher

performance level. But the majority of the animals do not. Furthermore, in the discrimination learning situation there is also a small percentage of animals that never discriminate better than chance; in the hurdle-jumping apparatus some animals never make a single avoidance response.

Consider the hurdle-jumping apparatus further. Most rats can learn to escape the shock by jumping the hurdle. Initially, the animal jumps up and down on the grid, tries to climb the walls and sonner or later jumps the fence. With successive trials of this sort all non-hurdle jumping responses drop out so that within 5-10 trials the animal is over the hurdle in less than 1-2 seconds. With continued experience, the animal takes a position on the grid which is optimal for jumping so that by, perhaps, 25 trials it is over the fence consistently in less than .2-.3 sec. from the onset of the shock. Yet, the same animal appears to have extreme difficulty in learning to avoid the shock. A successful escape response, of course, minimizes the shock exposure. And this fact is very important to us.

One more observation about the avoidance-learning problem is important. Even though the animal may show poor avoidance behavior, it does not follow that it has not made an association with the signal. In fact, both gross observation and physiological measures indicate that when the signal to avoid is presented, the animal prepares to jump. It crouches, tenses, shows changes in respiration and heart rate, etc., but nevertheless, it does not jump until the shock appears. The speed of this escape response is slightly faster than the escape response developed without a warning signal.

Two more common behavioral apparatuses should be described. One is the Skinner box or lever-pressing apparatus. It can be arranged so that bar-pressing delivers food, shock avoidance, or shock escape.

Again shock escape is an easy behavior to train. Shock avoidance is extremely difficult. Animals may learn to hover over the bar, but instead of pressing it when the signal arrives, they wait for the shock. A few animals learn relatively quickly, but most require hundreds of trials, as above, to a relatively low-level performance criterion.

Training to a food reinforcement in the Skinner box is a very tricky issue. There are two general approaches. In the older approach, still preferred by some psychologists, the animal is left to its own resources until it stumbles on the use of the lever. This kind of training is extremely slow and patience-trying for the experimenter. Today most psychologists "shape" the animal, i.e., on an individual animal basis, they train out or habituate the rat to the novel features of the apparatus and they guide it to the lever. For example, the experimenter waits until the animal is close to the food cup and then releases a pellet of food. The animal rarely accepts this food (even though very hungry). The sound of the food dropping into the cup is a startle stimulus, but with repeated experiences, the animal loses its fear of this sound and of the box and accepts the food. Then the experimenter releases a food pellet when the animal approaches the lever. By this means he shapes the animal, i.e., rewards it for coming closer and closer to the lever and finally for pressing it. This is a much quicker procedure overall than the other non-shaping method. It is not used by some psychologists because the shaping process depends upon the skill of the experimenter and, therefore, cannot be standardized from experiment to experiment or from rat to rat. Furthermore, there is no way to define a learning trial until the experimenter stops shaping so that the course of learning can be described from the beginning of the animal's experience with the apparatus. As a way to get a level of performance for evaluating the effects of

drugs, etc., this objection is probably minor. It could be an important question if interest is in the learning process which leads to that performance. In any case, in this apparatus, training with a food reward is not easy and learning in regard to it does not begin until the rat has lost its fear of the apparatus.

The straight runway is also frequently used as a behavioral apparatus. It consists of a starting box, a straight runway, and a goal box. Both boxes have doors so that the rat is detained before starting until a door is opened and detained after entering the goal box until it has eaten the food there. Again we find a very slow learning process. Early in training the animal may take many minutes (sometimes hours) to leave the starting box. It then explores the runway in great detail. It shows great hesitation in entering the goal box and, once in, may not accept the food at all. In time with many, many trials most animals will start and run very quickly, and eat quickly, but the asymptote of speed in the runway may take hundreds of trials to reach.

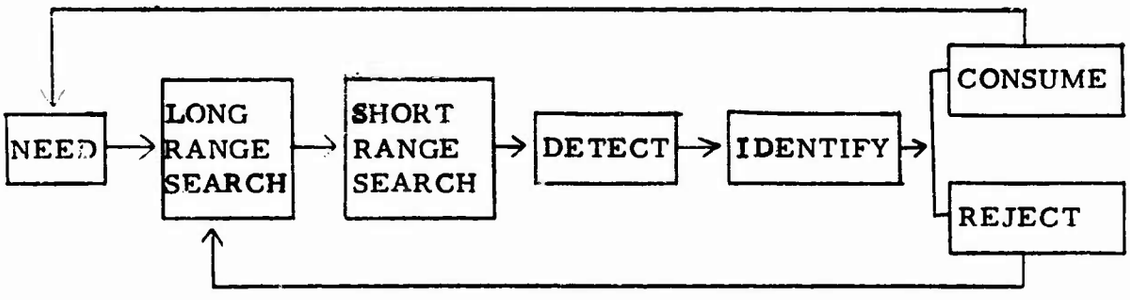
Finally, we must make the comment that before animals are used in these experiments, they are handled, gentled and every precaution taken to minimize general apprehension. In spite of this, it is apparent that no matter how hungry, even for these rats, the situation is one in which escape responses are more important and more readily made than food-seeking responses, and that the latter do not come into the picture until fear of the situation has gone. It is quite possible that the real food-locating learning is very rapid following a slow loss of fear and escape-seeking behavior. This, we believe, is the primary difference between the wild and the laboratory rat. The former is less tame; i.e., more afraid of the laboratory environment. We shall return later to the question of what constitutes a threat to the animal. For the moment, returning to the problem of

the wild rat in a real environment, we question the assumption that a "foraging" rat is exhibiting food-seeking behavior and propose instead that it is exhibiting escape behavior. Positive cue use associated with food rewards will be very slow until the animal no longer reacts to the environment as threatening. On this basis, then, we hypothesize that rather than being attracted to food by olfactory or other cues, the rat ends up at a food location only when it is guided there by an escape route, the end of which is a safe area which happens to contain food. What is learned with successive experience is the escape route.

Still another supporting consideration comes from a comparison of laboratory learning tasks and the real world of the wild rat. In the former the animal is put into an environment from which it cannot escape (unless escape behavior is being studied). After some time, the environment loses its threat value and the animal may explore for food. But the fact is that there is relatively little to be explored. There is a small volume of space which contains perhaps a lever as the only manipulable object, or a door to push, or a short space to traverse, etc. Furthermore, the arrangement is constant from occasion to occasion. Compare this to the situation faced by a field rat which may rarely have the same arrangement of its world from moment to moment. The location of food changes; the location of other animals and objects changes; even the weather changes. Such a rat would starve to death or be destroyed if it always took the same path to the same place. Such a rat, nevertheless, survives even though when it leaves the nest it has no way of knowing where food will be. The point is that even if the animal were primarily a learner of food-paths, it may have little that is constant enough in its environmental arrangement to be learned. We conclude again that the foraging rat is not primarily associating sensory experiences with food rewards.

An Alternative Interpretation

Consider the following paradigm as illustrative of the behavior of the rat:



The paradigm is based upon the following assumptions:

1. Food deprivation establishes a metabolic need state.

2. The need results in activities. The first activity is investigative; we are calling it long range search. The search is not for food, however. This is a long range search for danger or threat sources. It is characterized by head up looking and listening and intermittently by locomotion. That is, the animal searches the environment for signs of an attacker. If it finds none, it moves ahead a short distance in an apparently safe direction. If it suspects something, it freezes and after some time may gently sniff the air in addition to looking and listening. This is threat evaluation. If something is suspicious, it waits for it to attack. At the first sign of attack, the animal runs away (escapes) to another position. There the pattern is repeated until the animal is in an area in which there is no apparent threat. Support for this comes from the present investigation where we have found that the more the animal sniffs in the starting box of the runway, the more slowly it runs out of that box. In the Y-maze, the more the animal sniffed, the more slowly it ran. We note that the animals that learned the discrimination were the ones that sniffed

more and ran more slowly. We submit that these animals were more habituated, less afraid, of the apparatus, and using their sniffing mechanism for a different kind of evaluation. Similarly, in the runway and in the Y-maze the faster running animals were sniffing less and, at least in the Y-maze, learning less well to respond to food-associated signals. We suggest that these animals were less habituated and were making escape responses.

3. Once in the safe area, the rat engages in a short-range search. This is also a threat-evaluating procedure, but differs from the other in that the kind of threat being evaluated is different. This behavior is characterized by a search or investigation of small elements in the area. It is a head-down search. The ground or floor is felt and sniffed at. The path of locomotion is in the direction of the nearest stimulus. Locomotion is slow and stops at every object detected. Short range search leads to detection. If a new element enters the area, the animal reverts to long range search and that pattern of behavior. Barring this, it searches the area in detail.

4. Every object detected during the short range search is subjected to a threat evaluation process which leads to a simple identification of threat or non-threat. The identification process is characterized by a cautious approach to the object and by rapid sniffing. The object is then evaluated tactually with the vibrissae and olfactorily by continued sniffing. If up to this point, it appears safe, it is explored with the mouth by biting. If it passes this taste test, it is bitten and chewed on as a test of consumability. If there is not available a stronger or more preferred (established by previous taste associations) odor the object may be consumed <u>even if it is non-nutritious</u>. If a more preferred odor is available at the same time, the animal will leave the object and proceed to the next object although that one may not be the one having the preferred odor. That is, it

investigates everything it detects in the order of appearance during locomotion. These are all threat evaluations which lead to an identification. When it comes upon a preferred item, it will consume it. In general, consumption will be of that item, identified as safe, which has the most preferred taste and odor of those objects present. The animal does not look for a preferred food; it accepts the most preferred consumable object it detects. The object may not be nutritious, but as shown in the paradigm, if consumed it influences (usually reduces) the need state. Taste, touch, and odor are used only for identification. How much of the substance is eaten depends little or not at all on its odor and taste (if identified as non-threatening) and largely or completely on the metabolic requirement (Teitelbaum & Epstein, 1963).

5. If the object is identified as threatening (e.g., a poison or repellent), it is rejected for consumption and the animal will return immediately to a long range search mode. Again, it will freeze, look, listen, and if nothing happens, it will sniff gently. If no new threat appears and if the object does not attack, it may move on to the next detected object. Suppose it found it to be similar as would be the case if a chemical repellent were spread over an area. It will not run away unless attacked. If no more preferred substances were available, it would cautiously re-evaluate the object. Repetition of this process produces both an habituation (i.e., loss of fear reaction) and if the object produces discomfort, an adaptation (i.e., reduced sensitivity to the discomfort). As a result, the animal may penetrate the repellent barrier.

If the area is continuously without any threat except the repellent, it is now possible for the animal to begin to learn the cues to the location of food. If the dangers remain constant geographically, the

animal will escape, guided to the same area on successive forages. Situations of this sort exist, for example, in grain elevators and warehouses. In such cases, once in the safe area, the aversive odor might actually serve as a positive cue to the location of the food. That is, the odor of the repellent might become an attractant. The example of a warehouse is especially good because even though the escape route might vary from time to time, such a large area would be so occupied by consumables, that the rat will invariably be guided to them.

We cannot emphasize the idea too strongly that primarily the behavior of the hungry rat is oriented toward escaping threats and only secondarily toward food consumption. Threatened animals do not eat. In our laboratory, and in others, rats in cages have been known to starve to death with food available on an ad lib basis. We shall note shortly that the unavailability of an escape route in a novel situation constitutes a sufficient threat. Rats eat only in safe places and then they consume the most preferred substances of whatever happens to be there.

The Nature of a Threat and an Attack

We shall define a threat for the rat as an object which it has not yet identified. This means that for the rat there is uncertainty about whether the object will attack. An attack for the rat is an act which produces pain or which interferes with its normal functions and activities. Confinement in a cage is a state of being attacked, if not of seige, for the animal as is handling, insertion into an apparatus, or any other form of restraint. There is a wealth of data showing that the restrained rat goes into a high state of physiological arousal and the closely confined rat only less so. Even the gentler laboratory strains show physiological signs of fear when handled, even when they are used as pets.

Sources of attack may be either objects or places. The former may be another animal or it may be a pain-producing chemical. An important difference between these two examples is that a chemical repellent cannot follow a retreating animal. Thus, it is not a very effective attacker. That is to say that the escape routes are fewer in the case of another animal which can give chase. The rat need only back off from the repellent chemical.

A place may be an attacker in the sense used if the animal finds that its escape routes are reduced or altered after it has entered the place. The straight runway is a good example of this since the starting box door is closed behind the animal when it leaves that position. The runway area then attains the status of an attacker.

Attacks may vary in severity and, as noted, rats adapt to moderate physiological effects. If necessary they can even adapt remarkably to major effects. Adaptation must be distinguished from habituation. Habituation is what happens when the animal has reduced uncertainty about an object. A sudden noise is a threat to be identified. Repetition of the noise results in identification, i.e., habituation. In the hurdle-jumping apparatus, the animal learns that the grid may attack. However, unlike the experimenter who sees the signal as a warning, his question about this stimulus may be: Am I being attacked by this light? With repeated trials, the animal learns that the light is not an attacker. Therefore, why should it run away from it? It may also learn that the attack by the grid follows the occurrence of the light. But it is the grid which it is afraid of and from which it must run. That is, the rat's logic does not have to be the same as that of the experimenter! So the only information given by the light for the rat may be that it should prepare itself to run. And this it does.

