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BEHAVIORAL ACTIVATION AND LEARNING IN THE INFANT RAT

by

REGINA M. SULLIVAN

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ABSTRACT

BEHAVIORAL ACTIVATION AND LEARNING
IN THE INFANT RAT

by

Regina M. Sullivan

Adviser: Professor Gerald Turkewitz

The ability of an olfactory stimulus to modify behavior is dependent upon the pup's prior experience with that odor. The present experiments explore one way in which an odor may acquire this ability. Pups were exposed simultaneously to an odor and a stimulus which produces behavioral activation.

The first series of experiments involved orientation to the source of an odor and was designed to determine whether the simultaneous presentation of an odor and a behaviorally activating stimulus could modify orientation to an the odor. 3 to 6-day-old rat pups received a 10 min exposure to orange odor and a behaviorally activating stimulus (either tail pinching, stroking or odor of maternal saliva). 1 hr after treatment a choice test (orange odor vs. pine odor) was given. The results show that the orange odor is subsequenty capable of modifying orientation only when it has previously been simultaneously paired with a behaviorally activating stimuli.

The second series of experiments was designed to 1) explore the generality of the effects of this odor treatment procedure on other behaviors (huddling and feeding) and to determine whether the presence of the odor enhances responding of the experimental group or inhibits the responding of the control groups. 5-day-old rat pups were given a 10 min exposure to orange odor and stroking. A huddling or feeding test was given 1 hr later either with or without the presence of orange odor. Feeding was not modified. Huddling was only modified in the pups that were simultaneously exposed to orange odor and stroking and were tested in the presence of orange odor.

These results suggest that the simultaneous presentation of an odor and a behaviorally activating stimulus produces a general enhancement in responsiveness to stimuli encountered in the presence of that odor.

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Introduction

During the first week of a rat pup's life, it must make numerous adaptations to insure survival. In most cases, observation of the litter situation does not permit the targeting of the stimuli and responses responsible for these adaptations. Even more difficult is the determination of the mechanisms responsible for these adaptations. The aim of the present paper is to explore one way a pup's behavior may be modified by examining the effects of behaviorally activating stimuli presented with an olfactory stimulus on the subsequent behavioral responsiveness of the pup to that odor, and other stimuli in the presence of that odor.

Interestingly, although the rat pup's behavior changes during the first week of life, learning was not considered to be responsible for most of these behavioral modifications. In fact, it was believed that the rat pup was extremely limited in its learning abilities. However, recent research has provided numerous demonstrations of unsuspected learning capabilities in the rat pup less than 1 week of age. A number of factors have presumably contributed to this recent uncovering of the infant rat's learning capabilities. First, the principles of biological constraints and preparedness on learning in the adult rat (Seligman & Hager, 1972) were extended to the infant rat. Second, specific training and testing techniques were designed to accomodate the neural, sensory, and motor systems of the infant rat. Third, it was recognized that the infant rat has an ecological niche distinct from the adult rat. Thus, due to the consideration of these factors in the study of learning in infant rats, the types of learning that have been successfully demonstated in the rat pup of less than 1-week of age are nearly as extensive as those that have been demonstrated in the adult rat.

Habituation and Sensitization

The simplest form of learning is generally considered to be habituation (Thorpe, 1956), which is a decrement in responding that occurs after repeated presentation of a stimulus (Groves & Thompson, 1970). On the other hand, sensitization is the increase in responding that occurs after the repeated presentation of a stimulus (Groves & Thompson, 1970).

A number of researchers have demonstrated that habituation does occur in the infant rat. File & Plotkin (1974) and File & Scott (1976) found habituation of the head-raising and head-turning response elicited by a puff of air in 1 to 16-day-old rats. However, Bronstein, Neiman, Wolcoff & Levine (1974), who studied the developmenmt of habituation of the activity response in an open-field, did not find habituation in the rat pup until 21 days of age.