Implications for Rat Control

If our speculations are correct, no technique for repelling a rat will work if it can be identified (loses threat value) or if it provides a passive form of attack, i.e., hurts if contact is made, but does not pursue. Unless the effect of contact is debilitating, the animal can develop an acceptance of it. The task of any method for protection without killing the rat is to maintain the animal's uncertainty about: (a) whether it will be attacked, and (b) whether its escape routes are being reduced. In other words an effective technique is one which provides an unsolvable, uncertain threat. What is needed to develop such a technique is an understanding of how the rat searches and identifies. Given this knowledge, the answer will be tactical rather than embodied in any particular substance which is painful or aversive. Even a weak sound would be extremely effective if it were presented in such a way that it never lost its novelty.

The very first requirement is that the rat be detected so that it can be threatened and attacked. Secondly, when detected, it must be known whether the animal is engaged in long- or short-range searching. Presumably, the tactics will have to different for each. The next requirement is that it be very difficult to predict the behavior of the threatening object and that the routes for escape from the area be manipulable.

The problem of effective protection, then, will be solved by finding sources of threat to which the animal habituates slowly or not at all, and then by using these less to keep the animal away from an area or object than to steer it to one. This can be done in only two possible ways: (1) by environmental tactics which are based directly on the ongoing behavior of the rat, or (2) by interfering with those processes on which the rat depends for searching and identifying.

An example of the latter may be found in the results of experiments in which lesions have been made in the olfactory bulb. In rats, the loss of the sense of smell which is produced results in a marked reduction of food consumption even when the food is the normal diet given under usual conditions. Many animals starve to death. We have observed this with large lesions in our laboratory (not reported because no measurements were taken) and so have others. We interpret this to mean that the anosmic rat cannot reduce enough uncertainty about even familiar food to identify it as safe. It is very significant, we believe, that the same rat shows no reduction in sniffing behavior, and may even show an increase. That is, the short range search process involved in identifying the food-object is intensified.

We could go into greater detail in this manner of discussion, but we feel that our point is made. Further discussion should be in the context of experimental studies aimed at manipulation of the search processes and we recommend this strongly.

Recommendations for Further Research

We see the development of effective deterrents as requiring most importantly research aimed at the following:

1. An understanding of the rat's search processes and techniques to control it.

2. An understanding of the distinctions between aversion, repellency, and deterrency.

3. An understanding of the dependence of repellency on the ingestional factors including taste and sensations associated with digestion.

Relation Between the Olfactory System and Olfactory Behavior

The electrophysiological studies of Adrian (1951) have suggested that there may be a number of functionally distinct types of olfactory receptor cells and this view has received support histologically (Clark, 1956) and anatomically (Allison, 1957). An individual olfactory receptor cell has been reported shcwing graded differences in its morphology on the basis of mitochondrial content, in the number of olfactory hairs, size of the terminal swellings, length and cross-sectional diameter of the rods and argentophil reactions (Clark & Warwick, 1946; Clark, 1957; DeLorenzo, 1957; Sen Gupta, 1964).

Apart from these graded differences, Le Gros Clark (1957) divided the olfactory receptors into two major categories in roughly equal proportions - those which undergo almost immediate dissolution after destruction of the olfactory bulb and those which persist apparently unchanged for at least six months post-operatively. Nagahara (1940) found similar results in the mouse and postulated a complete reconstruction of the olfactory epithelium. These findings of Le Gros Clark (1957) and Nagahara (1940) have been accepted without question by several recent workers (Adey, 1959; Beidler, 1961; Moulton & Tucker, 1964). However, recent work by Sen Gupta (1964) has provided contrary evidence to the existence of two receptors of the type described by Le Gros Clark (1957) and Nagahara (1940). Instead, Sen Gupta found that all receptors underwent the same morphological changes following olfactory bulb ablations. Therefore, all that can be concluded about olfactory receptor cells is that they do exist and more work is necessary to determine their exact nature.

Since these receptor cells appear to be the initiators of olfactory input to the higher cortical centers, it might prove beneficial

4. An understanding of how odors develop attractant and aversive properties when associated with ingestion, the manner in which they function as signals, and the way in which they are evaluated.

5. The role of olfactory bulb and other brain functions in the control of food consumption and search behavior. We have not discussed the hypothalamus in the former regard, but it is obviously important. Whereas some information is available about hypothalamic stimulations which produce eating to obesity, little is known about the possibility of inhibiting eating. The olfactory bulb may be a possible route in this direction and, if established as such, the possibility of chemical control via this mechanism is wide open.

to assess their role in olfactory acuity or olfactory discrimination. It has been demonstrated by several investigators (Welker, 1964; Sen Gupta, 1964) that these cells degenerate following olfactory bulb ablations. However, the extensive connections between the primary olfactory nuclei and many other portions of the brain create problems in other modalities following bulb ablations. For example, Carr and Caul (1962) and Donovan and Kopriva (1965) have indicated that there are adverse effects on mating behavior following bulb ablations and Le Magnen (1959) and Novakova (1960) have provided evidence for a disruption of eating and drinking behavior following bulb ablations.

A possible solution to the problem of destroying these olfactory receptor cells without great damage elsewhere comes from the work of Smith (1938) and Schultz (1960). What these two investigators have found is that a 1% zinc sulfate solution, when flushed through the nares, destroys olfactory receptor cells with little regeneration up until six months afterwards. This procedure is not without difficulties due to the histological technique involved and the sparcity of experimental data available. Despite these difficulties, zinc sulfate treatment of the receptors appears to be less damaging to other areas than does olfactory bulb ablations. As a practical matter, however, we have not been able to use it successfully. That is, we find that a single injection (washing) of a one percent solution is usually lethal within seconds.

Although these findings provide a physiological basis for understanding the way in which odors are discriminated, the mechanism by which odorous substances excite the various olfactory receptors is still not clear. Moulton and Tucker (1964) have suggested that a possible approach is through an analysis of the relationship between the physico-chemical properties of an odorant and it's relative detectability. In recording

from five sites in the olfactory bulb of a rabbit, Moulton found that various odorants differed markedly in their relative effectiveness at each site. He also found differences in the temporal evaluation of responses to a given odorant.

Mozel (1964) offered another approach to the problem by suggesting that the receptor sheet as a whole might elicit differential time-spaced discharges in response to a separation of the chemical vapors in a manner analogous to gas chromatography. Some support for this approach has been demonstrated by Mozel (1964) but as yet his evidence is rather sketchy.

Despite Moulton and Tucker (1964) and Mozel (1964), there still appears to be no direct evidence that different parts of the mucosa respond differentially to different odors. Although the type of approaches outlined by Moulton and Mozel have promise for the future, the majority of work concerning olfactory perception has been behaviorally oriented.

The use of macrosomatic laboratory animals as test subjects in olfactory discrimination and acuity studies has gotten off to rather a slow start. A probable reason for this, at least as far as the rat is concerned, is the initial difficulty experienced by early investigators (Ligget, 1928; Swann, 1933; Brown & Ghiselli, 1938) in developing satisfactory habits based on olfactory discrimination.

Prior to 1960 the only success in establishing rapid learning of olfactory discrimination has been when the odor of food itself has provided a major component of the positive stimulus. French (1940) designed an apparatus requiring the animal to run up a tunnel to the center of a cage, the floor of which had small holes leading to food

boxes. The holes were just large enough for the animal to get its paw into the food box. Animals learned to associate an odor with food and could discriminate. Stone (1941) found rats able to discriminate dishes of standard Purina Chow from those containing Purina Chow plus a mixture of quinine sulfate, without tasting the contents of the dishes.

Le Magnen and Rappaport (1951) trained rats to discriminate between drinking bottles on the basis of an odorous substance smeared on their spouts, and Gruch (1957) trained rats to discriminate between three boxes on the basis of odorous-non-odorous air flowing from a tube. However, all of these studies can be criticized on the ground that visual cues and smell of the reward were not properly controlled for. Our Experiment XII probably represents the most carefully-controlled situation yet available. It does support these older findings in showing that odor may be used as a discriminative cue.

Moulton (1960) used three different experimental techniques and found that the ease with which olfactory discriminations are learned depends to a large extent on the method of odor presentation. A major factor determining the rapidity of learning appears to be the degree of contiguity of stimulus and reward. When contiguity of stimulus and reward were remote, as in the Y-maze, learning did not occur, whereas when the odor was directly attached to the drinking spouts and a shock given for an incorrect choice, the animals had little difficulty learning. These results might help explain the lack of success of Swann (1933) who used a modified Lashley jumping stand and Ligget (1922) who used a T-maze and Yerkes discrimination apparatus.

A method for testing olfactory discrimination in dogs was described by Becker, King, and Markee (1962) who comment on the difficulty of constructing a situation in which the dogs can be forced to use odor cues exclusively. From their observations they suggest that the animals will use any other cue if possible.

If one looks at the extensive anatomical connections between the olfactory system and the hypothalamus, it would appear logical to investigate the behavioral significance that these connections might subserve. Therefore, in recent years there has been an extensive amount of research concerning the role of olfaction in the sexual behavior of lower animals. Stone (1922, 1923) and Beach (1942) showed that copulatory patterns survive olfactory bulb ablations and that as long as the female and male are in proximity mating can occur. Heimer's (1967) findings agree with the above but specify that while mating can occur, the frequency of mounting, intromission, and ejaculation latency are definitely affected. Furthermore, Calhoun (1962) in observing the behavior of the wild Norway rat living under semi-natural conditions, observed that as the female ranges from the nest she leaves a scent on the ground. These scents are examined by the male and may be used in locating the female.

In recent years evidence has accumulated that odor can constitute an exteroceptive factor affecting the oestrous cycle, mating behavior, and probability of pregnancy of vertebrates (Lee-Boot, Whitten, and Bruce Effects). These observations have led to the development of the concept of pheromones, external chemical secretions which are capable of producing specific reactions within nonspecific receiving organisms.

The possible implication of the above concept on measuring the performance of the rat in the typical runway or maze is suggested by Ludvigsen (1967) who found that he could significantly predict the path one rat would take based on the path taken by the previous rat.

Since a thorough cleaning of the runway or maze between animals eliminated his success in predicting, Ludvigson concluded that the second rat is influenced by the odor of the first.

Further experimental evidence showing olfactory discrimination using biological odors has been conducted by LeMagnen (1952) who delivered the odor of estrous and nonestrous females to separate sides of a T-maze and showed that the adult, male rat could discriminate and locate the receptive female. However, castrated males showed no preference. In further investigation, LeMagnen found that the prepuberal or castrated male rat can discriminate between the odors from receptive vs. nonreceptive females and that gonadal insufficiency influences the animal's preference for the two odors.

Support for this view was given by Carr and Caul (1962), who showed that both the normal and castrated male rat can discriminate between the odors from receptive vs. nonreceptive females if the males are reinforced for responding discriminatively to the two odors. Moreover, Carr and Pender (1958) found that both the normal and castrated male rat can discriminate between the odors of urine excreted by receptive vs. nonreceptive females.

Although the above lines of research differ markedly in terms of the subjects used, apparatus, method of stimulus delivery, and results, it seems clear that animals can make use of odorants as cues when forced to discriminate. The major questions concern the conditions necessary for the discrimination to take place and the importance of odor cues as compared to other sensory cues. See our discussion above in which we suggest the conditions and manner in which the rat may employ sensory information.

Studies of odor discrimination have involved such responses as pressing a bar, drinking from an odorized water spout, running

a maze, etc. None of these responses can be used to make inferences about associated olfactory-oriented behavior. It is for this purpose that sniffing behavior is especially useful. We are not the only ones who have taken advantage of this. We believe that our studies have provided the first model and systematic set of empirical relationships.

Berlyne and Slater (1957) noticed that rats in a Y-maze sniff more when novel stimuli are presented and Stuver (1958) noted that the location of the nasal mucosa is such that for a molecule of odor to reach the mucosa, the animal must sniff. Sniffing draws the air up into the mucosa while normal breathing does not.

So far as we know, aside from our work, the only actual investigation of the sniffing response has been that of Welker (1964). Welker employed a motion picture camera running at 32 or 64 fps. to provide a systematic, single frame, time-motion analysis of the response. The movie records were analyzed in terms of three distinct actions: (1) sniffing movements, (2) latency of response and contact frequency, and frequency of occurrence of sniffing at two standard test objects, (3) measures of duration of sniffing contact with a concrete block smeared with various edible substances. The technique appears to be excellent, but limited in terms of the freedom of movement of the animal.

Welker's results indicated that mildly novel visual, auditory, tactile or olfactory stimuli will evoke a sniffing response from the rat. This sniffing response can be divided into four major behavioral sequences: (1) polypnea, (2) protraction and retraction of mystacial vibraisae, (3) head movements and fixations, and (4) protraction and retraction of the tip of the nose. Ontogenetically, Welker found that these sniffing responses appear in newborn rats at eight days after birth but are not fully developed until the eighteenth day.

To study the relation between sniffing and olfaction, Welker ablated various parts of the olfactory systems and observed the effect on sniffing. His film records showed that bulb ablations appeared to have no effect on sniffing. Although there was a lack of any noticeable effect in sniffing behavior, in terms of other behavior there were some obvious differences between operated and non-operated animals. Normal animals approached a xylol-impregnated cotton ball hesitantly and sniffed from a distance of 1-2 inches away, while operated animals approached and touched the cotton ball. Also, the animals having bulb ablations had difficulty locating food pellets. The only time sniffing behavior appeared disrupted was in those animals having damage to the frontal cortex.

Welker concluded that sniffing is a relatively fixed and stable response pattern which appears early in ontogeny and persists after bulb ablation, but disappears after frontal cortex damage. He suggested that sniffing is more than an olfactory response since visual, tactile, and auditory stimuli may induce its occurrence. However, too strong a stimulation in any modality appears to inhibit sniffing. Our results certainly agree as far as they are comparable, except for observations which suggest that if the animal is in a situation in which it has experienced an aversive odor, it sniffs more than normally and that it requires more sniffing to identify an odor.

All things considered, sniffing appears to provide an excellent response for studying habituation and the attentional behavior of the rat in regard to any stimulus modality. To the degree that sniffing is associated with subsequent activities of the olfactory bulb, this suggests that the bulb directly, or indirectly via its (not well-traced) inter-connections with the rest of the brain, functions as part of an attentional mechanism. The frequent, casual observation of a reduction in food consumption following olfactory bulb lesions suggests

possible important involvements in the control of eating. Clearly, a better understanding of the olfactory bulb of the rat and its functions, of the sense of smell, and of sniffing in relation to these and to the behavior of the rat in general, would lead to a great advancement in our understanding, and therefore, our ability to control the rat.

The Olfactory Bulb of the Rat

The olfactory bulb in the rat is an anterior extension of central nervous tissue which ostensibly functions to transmit olfactory information from the specialized receptors in the nasal epithelium to the secondary olfactory areas of the cerebral hemispheres and limbic structures. The bulbs are located from 8.5 mm. anterior Bregma, to 11.5 mm. anterior Bregma, and from the midline to the supraorbital bone. Each olfactory receptor gives rise to a nerve fiber which, after aggregating in a bundle of about 1000 such fibers, is enveloped by a Schwann cell and passes through the cribriform plate and enters the olfactory bulb.

The early electrophysiological studies of Adrian (1950, 1956) show that there is a rather crude, though logical, topographical correspondence between these primary sensory fibers and their termination in the bulb with the upper and back areas of the mucusa projecting mainly (precisely) to the upper surface of the bulb, while the lower regions of the epithelium project mainly (less precisely) to the lower surface of the bulb.

The bulb, itself, is composed of both central nuclear areas and superficial fiber tracts. The primary neurons upon entering the bulb spread out over the surface of the bulb and form an elaborate network in its outer layers. These fibers then descend deeper into the bulb

where they end in synaptic contact with the primary dendrites of the secondary neurons in discrete spherical bodies: the glomeruli. This glomeruli formation is the most distinctive feature of the olfactory bulb since they contain the only synapse in the direct path between receptor and cerebral cortex.

The primary fibers entering the bulb do not divide until they enter a glomerulus, but here their densely branching terminals spread out and terminate on mitral cells. The axons of the mitral cells form the bulk of the lateral olfactory tract, which passes back to the brain. However, it is important to note that this tract also contains centrifugal fibers conveying impulses in the opposite direction -- from the brain to the bulb.

In addition to the mitral cells and glomeruli there are two main cell types in the olfactory bulb: the tufted cells and the granule cells. The tufted cells lie deep to the glomeruli in what is called the external plexiform layer and their processes form a dense plexus with the dendrites and recurrent axon collaterals of the mitral cells and the peripherally directed processes of the granule cells. Unlike the tufted cells, the mitral cell axons do not give off collaterals until they reach the granule cell layer. This is a complex structure having, in addition to many sheets of granule cells, three types of short axon cells.

Also, within the bulb and continuing posteriorly into the base of the cerebral hemispheres, is the anterior olfactory nuclear area whose axons enter the olfactory tracts. Experiments by Lohman (1969) in the guinea pig seem to indicate that the commissural fibers terminating in the olfactory bulb have their origin in the anterior olfactory nucleus rather than in the contralateral olfactory bulb. The lack of evidence for commissural connections between the two olfactory bulbs in recent studies by Heimer (1968), Powell, et al. (1965), and by White (1965) seem to indicate that the same conclusion is justified

in the rat. In hindsight, it appears that earlier descriptions of an interbulbar connection may have referred to a small number of degenerating fibers that can be followed across the midline in the anterior commissure in rats that have survived an olfactory bulb lesion for more than two weeks.

It is generally accepted that the fibers from the olfactory bulb project on four main regions: (1) the olfactory peduncle, (2) the olfactory tubercle, (3) the prepyriform and periamygdaloid fields, and (4) the cortico-medial amygdaloid complex.

Although most investigators seem to restrict the projection filed of the olfactory bulb fibers to the primitive cortex of the ipsilateral periform lobe including the cortical amygdaloid nucleus, there is still disagreement regarding the limits of the projection. With respect to the rostral part of the olfactory cortex, most investigators (Heimer, 1969; Powell, et al., 1965) limit olfactory bulb projections to the anterolateral part of the olfactory tubercle. There also appears to be some controversy as to whether or not bulbofugal fibers terminate in the ventral entorhinal area with Heimer (1969) and White (1965) getting positive results and Powell, et al (1965) getting negative results.

The results of recent physiological and behavioral experiments have indicated a close functional relationship between the olfactory apparatus and the hypothalamus -- particularly in reproduction. These close ties between posterior-medial hypothalamus and the olfactory system can be demonstrated anatomically by describing the projections: (1) from stria terminalis originating in the cortico-medial amygdaloid region to the hypothalsmus, (2) from the olfactory tubercle to the rostral part of the medial forebrain bundle, which then forms the oligosynaptic pathway between the bulb and the lateral hypothalamus, (3) from the prepyriform cortex to the medial forebrain bundle and then to the hypothalamus.

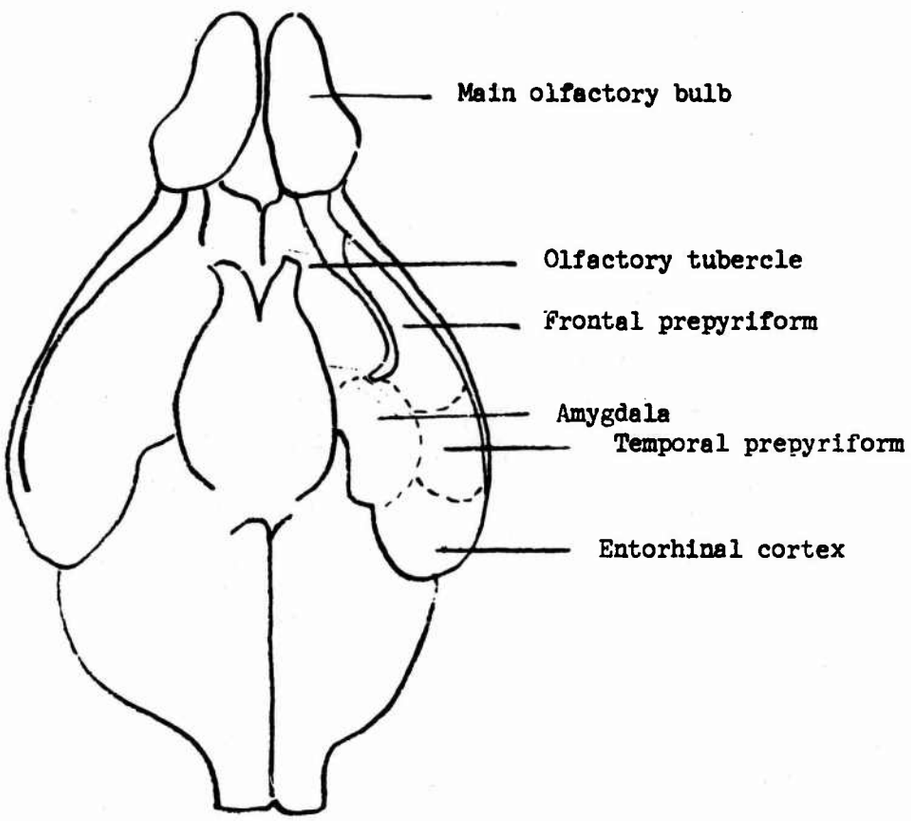
There is also evidence for direct projections, however moderate, of olfactory bulb fibers to the ventral entorhinal area. Since the entorhinal area sends a massive projection to the hippocampal formation, this suggests that, in rats at least, the olfactory bulb stands in proximity to the hippocampal mechanism.

Receiving the vomeronasal nerve from the vomeronasal organs is the accessory olfactory bulb located on the dorso-medial aspect of the main bulb. This area has complete representation of layering from glomerular to mitral layers as the main bulb does.

Histological sections of the normal olfactory bulb are distinctive in appearance and totally different from cerebral sections. Since very few atlases include either schematic drawings or photographs of the bulb, a brief description may be helpful.

The different cell types and fibers of the bulb are arranged in concentric ovals. The outer layer is composed of nerve fibers from the three afferent (to the bulb) cranial nerves. The second layer is glomerular. The third band is lightly stained gray matter. The next darkly stained ring is of mitral cells, followed by the inner core of granule cells. This concentric pattern is observable from the anterior tip cross-sections to the cross-sections just preceding the accessory bulb. At that point, the glomeruli discontinue on the dorso-lateral surface and are replaced by the lateral stria. Also, the pattern of mitral and granule cells is replaced by the accessory bulb and the anterior olfactory nucleus which proceeds posteriorly into the cerebral hemispheres.

Figures 34 through 38 are of representative cross sections of the bulb at the anterior tip, in the main bulb, in the area of the accessory bulb, and at the point of projection into the cerebrum, respectively. The only individual differences observed among animals was in overall sizes of the bulb.



Ventral View of rat brain

Figure 34. Primary Olfactory Cortex in the Rat.