Habituation and sensitization of the forelimb withdrawal response to shock has received considerable and thorough attention from Campbell and his colleagues. Stehouwer & Campbell (1980) have studied the time course of habituation and sensitization of the leg flexion response as a function of age (3,6,10, and 15 day old rats), shock intensity, and interstimulus interval (ISI). Their results generally agree with Thompson & Spencer's (1966) defining characteristics of habituation at all four ages: 1.) The percentage of leg flexions elicited by the forelimb shock decreased as a function of repeated shocks: 2.) The amount of habituation (percentage of responses) declined over repeated stimulations more quickly at shorter ISI's: and 3.) A strong shock, which was delivered after the leg flexion response had been habituated, produced dishabituation. However, they did find differences in the leg flexion response among the different ages: 1.) Although increasing intensities of shock initially produced a higher percentage of leg flexion responses at all ages, the rate of habituation at strong shock intensities took longer in 3 and 6-day-old rats than in 10 and 15-day-old rats, 2.) Increasing frequencies of shock delivery (shorter ISI's) were habituated to more quickly in 3 and 6-day-old rats than in 10 and 15-day-old rats. 3.) After presentation of the dishabituating stimulus, the 3 and 6-day-old rats showed a dramatic increase in responding to the habituating stimulus, while 10-day-old rats showed only a slight increase and 15-day-old rats showed no increase in responding. Campbell & Stehouwer (1980) refer to this transient increase in responding as short term sensitization.

Campbell & Stehouwer (1980) do not, however, consider the response differences among the various ages as a reflection of alterations of the habituation system. Rather, these response differences were considered to be due to the development of an antagonistic long term sensitization system at about 6 to 10 days of age that began interacting with the habituation system. Campbell & Stehouwer (1980) and Stehouwer & Campbell (1978) found that sensitization of the forelimb reflex was not transient and lasted for at least 24 hours.

Thus, habituation, short term senzitization, and long term sensitization have been demonstrated in the infant rat. The reason for the differences in the results of the various habituation studies is probably due to a number of factors. The functioning of the sensory and motor systems for various behaviors may change during the course of development. For instance, the head raising response in the File & Plotkin (1974) and File & Scott (1976) studies are initialy different at the various ages used in their study. Also, the exact stimulus and response under study is not always clear. For instance, in the Bronstein, Neiman, Wolcoff & Levine (1976) study it is unclear what stimuli in the open field were producing the activity or what behaviors constitute activity at any given age. Thus, it appears that the rate and time course of habituation varies as a function of the stimuli and response studied. Although sensitization has not received comparable experimental attention and cannot be evaluated to the extent that habituation has been, it is likely that the same variability in sensitization of different responses will emerge when behaviors other than the leg flexion response are studied.

Classical Conditioning

As early as the 1960's, classical conditioning techniques were used to study the learning capabilities of the infant rat. Caldwell & Werboff (1962) were able to demonstrate that the simultaneous presentation of a vibrotactile stimulus (CS) and a shock (UCS) produced conditioned responding to the CS. Although the percentage of CRs in the experimental group was only 32%, it was significantly higher than in the semi-randomized control group, which only showed CRs 5% of the time. Similar results were found in a developmental study by Gray, Yates & McNeal (1967), who found both that 1-day-old rats had a significantly higher number of CRs and that the number of

CRs increased as a function of the age of training. Bachevalier & Blozavski (1980) repeated the Caldwell & Werboff (1962) study but included a 24 hr retention test and an extinction test. Their results, which confirmed the earlier studies of classical conditioning, demonstrated that the same percentage of CRs were retained for 24 hr, and extinction curves were obtained. Thoman, Wetzel & Levine (1968) found CRs to stimuli associated with tube feeding after 3-day-old rat pups were tube-fed milk a number of times, although a difficultly with this study is that only the experimental group was deprived of contact and nutrients from the mother. In summary, however, it appears that classical conditioning can be demonstrated in rat pups during the first week of life.