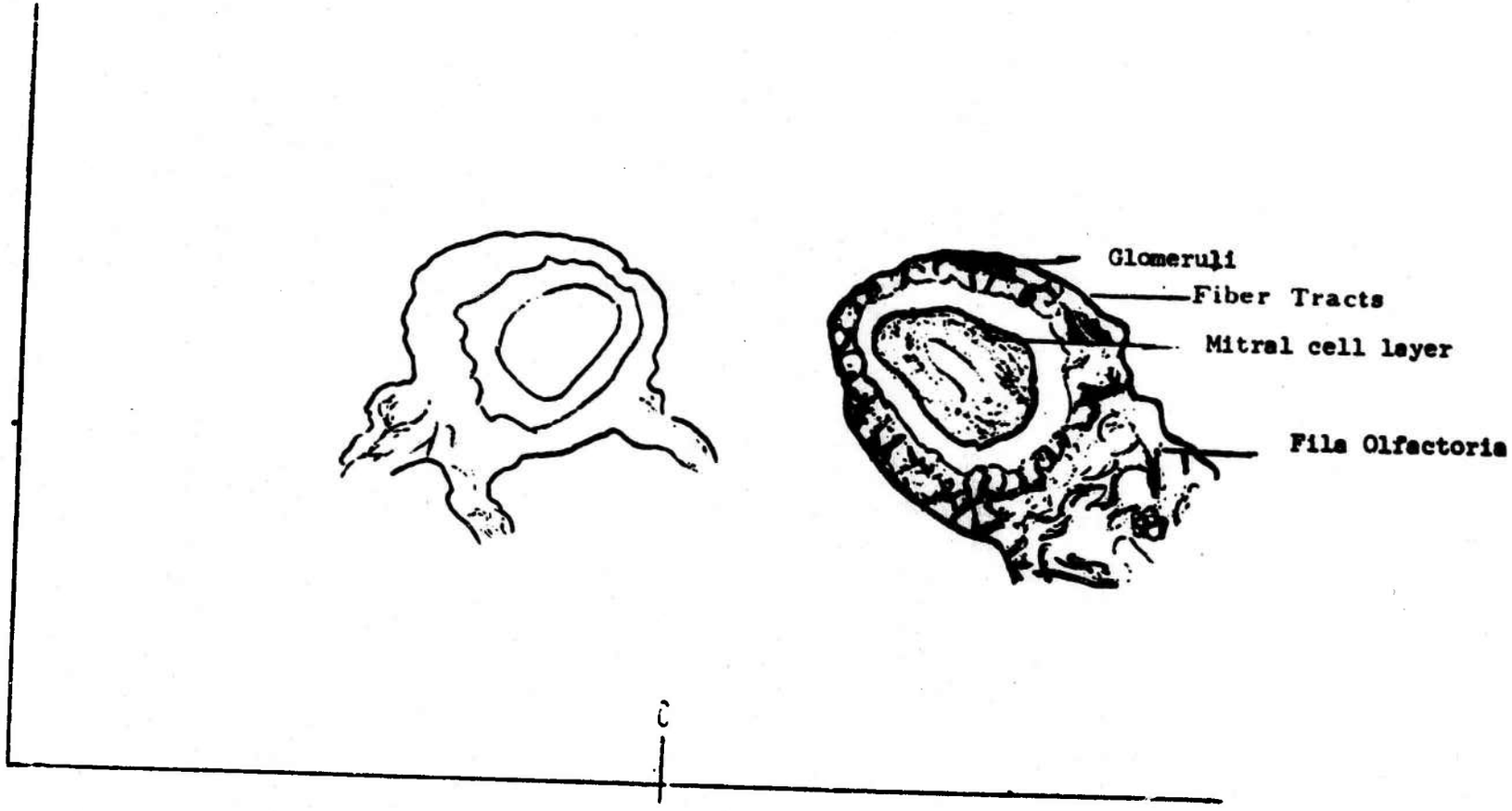


Figure 35. Cross Section of Anterior Tip of Bulb

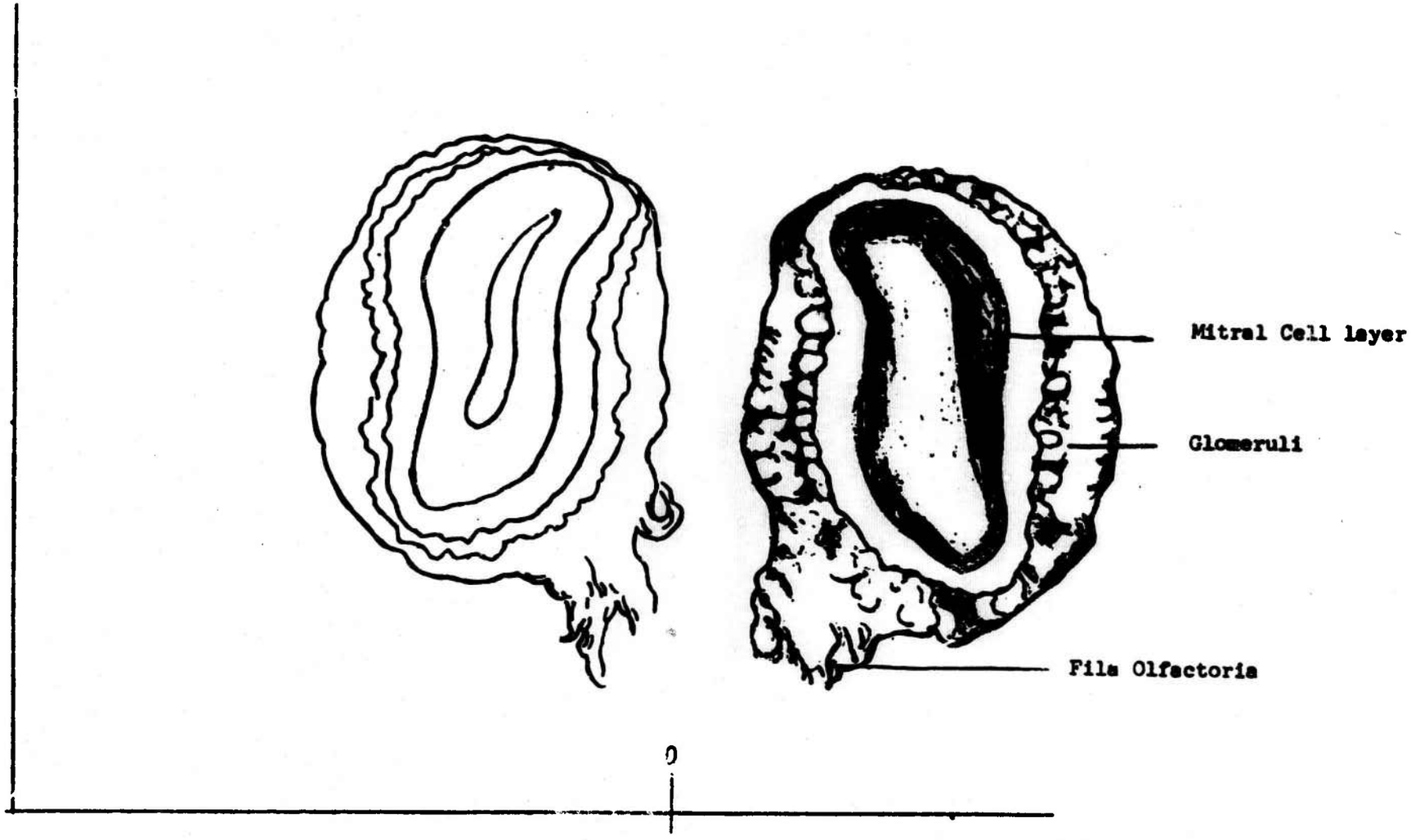


Figure 36. Cross Section of Main Bulb

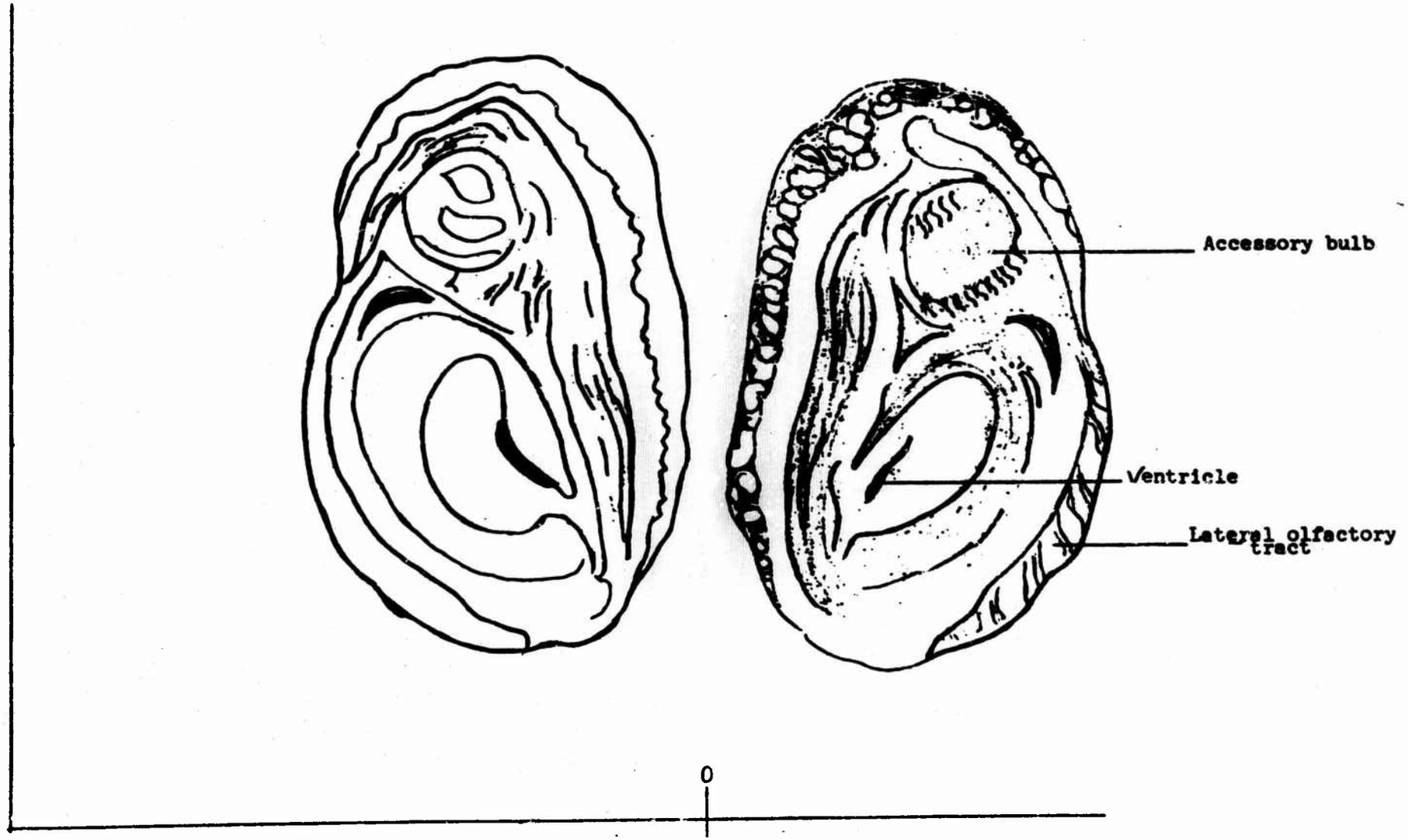


Figure 37. Cross Section Through Accessory Bulb

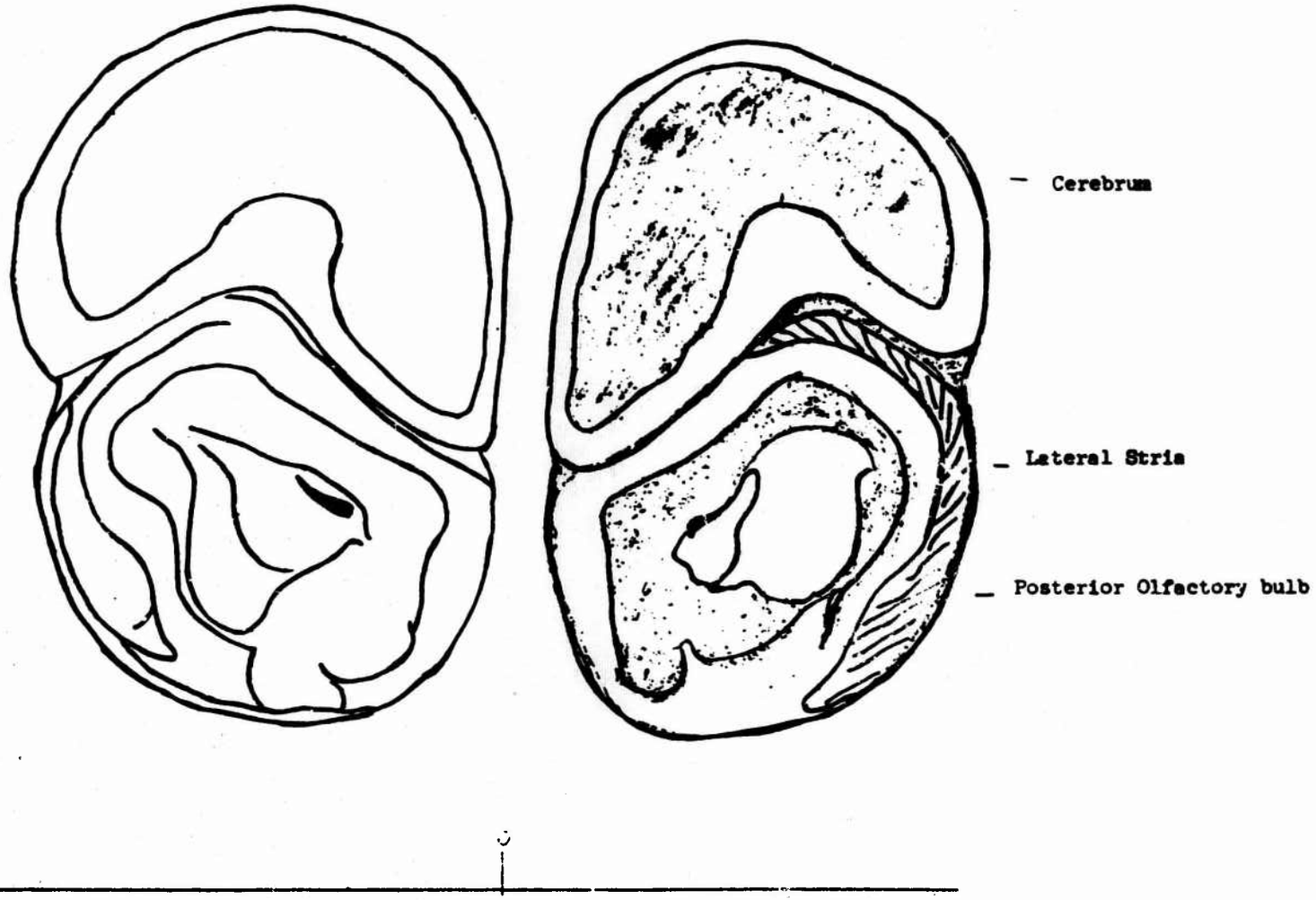


Figure 38. Cross Section of Bulb at Juncture with Cerebrum

Three primary cranial nerves enter the bulb from the nasal cavity. The vomeronasal nerve proceeds from the vomeronasal organ (or organ of Jacobson, a specialized epithelium of the nasal system) to the accessory olfactory bulb. The nervous terminalis originates in the nasal area close to the vomeronasal nerve and enters the cerebral hemispheres in the region of what, until recently, was called the medial olfactory tract. This tract is now suspected to be a thickening of the lateral olfactory tract. The function of these two nerves is not yet fully understood, but they may be components of the autonomic system innervating blood vessels in the mucosa or carrying cutaneous sensory components from the nasal septum. Better understood is the fila olfactoria, the shortest of the three nerves, which travels from the receptor cells in the mucosa to the inferior surface of the olfactory bulb where its fibers branch freely inside the glomeruli. Any individual axon terminates in only one glomerulus. Thus, each glomerulus receives impulses from a distinct receptor field. There are no synapses between the receptor cells and the glomeruli.

Also in this area, though not concerned directly with olfaction, is the trigeminal nerve which sends somatic afferents to the skin and mucous membranes of the head.

Surgical Techniques

The details of surgical and histological techniques are not usually reported, especially the details of their development. However, since few investigators will be familiar with the olfactory bulb (at this writing) a description of our experiences in performing and evaluating various methods may be of value.

A flip-back stereotaxic instrument (Model F, Scientific Prototype Mfg. Co.) for small mammals (rats and mice) was used to stabilize

the animals' head for electrode placement. It was equipped with an infinitely variable micro-manipulator and electrode holder. If the tips of the ear bars of the stereotaxic are not rounded down, ear plugs may be inserted in the animals' ears for protection. A Fordham Model No. 21 dental drill with variable speed control was used to drill the holes in the skull. Size 2 to 5 S.S. White burs are suitable for most electrode sizes. An electrolytic lesion maker was the source of current for lesioning. The amperage output was adjustable from .1 to 10 m.a. One lead from the current source was attached to the lesioning electrode at the uninsulated upper tip. The other lead served as a ground and could be placed anywhere on the body of the rat (usually the ear) or onto another grounding electrode which was implanted in the brain. Current was delivered as long as the switch was depressed (i.e., there was no automatically timed device for delivery of current).

Many types of electrodes were tested and used in different phases of the experimentation. A commercially purchased bipolar electrode was first tried which produced a desirably sized lesion when tested in egg white. The electrode, distributed by the David Kopf Co., was made of stainless steel wire coated with physiologically inert epoxylite. The tips of the electrode, the lesioning tip and grounding tip, were 0.5 mm. apart and exposed for 0.5 mm. at the tip. Two disadvantages, however, discouraged the use of this electrode at the beginning of the experimentation. First, the size of the hole in the skull necessary to accommodate this electrode was too large to be feasible at that time. Preliminary animals died because of excessive bleeding. Secondly, after two or three uses, the lesioning tip of the electrode burned away and was no longer functional. Although the second disadvantage remained throughout the studies, the first was overcome by refinement of surgical technique. Thus, bipolar electrodes were used later on some animals.

Monopolar electrodes were next tested and found to have the advantages of ease of placement, ease of replacement (they were easily made by hand) and variety of ground placement (on the ear, in the bulb with the lesioning electrode, or in the opposite bulb). To make the lesioning electrode (the grounding electrode was made the same way), a piece of Teflon - coated stainless steel wire, 0.2 mm. in diameter and approximately 4" long, was cut from a roll. The Teflon was scraped 3/4" off the upper end. Masking tape was placed around the lower end, exposing 1/4" of Teflon - coated wire and then the exposed Teflon was scraped off. The tape was removed, leaving a relatively straight demarcation between coated and uncoated wire. The uncoated 1/4" tip was carefully measured and cut down to 1 mm. The only problem of the monopolar electrode was keeping it straight and steady during the implantation. One solution was to place it inside a hypodermic needle.

In preparation for surgery, the animal was first weighed to determine dosage of anesthetic. The anesthetic used throughout was Equithesin from Jensen-Salisbury Labs. Equithesin must be kept out of light and because of decomposition and precipitation, cannot be used reliably if over one year old. The dosage was 0.003 cc/gram body weight and the injection, always using sterile syringes, was intra-peritoneal at the lower abdominal midline. This treatment was usually sufficient to keep the animal unconscious for 1-3 hours. When the original dose was not sufficient to subdue the animal, the animal was returned to its home cage and re-tried the next day at a slightly increased dosage.

When preparing the wild rats for surgery, a different procedure was used because of the inability to safely handle them. The animals were dropped from a transfer cage into a large vacuum sealed jar containing ether-moistened cotton. When the animal was incapacitated

enough to be handled, it was placed in a restraining box which had an aperture for insertion of the hypodermic needle. When the animal fully recovered from the ether, it was injected with Equithesin and removed when unconscious for surgery. Any animal was considered ready for surgery when pinching the ear or inserting the ear bars produced no physical reactions.

Before placement in the stereotaxic apparatus the following preparations should be made. Using scissors or a razor, remove as much hair as possible from the top of the head from $\frac{1}{2}$" in front of the eyes to $\frac{1}{2}$" behind the eyes. After washing the area with benzalkonium chloride, the initial incision is made with a scalpel on the midline from $\frac{1}{4}$" in front of the eyes to $\frac{1}{4}$" behind the eyes. Again, with the scalpel, cut the underlying fasciae the same way. Using scalpel or forceps, scrape away the fasciae until a clear view of dry bone is seen. The animal is then placed in the stereotaxis and the head is firmly fixed with ear bars, and nose and teeth clamps. Using four hemostats, clamp back the scalp and fasciae so that the skull markings over the olfactory bulbs are completely visible. More scraping, cleaning, and drying may be necessary. Experience with just one or two animals enable the surgeon to accurately delineate the area of the skull over the olfactory bulbs.

No stereotaxic coordinates were used to select the point for drilling. Instead, the surgeon approximated the longitudinal center of the bulb (approximately 10 mm. anterior Bregma) and drilled as far to the side of the midline at that point as possible (approximately 1.5 mm. from midline). NOTE: At approximately 2.0 mm. to the side of the midline is the eye socket. Therefore, there is very little space to choose in the lateral direction.

Although this choice of locations seems haphazard, there was virtually no variability in the site of the lesions made by one surgeon or many surgeons.

After marking the point with a pencil, a size 2 bur was used to make a small hole over the olfactory bulb suitable for a monopolar electrode. To accomodate a bipolar electrode, the same small hole was first drilled, then enlarged with a size 5 bur. The danger of the larger hold is drilling too near the midline, severing a major blood vessel, and causing fatal bleeding. The skull is approximately 1 mm. thick and again great care must be taken when approaching that depth to break through without damaging the bulbs underneath. It is recommended that drilling be done at high speed and the bone be kept very dry. If properly done, the entire surgical procedure produces little if no bleeding.

When the proper hole has been made, the lesioning electrode may be lowered into the bulb to the desired depth. The convention used in these studies was to start the measurement 1 mm. below the surface of the skull, that is, approximately at the surface of the bulb.

The lesioning electrode did not always enter the bulb perpendicular to the skull. Some lesions were made by implanting the electrode at a 50-60^{o} angle from the horizontal plane. When lesioning with a 90^{o} electrode, the range of depths was 2.5 - 4.0 mm.; when lesioning with a 60^{o} electrode, the range of depths was 1.5 - 2.0 mm. These two different methods resulted in equivalent lesions in the same area of the bulb. After the lesioning electrode had been placed and the ground electrode attached to some point (varying according to type of lesioning electrode and size of lesion) the current was delivered. In the studies reported, the range of amperage used was 1.0 - 10.0 m.a. and the duration was always 30 seconds. When one lesion had been made, the usual procedure was to reverse the position of electrodes and lesion the other bulb.

After removing electrodes the entire skull and scalp area was wiped with benzalkonium chloride. Three to four stitches of size 4

suture were used to close the incision. Although only a semi-sterile technique was used throughout, no problem of infection ever occurred. A few animals received a penicillin injection (0.1 cc I.M.) post-operatively, but this precaution was not deemed necessary and the injections were discontinued.

Typical recovery behavior after surgery was as follows. Approximately 30 minutes after suturing, the animal would begin to regain sensitivity to the environment, i.e., eye blink to air puff, or retraction of limb when touched. One to two hours later, the animal would begin to attempt to stand although still unsteady. Four to five hours after surgery, the animal may be in full motor control, but remain tense and sensitive. By the following day the animal should appear normal in all respects including eating and drinking behavior and response to the experimenter's handling. The sutures fall out after about eight days and when the fur grows back, the animal appears normal.

Experimental Results

Six studies for which histological data are available will be reported.

Table 7 lists the parameters used in the first study. Histology was done by the paraffin method and no behavioral measures were taken from these animals. The missing numbers were animals which died either during surgery or before perfusion was possible. Deaths during surgery at this time were usually due to the fact that all the preceding surgical procedures were still in the developmental stage and refined techniques had not been acquired.

The results of this study are tenuous. Histological techniques were also being developed at this time and some data were lost or distorted in the process. The only conclusion which seemed unquestionable

TABLE 7

Study I [1,2,3]

Animal number	current in m.a.	depth in mm.	electrode	ground	result
3	3	3	m	clip on ear	questionable
5	3	2.5	m	"	"
6	2	2.5	m	"	"
7	2	3	m	"	"
8	4	2.5	m	"	"
9	3	4	m	"	"
10	3	4	m	"	"
11	2	4	m	"	"
12	3	3	m	"	"
13	1	4	m	"	"
14	3	3	m	"	"
15	3	3	b	"	evidence of lesion
17	3	4	b	"	"
19	4	4	b	"	histology incomplete
20	3	3	m	"	questionable
21	3	3	b	"	evidence of lesion
22	3	3	m	"	questionable

[1] animal #1, 2 not perfused
" #4 died in surgery
" #16 died in surgery
" #18 died after surgery

[2] current duration was 30 sec. for all animals

[3] m = monopolar
b = bipolar

was that bipolar electrodes produced some, though not radical, lesions. Monopolar electrodes produced, at the most, questionable lesions. In other words, in every case of monopolar lesioning, tissue damage either definitely was or could have been artifact.

Gross observations before histology revealed slight abnormality of tissue for the bipolar lesioned animals only. After histology, the evidence of bipolar lesioning was deduced from several irregularities. First, in two of the four animals there was a distortion of the shape, though not content, of the mitral cell layer so that it appeared "pinched" in the middle. However, this type of evidence is supportive rather than conclusive. More significant is the fragmentation of the lateral glomeruli and the "smeared" appearance of some areas. They are characterized by a lack of extant cells and resemble scar tissue (See Figure 39). All these irregularities occurred at the anterior tip or on the anterior lateral surfaces. It may be noted here that in all six studies damage found was always more anterior than posterior, and more lateral than medial. This, of course, was the result of consistent, if not accurate, electrode placement. However, the intent of the lesion was to interfere maximally with input from the receptors, so this electrode placement was fairly acceptable. More medial placement was impossible until the angled entry (discussed later) was developed.

Normal histological procedures subject brain tissue to physical and chemical stresses which, in turn, produce irregularities not related to the lesioning treatment. These irregularities are sometimes very difficult to distinguish from lesions and may lead to erroneous conclusions concerning experimental results. A discussion of such artifacts may aid in interpreting the r ılts of these six studies.

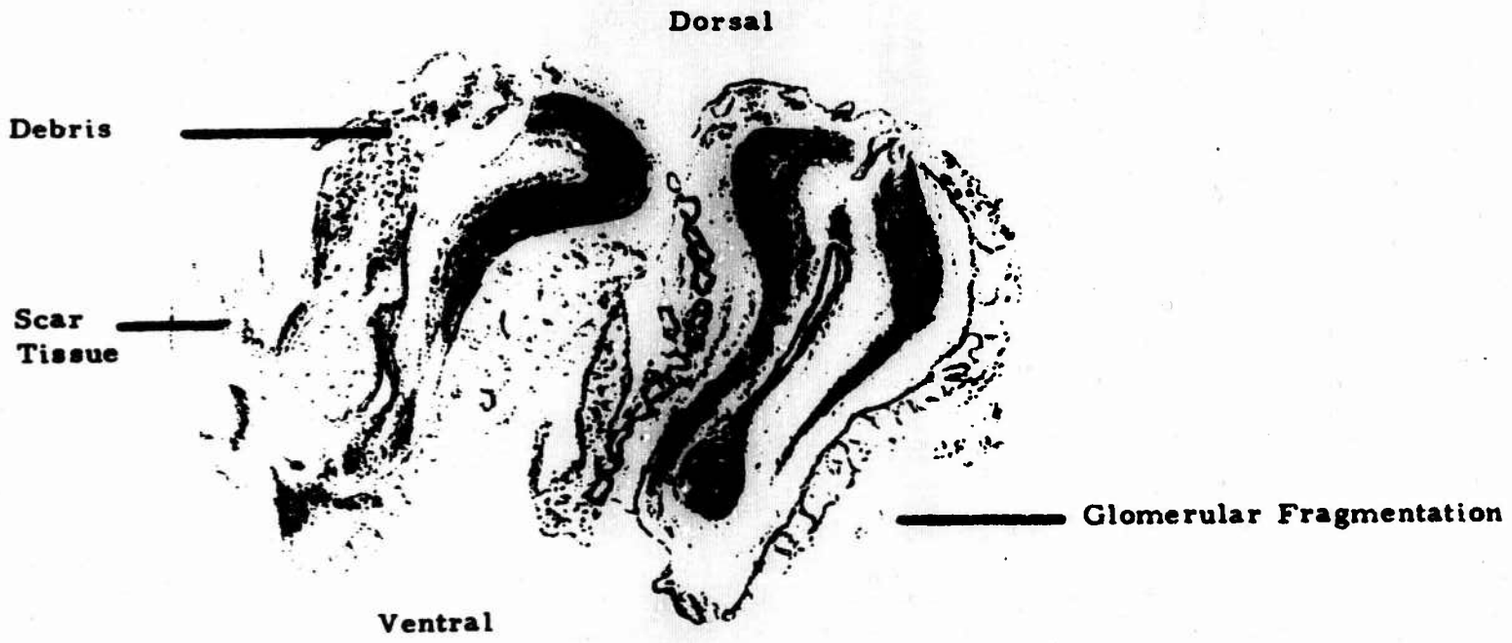


Figure 39. Study I; Animal 17: Anterior Section

The first and major source of artifact is the dissection of the brain from the skull. It is very difficult, especially in the area of the olfactory bulb, to chip the surrounding bone without nicking or cutting the tissue beneath. Secondly, any lesioned tissue tends to adhere to the dura or other connective tissue and thus separates from healthy tissue when removed from the skull. Therefore, the part of the bulb that is left for embedding appears normal and the extent of lesion in the missing tissue cannot be determined.

After dissection, the brain is immersed for several days in a series of chemical preservatives, mostly formaldehyde, which either shrink or swell certain areas and leave the cells little resembling live tissue. In the paraffin method of histology the entire brain is further infiltrated with wax so that what is observed under the microscope is more of a fossil than a specimen. The frozen method is more gentle in that the tissue is left relatively unmanipulated from dissection to embedding to slicing, but the very nature of the method produces what is called ice artifact. This irregularity is manifest in crystalization of tissue, cracking, and other types of fragmentation. Even the knife used to slice the tissue may cause artifact because of small nicks in the blade.

Finally, the staining procedure subjects the tissue to still more caustic chemicals which produce further shrinking, peeling, and loss of fragilely connected sections which are indicative of olfactory bulb lesions.

Some artifact, it is true, can be prevented, and some can be detected as such and not interfere with observing the lesion. But other types are inherent in the histological process and cannot be distinguished from lesioning effects. In all the studies here reported, only those irregularities which were <u>unquestionably</u> the result of lesioning were termed such. All other irregularities were considered artifact.

The determination of a lesion depended on several criteria. First, there is usually an observable darkening or disintegration of tissue apparent in dissection. Second, low power microscopic observations reveal scar-like smearing of cells, disorientation, or obliteration of the concentric layers, and clumps of debris which stain more darkly than healthy tissue. Finally, the most important criterion is that the same type or pattern of irregularity must be repeated in a series of consecutive slices and not confined to just one section. Any irregularity for which an artifactual interpretation was possible, was not called a lesion.

The second surgical study was on those animals used as subjects in Experiment IX. Ten animals served as controls and experienced all the surgical treatments except for electrode implantation and lesioning. The nineteen experimental animals were lesioned with a monopolar electrode at a depth of 3.0 mm., amperage of 3.0 m.a., and duration of 30 seconds. Although this combination of parameters did not produce the desired lesion in the first study, the use of bipolar electrodes was still unfeasible because of lack of technique, and a higher amperage could not be experimented with at that time. Furthermore, the experimental design could not allow these animals to delay any longer before completing the experiment. Consequently, as might be expected, the paraffin histology on these seventeen animals (one was not dissected safely, and one died before perfusion) did not reveal blatant lesions. For three animals no evidence of lesion could be found. Five animals displayed questionable lesions which were difficult to distinguish from artifact. The remaining nine animals showed very slight damage to the anterior lateral glomeruli in an area not more than 100 microns wide (See Figure 40). Behavioral data on these animals, however, described in the main text of the report, may be interpreted as showing an effect of lesioning not observable by histology. It must be noted that there was no quantitative relationship described between damage to bulb structure and behavior.

TABLE 8

Study II*

Animal number	Result
B3	Questionable
A10	Questionable
B11	Questionable
A12	Slight damage on dorsal glomeruli halfway through 1 bulb.
B15	Slight damage to anterior-lateral surface of one bulb confined to small area
A16	Questionable
A19	Questionable
B20	Questionable
B23	Questionable
B24	Questionable
B25	Lateral damage in form of distortion to shape of layers
B26	Questionable
B27	Questionable
B28	Distortion of concentricity, but no evidence of damage
B29	Questionable
B30	Questionable

*All animals were lesioned with a monopolar electrode, ground clip on the ear, at a depth of 3 mm. amperage of 3 m.a. for 30 sec.

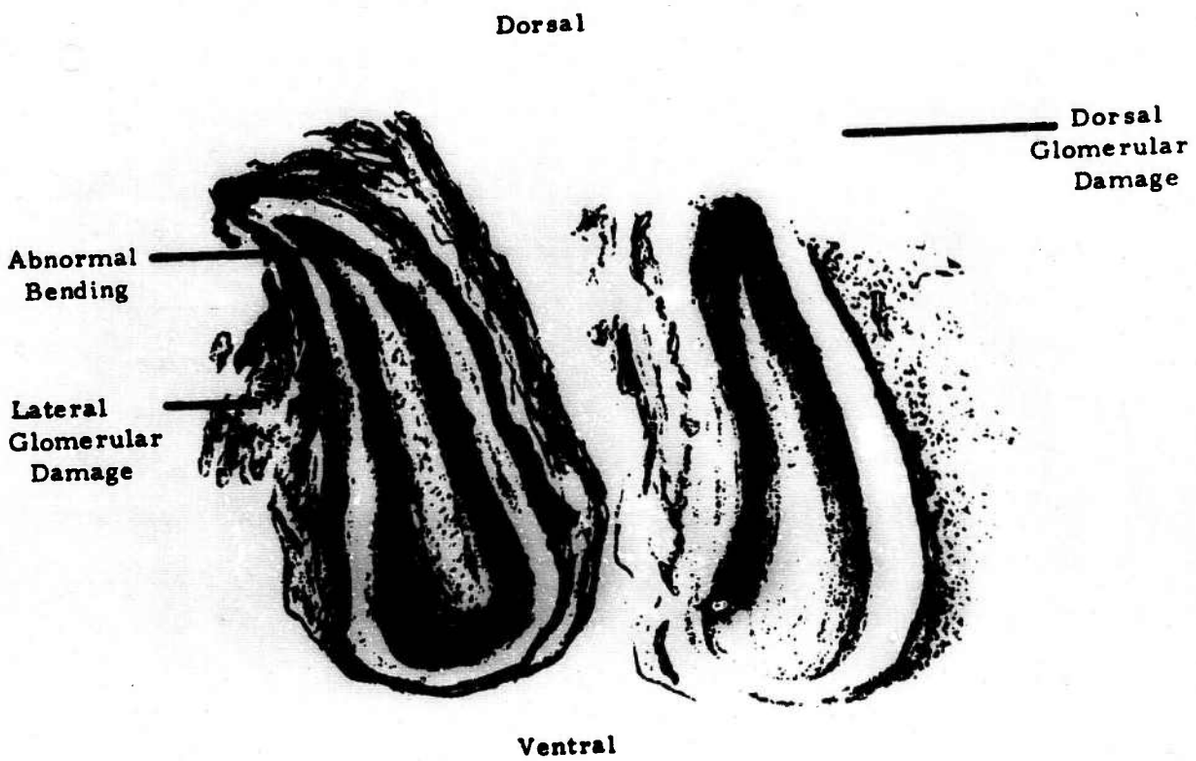


Figure 40. Study II; Animal B25; Anterior Section

It well may be that subtle anatomical changes not apparent under the microscope produce marked behavioral changes.

The third study performed at the same time as the second was an exact duplicate in surgical design, but had as its subjects six Norway rats trapped in the wild. The experiment involving these rats is also described in the main text. Histological results were similar and the only noteworthy comment was that one animal died during surgery.

The fourth study was with three animals not involved in any experiment. The discouraging results of the previous studies prompted experimentation with more severe measures. The technique for implanting the bipolar electrode safely was perfected and all three animals received bipolar lesions, 3 m.a., for 30 seconds, and at a depth of 4.0 mm. The animals were perfused a week after surgery and the brain tissue was prepared for the frozen method of histology. For the first time, the information provided by the histology was unquestionable.

Although the parameters of this study were not different from the bipolar parameters of the first study, a great amount of surgical skill and confidence had been acquired in the interim. More accurate placement and fewer errors in judgment resulted in greater damage from the same electrode. Also, the frozen method of sectioning, producing 40 micra, rather than 8 micra slices, proved to be more helpful in detecting lesions.

The evidence of lesions was similar in type to the lesions of the first study, but more severe and extensive. Gross observation revealed darkened tissue, blood coagulation around the bulbs (especially in the dura), and complete disintegration of some anterior tips. Animal S-1 showed lesioning effects in 50% of each bulb (See Figure 41). When

TABLE 9

Study III*

Animal number	Result
2W	Incomplete histology; no glomeruli on anterior dorsal surface
3W	Electrode tracts clearly visible at anterior tip, but very little damaged tissue around them
4W	Questionable
5W	Lateral glomeruli on one bulb torn away
6W	Bending; some lateral damage to one bulb

*All animals were lesioned with a monopolar electrode, ground clip on the ear, at a depth of 30 mm. and amperage of 3 m. a. for 30 sec.

TABLE 10

Study IV

Animal number	current in m. a.	depth in mm.	electrode
S1	3	4	bipolar
S2	3	4	bipolar
S3	4	4	bipolar

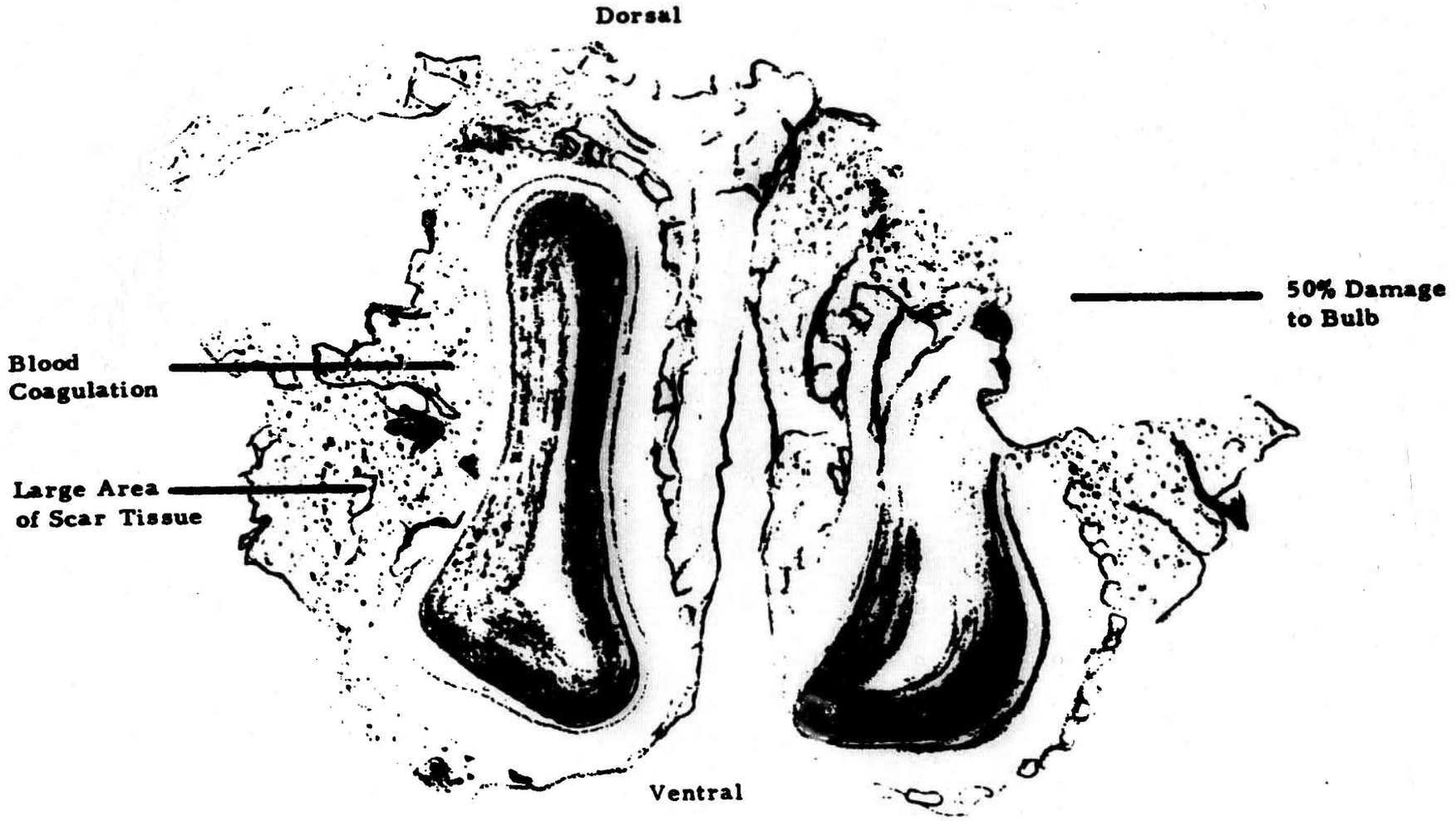


Figure 41. Study IV: Animal S1: Mid-Bulb Section

examining the slides from anterior to posterior sections, damage fir t appears slightly beyond the tip as a disruption in the lateral olfactory tract and adjacent glomeruli. Proceeding posteriorly, the damage extends to the mitral layer and at some points obliterates half the bulb. The last third of the bulb is normal in structure. The other two animals showed similar damage amounting to 40-50% of each bulb and confined to the anterior and lateral aspects. In one animal it was possible to see the electrode tract and the cell damage around that area.

Although all the previous studies described were helpful in assessing the progress in developing a suitable lesioning technique, the next study provided the most information about the range of feasible variables.

In the fifth study, it was decided to maximize all variables to determine the limit of tolerance to bulbar damage. In addition, the 60^{o} angled electrode placement was developed. The purpose of trying out this method was to allow more medial placement of the lesioning tip. Eight male albino rats were used and the histology was done by the frozen method.

Table 11 lists the parameters used for each animal and briefly evaluates damage. In this study, damage ranged from 40-90% of the bulb. The largest lesions in animal P-8 resulted in complete disruption of the main bulb to the accessory bulb (See Figure 42). This was probably due to the unusual ground placement. Unfortunately, however, this method is not feasible since it can easily result in short circuiting, and the distance between electrode tips is neither calculable nor exactly reproducible.

Characteristic of all the animals was the accumulation of blood in many areas both outside the brain tissue and in the spaces created by the lesion. It has been suggested that this blood is the result of

TABLE 11

Study V

Animal number	Current in m. a.	Depth in mm.	Electrode	Ground	Result
P2	7	1. 5	60°m	opposite bulb	70% of each bulb shows severe damage
P4	10	3. 5	90°m	" "	One bulb was completely lost in dissection because of deterioration. Some of the other bulb was also lost so that only 10% of the remaining tissue showed damage.
P5	5	1. 7	60°m	" "	40% of each bulb showed damage
P6	8	4	90°b		There was only slight damage, but the anterior half of one bulb was lost in histology
P8	7	4	90°m	same bulb	Unilateral lesion - 90% of that bulb was destroyed
P10	7	4	90°m	opposite bulb	Poor histology - no conclusions
P28	5	4	90°m	opposite bulb	Anterior 25% of one bulb destroyed. The other bulb had equivalent damage which came off in dissection.

m = monopolar

b = bipolar

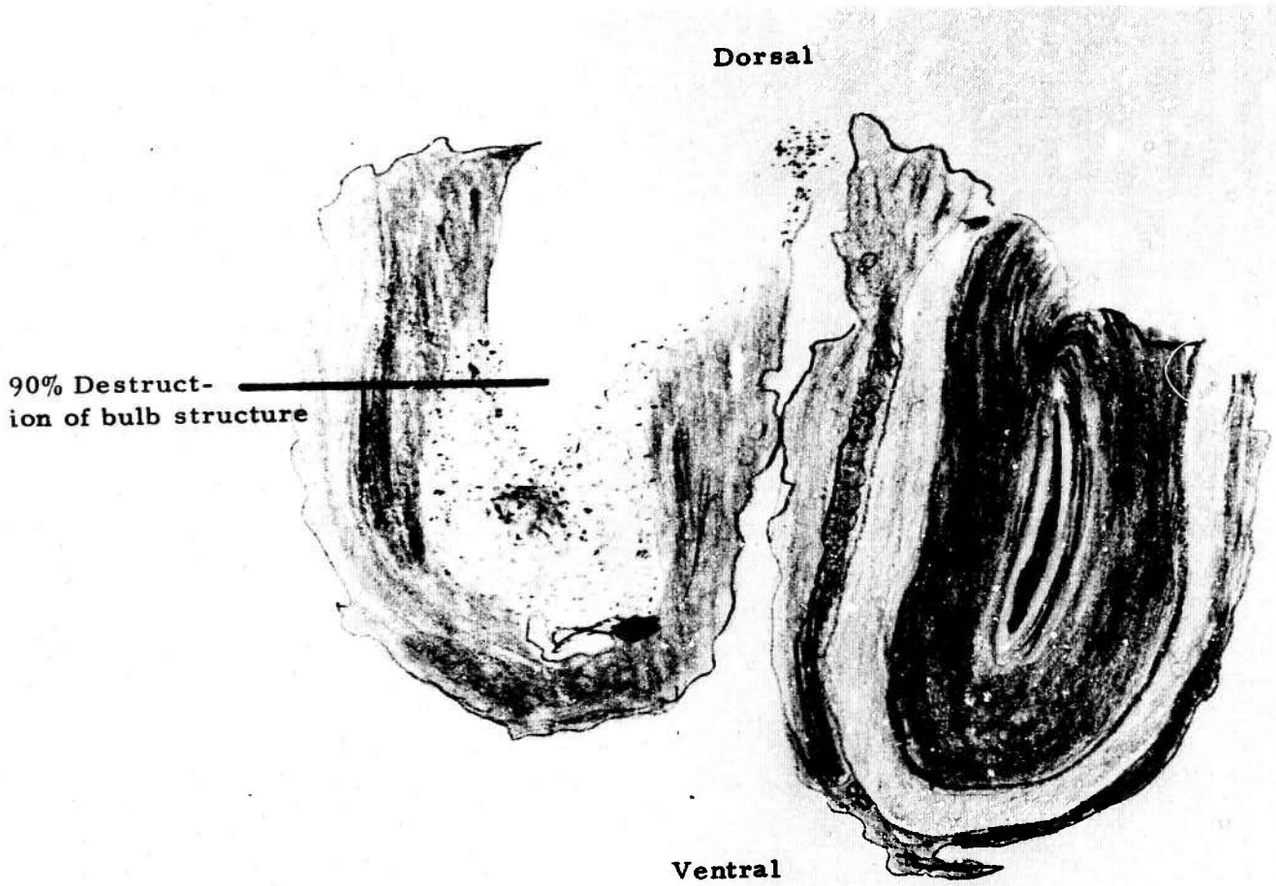


Figure 42. Study V; Animal P8, Mid-Bulb Section

damage to the richly vascularized nasal area anterior to the bulbs. In one animal, the angled electrode placement yielded total destruction of both bulbs in a series of four cross-sections from the anterior half of the bulb. This seems to indicate that angled placement does damage more medially than the straight placement, and in fact, can affect the total cross sectional area of the bulb instead of just the lateral surface.

One disappointment was the lack of conclusive data on the effect of varying amperage. Those animals lesioned with 5 and 7 m.a. seemed to show more damage than the 10 m.a. The small number of animals, however, precluded definitive statements. The major conclusion from this study, therefore, is that some animals could survive the most radical measures administerable. Having established this limit, the objective of the next study was to find the one combination of parameters that would yield the largest lesions and at the same time ensure the survival and normal recovery of all animals.

In Study VI, 20 male albino rats were assigned to the conditions shown in Table 12. The surgery was done in 5 days, the animals were allowed to recover for 5 days, and perfusions were done on the next three days. One rat died during surgery and two died after surgery but before perfusion. As far as possible, extra rats were used to replace them so that there was one surviving animal for each condition except one.

All animals clearly showed lesioning affects. The typical appearance of the bulbs after dissection and before embedding was that at least the anterior tips of the bulbs were completely destroyed. In most animals, damage extended at least halfway through the bulb. In a few animals the whole bulb was darkened and damaged, and in two animals there seemed to be degeneration of fibers and tracts well back into the primary olfactory cortex up to the entorhinal cortex.

TABLE 12

Study VI

Animal number	Current in m. a.	Depth in mm.	Electrode	Ground	Gross Result
P11	7	3	90°m	opposite bulb	3
P19	8	3	90°m	" "	4
P17	9	3	90°m	" "	2
P21	10	3	90°m	" "	2
P20	7	4	90°m	" "	1
P14	8	4	90°m	" "	4
P29	9	4	90°m	" "	4
P25	10	4	90°m	" "	3
P15	7	1.5	60°m	" "	3
P27	8	1.5	60°m	" "	2
P24	9	1.5	60°m	" "	1
P30	10	1.5	60°m	" "	4
P16	7	2	60°m	" "	3
S(died)	8	2	60°m	" "	
P26	9	2	60°m	" "	1
P9	10	2	60°m	" "	4

m = monopolar

Because of this extensive damage, dissection was difficult. In a few cases, the damaged tissue broke off completely so that only the posterior bulb was left. In other cases, damage was so severe that removal of the bulbs from the skull was impossible without severing them from the hemispheres. In both cases, histology could not be done because there was no support or orientation of tissue for sectioning. In all cases, tissue damage was extensive so that only visual observations were made.

In the result column of Table 12 the extent of lesion for each animal is evaluated on a 1 to 4 scale, 1 having the most damage and 4 the least. The characteristics of Type 1 lesions were that usually half of each bulb was darkened or deteriorated; sometimes degeneration extended to the primary olfactory cortex and damage always affected the whole cross sectional area of the bulb from dorsal surface to ventral tracts. A typical Type 2 lesion showed damage to half of one bulb but only a quarter or less of the other. Only occasionally did the lesion extend to the ventral tracts. In Type 3 lesions, only the anterior quarter or less of both bulbs was destroyed; the damage was confined to the dorsal surface. The final classification is for those animals in which one bulb had lesion of $\frac{1}{4}$ or less of its area, and the other bulb had no lesion. The case of no lesion resulted from placing the electrode anterior to the bulb and in the nasal cartilage. This placement caused a great amount of bleeding and coagulation around the bulbs, but no damage to the bulb itself.

As can be seen from Table 12, there was not a systematic effect related to amount of current. Also, although the angled electrode placement yielded more medial damage as determined in Study V, the difference between 60° and 90° placement could not be differentiated in the gross observation of Study VI lesions.

Although these six studies are incomplete in many ways, and the number of subjects was small, some tentative conclusions may be drawn concerning the feasibility of olfactory lesioning and the acceptable range of parameters for lesioning.

First, rats can easily tolerate the surgical trauma involved providing the anesthetic is not in itself fatal and no complications arise. No permanent debilities result, recovery is rapid, and feeding and drinking behavior is not ostensibly changed. Neither does the animal become aggressive or docile, but retains its previous disposition. In short, olfactory bulb lesioning is practicable and easily incorporated in an experimental design.

Conclusions on parameters are broad and subject to refinement by future studies. Amperage lower than 7 m.a. is always tolerable but results in small lesions. Amperage from 7-9 m.a. is reasonably tolerated and results in destruction of approximately 50% of each bulb. Above 9 m.a. the risk of losing animals from shock is too great to be warranted. Thus, it seems that, with electrolytic methods, the largest lesion the animal can tolerate affects only half of the olfactory bulb. The values of depth and electrode angle used in Study VI are all acceptable in the sense that they will produce approximately equivalent lesions. Any differences in lesion, for example the more medial damage that results from the 60° placement, are not great enough for one placement to be considered more advantageous than the other. The use of the monopolar electrode with ground in the opposite hold is easy to accomplish and least hazardous to the animal. But a bipolar electrode correctly placed will yield the same amount of damage, so both techniques are acceptable.

Our hesitancy to recommend one combination of variables over another stems from the feeling that the size of lesion depends as much on extraneous variables which cannot be controlled as it does

on depth, angle, and current. Because of slight individual animal differences in position and size of bulb, two ostensibly identical placements may yield radically different lesions. Low amperage may cause more damage than high amperage if the electrode tip is in a particularly conductive area of the tissue. The electrode may be deflected by a protruding piece of skull and not enter the bulb, but rather a sinus to the side. It is for these reasons that those parameters just discussed are all acceptable, for they can produce a maximal lesion depending on the extraneous variables. More definitive conclusions on parameters cannot be made until surg cal techniques are designed to compensate for random errors, or until a method of more quantified observation is developed.

Zinc Chloride Treatment

Besides electrolytic lesioning of the olfactory bulb, another method of interfering with the transmission of odor information was briefly attempted. This method involved bathing the nasal cavity with zinc chloride, a chemical known to destroy mucous membranes.

The procedure involved anesthetizing the animal with Equithesin to the same depth of unconsciousness as used for surgery. The animal was then strapped to a board, ventral side up, and hung upside down. The zinc chloride solution was injected from a syringe capped with a plastic nozzle approximately 2.0 mm. in diameter at the tip. The tip was inserted into one nostril while the tongue was pulled aside to prevent swallowing the solution. The intention was for the solution to bathe the mucosa and run back out the nostril or mouth.

Ten albino rats experienced this treatment with a few modifications. Some animals received a total dosage of 1 cc. of solution in each nostril, injected .33 cc. at a time. Others received smaller doses,

sometimes as little as .2 cc. total. In some animals, both nostrils were treated at the same time, in others, just one nostril was done with the intention of treating the other a few days later.

The results were that all but two animals died during the treatment. In most cases, breathing stopped as soon as the solution was injected. Other times the animal died immediately after the injection. The cause of death remains unknown. Very small amounts of the solution may have entered the lungs or stomach, but the mechanism by which that may have caused death is unknown. Also, the awkward position of the animal and the temporary blockage of the air passages in conjunction with the anesthetized condition may have been a factor. The two surviving animals both received small doses, one just in one nostril, the other in both nostrils.

Appendix I

Apparatus

Air Flow System. Details of the flow system are shown in Figure A-1. Within this Figure dashed lines enclose those components housed within a temperature-controlled water tank. Chemicals used as odor sources were contained within the odorant flasks. The output of each channel mixture was maintained at a constant flow rate by exhausting the surplus flow in the odorant line as the flow in the fresh air line was decreased. Arrows at the bottom of the diagram indicate the flow to the animal apparatus.

Behavioral Apparatus. Details of this apparatus are shown in Figure A-2. As noted, the apparatus was convertible from the Y-maze illustrated to the short runway by moving the starting box. Grooved fittings maintained an airtight seal. The entire apparatus was lined with Teflon. The three doors were guillotine-type operated by the experimenter. The light source for the cadmium cells was located between the two arms of the Y. A single source was directed to both cells via a prism and mirrors. Raising of the starting box door started a Hunter electronic counter which was stopped whenever the animal broke the light beam. The sensor was a current detector in series with the cell. A holding circuit prevented retriggering. The audio outputs were amplified, rectified and recorded on a Beckman Offner Dynograph. The microphones and amplifier were both inexpensive, low fidelity items with an upper spectral limit of about 4000 cps. This selection was deliberate as our early experience with high fidelity equipment indicated the need for filtering out the higher frequencies associated with other animal sounds such as the rubbing of fur, etc. The components used did not filter out unwanted sounds, however but merely reduced them. An experimenter was still required as a monitor of the record to indicate the presence of sniffing and non-sniffing traces.

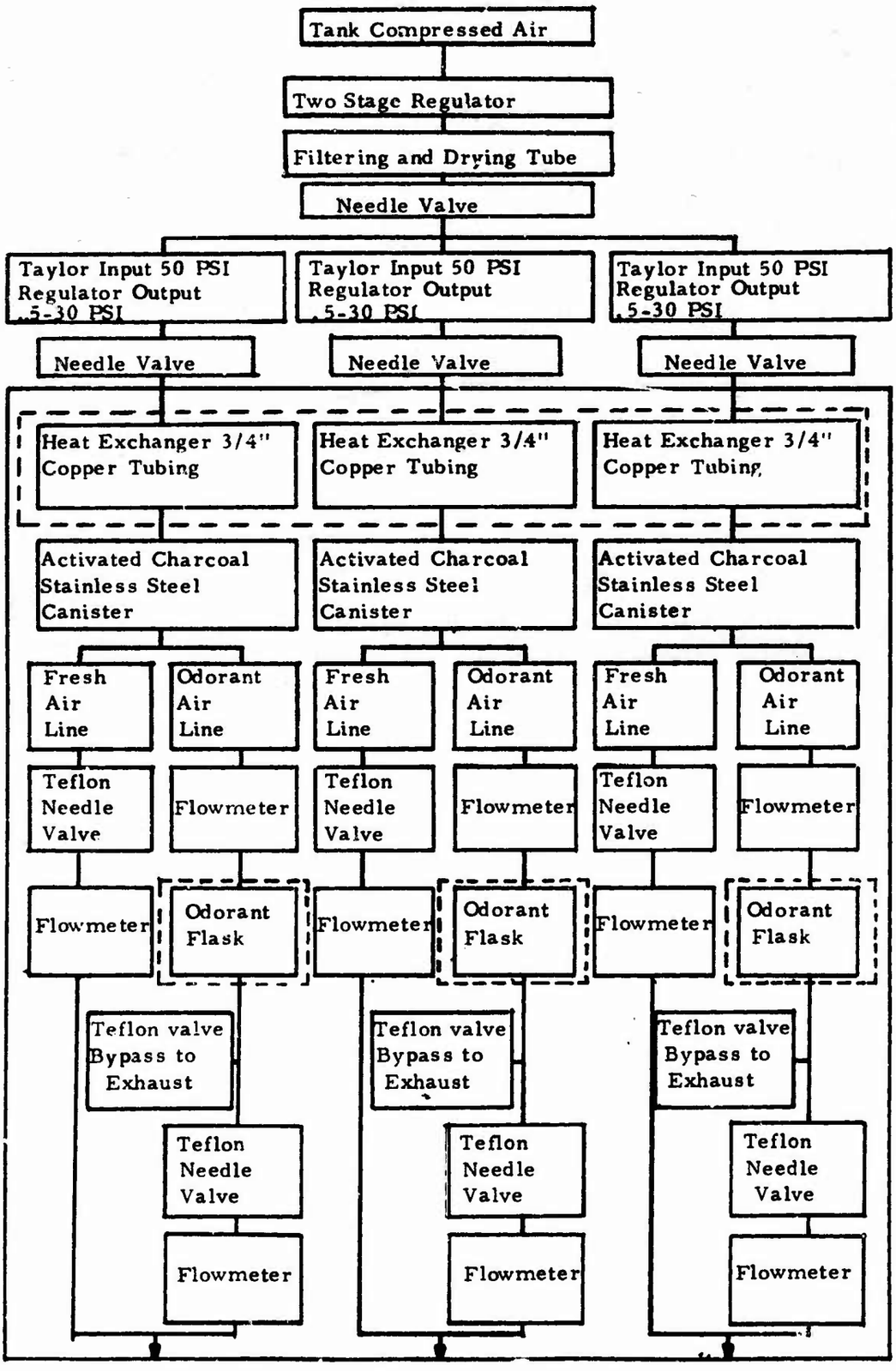
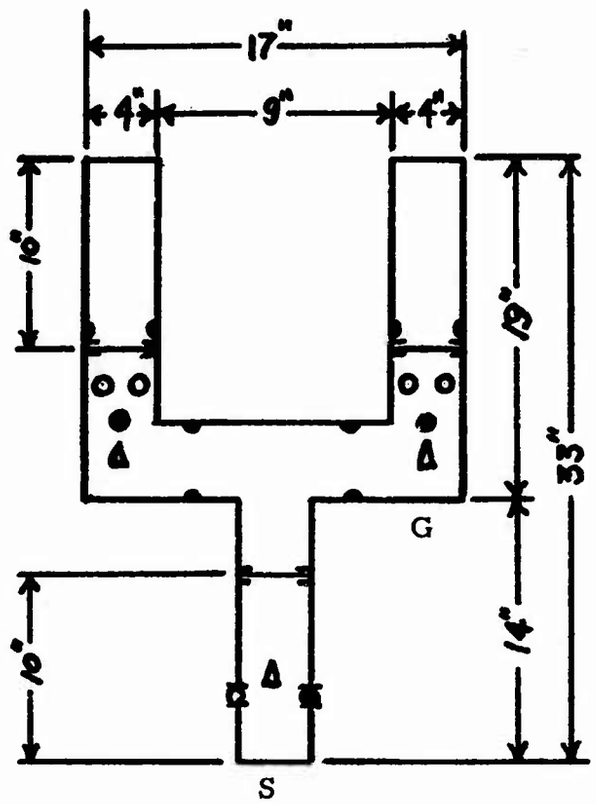


Figure A-1. Block Diagram of Three-Channel Flow System.



● Air Outflow

○ Air Inflow

Cadmium Photocell

Δ Microphone

Figure A-2. Behavioral Apparatus. Y-Maze as Shown. Removal of Starting Box (S) from Illustrated Position and Connection of S to G Converted the Y-Maze to a Short (SG) Runway.

The behavioral apparatus was housed in an electrically shielded cage, although that was not a necessary condition. The cage was in a larger, sound-damped room within which the air flow system was housed. The recorder was outside of the sound room. To operate the behavioral apparatus, one experimenter was inside the shielded cage with the animal; the other was outside with the recorder. When necessary, communication from inside to outside was accomplished via the microphones to the monitor's earphones.

References

Adey, W. R. The sense of smell. Handbook of Physiology, 1959, Section I, p. 536. American Physiological Society, Washington, D. C.

Adrian, E. D. Olfactory discrimination. Annual Review of Psychology, 1951, 50, 107-113.

Allison, A. C. The morphology of the olfactory system in vertebrates. Biological Review of the Cambridge Philosophical Society, 1953, 28, 195-244.

Beach, F. A. Analysis of the stimuli adequate to elicit mating behavior in the sexually inexperienced male rat. Journal of Comparative Psychology, 1942, 33, 163-207.

Becker, R. F., King, J. E., & Markee, J. E. Studies on olfactory discrimination in dogs: II. Discriminatory behavior in a free environment. Journal of Comparative and Physiological Psychology, 1962, 55, 773-780.

Beidler, L. M. The chemical senses. Annual Review of Psychology, 1961, 12, 362-388.

Berlyne, D. E. & Slater, J. Perceptual curiosity, exploratory behavior, and maze learning. Journal of Comparative and Physiological Psychology, 1957, 50, 228-231.

Bindra, D. & Spinner, N. Response to different degrees of novelty: The incidence of various activities. Journal of the Experimental Analysis of Behavior, 1958, 1, 341-350.

Brown, A. W. & Ghiselli, E. E. Subcortical mechanisms in learning. IV. Olfactory discrimination. Journal of Comparative Psychology, 1938, 26, 109-120.

Calhoun, J. B. The ecology and sociology of the Norway rat. (USPHS Publication No. 1008), Washington, D. C., 1962. Cited in W. J. Carr, L. S. Loeb & M. L. Dissinger. Responses of rats to sex odors. Journal of Comparative and Physiological Psychology, 1965, 59, 370-377.

Carr, W. J. & Caul, W. F. The effect of castration in rat upon the discrimination of sex odors. Animal Behavior, 1962, 10, 20-27.

Carr, J. W. & Bender, B. The effect of castration of the male rat upon olfactory sensitivity to estrous female urine. Paper read at Eastern Psychological Association, 1958. Cited in W. J. Carr, B. Solberg, & C. Pfaffmann. The olfactory threshold for estrous female urine in normal and castrated male rats. Journal of Comparative and Physiological Psychology, 1962, 55, 415-417.

Clark, W. E. Le Gros. Inquiries into the anatomical basis of olfactory discrimination. Proceedings of the Royal Society (London), Ser. B, 1957, 146, 299-319.

Clark, W. E. Le Gros & Warwick, T. The pattern of olfactory innervention. Journal of Neurological Neurosurgery and Psychiatry, 9, 101-111. Cited in F. Sen Gupta. Olfactory receptor reaction to the lesion of the olfactory bulb. Olfaction and Taste II, Proceedings of the Second International Symposium (Y. Zotterman, Ed.) Pergamon, Oxford, 1967, 193-203.

DeLorenzo, A. J. Electron microscopic observation in the olfactory epithelium of the rabbit. Anatomical Record, 1957, 127, 284. Cited in F. Sen Gupta. Olfactory receptor reaction to the lesion of the olfactory bulb. Olfaction and Taste II, Proceedings of the Second International Symposium (Y. Zotterman, Ed.) Pergamon, Oxford, 1967, 193-203.

Donovan, B. T. & Kopriva, P. C. Effect of removal or stimulation of the olfactory bulbs on the estrous cycle of the guinea pig. Endocrinology, 1965, 77, 213-217.

French, R. L. A method and apparatus for demonstrating olfactory sensitivity in the rat. Journal of Comparative Psychology, 1940, 29, 307-313.

Gellerman, L. W. Chance orders of alternating stimuli in visual discrimination experiments. Journal of Genetic Psychology, 1933, 42, 207-208.

Heimer, L. Synaptic distribution of centripetal and centrifugal fibers in the olfactory system of the rat. An experimental anatomical study. Journal of Anatomy, 1968, 103 (3), 413-432.

Le Magnen, J. Le role des stimuli olfacto-gustatifa dans la regulation du corportement alimentaire du mammifere. J. Psychol. Norm Pathol., 1959, 56, 137-160. Cited in B. Wenzel & M. H. Sieck. Olfaction. Annual Review of Physiology, 1966, 28, 381-434.

Le Magnen, J. & Rapaport, A. Essai de determination du role de la vitamine A dans le mechanisme de l'olfaction chez le rat blanc. C.R. Soc. Biol. Paris, 1951, 145, 800-803. Cited in J. T. Eayrs & D. G. Moulton. Studies in olfactory acuity. II. Relative detectability of n-aliphatic alcohols by the rat. Quarterly Journal of Experimenal Psychology, 1960, 12, 99-109.

Liggett, J. R. An experimental study of the olfactory sensitivity of the white rat. Genetic Psychological Abstracts, 1928, 3, 1-64. Cited in J. T. Eayrs & D. G. Moulton. Studies in olfactory acuity. II. Relative detectability of n-aliphatic alcohols by the rat. Quarterly Journal of Experimental Psychology, 1960, 12, 99-109.

Lohman, A. H. & Mentink, G. M. The lateral olfactory tract, the anterior commissure and the cells of the olfactory bulb. Brain Research, 1969, 12, 396-413.

Ludvigson, H. & Sytama, D. The sweet smell of success: Apparent double alternation in the rat. Psychonomic Science, 1967, 9, 283-284.

Moulton, D. G. Studies in olfactory acuity. III. Relative detectability of n-aliphatic acetates by the rat. Quarterly Journal of Experimental Psychology, 1960, 12, 99-109.

Moulton, D. G. & Tucker, D. Electrophysiology of the olfactory system. Annals of the New York Academy of Science, 1964, 116, 380-428.

Mozell, M. M. Olfactory discrimination; electrophysiological spatio-temporal basis. Science, 1964, 143, 1336-1337.

Nagahara, Y. Experimentalle Studien uber die histologischen Veranderungen des Geruchsorgans nach de Olfactoriusdurchachneidung. Japan J. Med. Sci. Pt. V. Pathol., 1940, 6, 165-199. Cited in D. M. Wenzel & M. H. Sieck. Olfaction. Annual Review of Physiology, 1966, 28, 381-454.

Novakova, V. & Dlouha, H. Effect of bulb lesions on intake and excretion of water in rat. Nature, 1960, 186, 638-639.

Powell, T. P. S., Cowan, W. M., & Raisman, G. The central olfactory connexions. Journal of Anatomy, 1965, 99, 791-813.

Schultz, E. W. Repair of olfactory mucosa with special reference to regeneration of olfactory cells. American Journal of Pathology, 1960, 37, 1-19.

Sen Gupta, P. Olfactory receptor reaction to the lesion of the olfactory bulb. In Y. Zotterman (Ed.), Olfaction and Taste II, proceedings of the Second International Symposium. Oxford, England: Pergamon Press, 1967, p. 193-203.

Smith, C. G. Changes in the olfactory mucosa and the olfactory nerves following intranasal treatment with one percent zinc sulfate. Journal of the Canadian Medical Association, 1938, 39, 138-141.

Stone, C. P. The congenital sexual behavior of the young, male, albino rat. Journal of Comparative Psychology, 1922, 2, 95-193.

Stone, C. P. Further studies in the sensory functions of the sexual behavior in the young, male, albino rat. Journal of Comparative Psychology, 1923, 3, 469-473.

Stone, C. P. A simple method for developing olfactory discrimination habits in rats. Journal of Genetic Psychology, 1941, 58, 419-421.

Stuiver, M. An olfactometer with a wide range of possibilities. Acta Oto Laryngologica, 1960, 51, 135-142. Cited in D. M. Wenzel & M. H. Sieck. Olfaction. Annual Review of Physiology, 1966, 28, 381-454.

Swann, H. G. The function of the brain in olfaction. I. Olfactory discrimination and an apparatus for its test. Journal of Comparative Psychology, 1933, 15, 229-241.

Teichner, W. H. Runway performance as a function of hunger level measured as food acceptance. Journal of Psychology, 1966, 63, 179-195.

Teichner, W. H. A method for studying olfaction in the unrestrained rat. Journal of Psychology, 1966, 63, 291-297.

Teichner, W. H., Price, L. M., & Nalwalk, T. Suprathreshold olfactory responses of the rat measured by sniffing. Journal of Psychology, 1967, 66, 63-75.

Teitlebaum, P. & Epstein, A. N. The role of taste and smell in the regulation of food and water intake. In Y. Zotterman (Ed.), Olfaction and Taste. New York: MacMillan, 1963.

Tucker, D. Olfactory, vomeronasal and trigeminal receptor responses to odorants. In Y. Zotterman (Ed.), Olfaction and Taste. Oxford, England: Pergamon Press, 1963, p. 45-69.

Welken, W. I. Analysis of sniffing in the albino rat. Behaviour, Leiden, 1964, 23, 224-244.

White, L. E., Jr. Olfactory bulb projections of the rat. Anatomical Record, 1965, 152, 465-480.

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13. ABSTRACT

The mode of action of rat repellents was investigated in a series of experiments concerned with food consumption, olfactory sensitivity and discrimination, adaptation and habituation, and performance. A variety of chemical repellents were used both in the animal's food and in the air. Comparisons were also made among laboratory strains and the Norway rat (both wild and tame), with and without lesions in the olfactory bulb. It was concluded that the odor of a chemical makes little or no contribution to the repellency of a chemical or to its value as a deterrent except when it has a signalling value from association with a painful taste or when it is a novel stimulus. Additional conclusions relate to methodology for testing the effectiveness of repellents, theory, and needs for future research.

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