During the past decade, research on classical conditioning has extended our knowledge of the stimuli that can function as a CS and UCS, as well as our knowledge of the parameters that can influence classical conditioning. This is significant because it illustrates the unique capabilities and limitations on learning in the infant rat. For example, a preference for an odor can be produced in 3 to 6-day-old rats but not in 9-day old rats by brief simultaneous pairings of odor with the presentation of milk (Johanson & Hall, 1982; Johanson & Teicher, 1980). Furthermore, conditioned appetitive milk consumption responses to an odor CS can be elicited in 3, 6, and 9-day-old rats utilizing similar simultaneous presentations of the odor CS and milk UCS (Johanson, Polefrone & Hall, 1983). Johanson's studies have also illustrated a number of factors which appear to be important in successfully demonstrating learning in infant rats, such as ambient temperature, deprivation level of the pup, and the necessity of a simultaneous presentation of the odor and the reinforcer. Another exampla is a series of experiments from Blass's lab. Pedersen, Williams & Blass (1982) and Pedersen & Blass (1982) were able to elicit nipple attachment on a chemically lavaged female (to which pups will not normally attach) in the presence of an artificial odor, provided the pup had previously been stimulated (by vigorously stroking the pup or by the administration of amphetamine) in the presence of that odor. Furthermore, Pedersen et al (1982) found that the pup's performance in the nipple attachment test was an inverted U-shaped curve derived as a function of increases or decreases in odor concentration or ambient temperature. Thus, provided the treatment and testing conditions are within a particular range of values, modification of a rat pup's behavior can be successfully demonstrated using a classical conditioning paradigm.

Most of the studies cited above as examples of classical conditioning contained control groups in which learning did not seem to occur. Although the use of these control groups - CS alone, UCS alone or negatively contingent presentation of the CS and UCS - may be sufficient to verify learning in adult rats, they may not be sufficient as learning controls in infant rats. A rat pup spends most of its time asleep, especially when it is placed in isolation (Gramsbergen, Schwartze & Prechtl, 1970; Jouvet, Astic & Lacote, 1970; Shair, Brake & Hofer, 1981), and the rat pup seems to unpredictably fluctuate from one behavioral state to another. Furthermore, it is difficult to determine exactly which behavioral state a pup is in at any given time. However, it is not unreasonable to say that an active pup is probably awake and an inactive pup is probably asleep. A rat pup that has been placed in isolation with little or no stimulation is usually inactive and a rat pup that is receiving stimulation is usually active. In contrast, an adult rat does not usually become inactive when placed in isolation; it usually explores the area, even when there is little obvious stimulation in the environment. It is in this framework that the inadequacy of adult learning controls becomes obvious when they are extended to the verification of learning in infant rats. In a classical conditioning experiment using infant rats, the training phase usually contains an experimental group that receives the simultaneous presentation of a CS and UCS, and a number of control groups, such as a group that receives the presentation of only the CS, a group that receives the presentation of only the UCS, and a group that receives a random presentation of the CS and UCS or a negatively contingent presentation of the CS and UCS. Generally, a CS should have a relatively "neutral" value to the rat, while a UCS should have some "positive" or "negative" value to the rat. The consequences of the CS and UCS's properties on the behavior of the rat pup are in accordance with their value. During the presentation of a CS, the rat pup shows very little observable behavioral response, while during the presentation of the UCS, the rat pup becomes behaviorally active. Thus, it does not seem unreasonable to suppose that a rat pup may be asleep during the presentation of a CS and awake during the presentation of a UCS. When this supposition is extended to the behavioral consequences of the treatment of experimental and control groups in a classical conditioning experiment, the following results might occur: The experimental group (CS-USC simultaneously presented) would be awake during the presentation of the CS, while all of the control groups, which do not receive a behaviorally activating UCS during the presentation of the CS, would be behaviorally inactive and perhaps asleep during the presentation of the CS. The test to verify whether classical conditioning has occurred is usually a measure of the pup's response to the presentation of the CS alone. However, due to the possible differences in the pup's perceived exposure time to the CS during treatment, either due to the pup's sleep state or inattentiveness, the modification in an experimental rat pup's behavior during the test for classical conditioning has a number of explanations: First, associative learning may actually have occurred; second, due to differences in perceived exposure time among the various treatment groups, there may be a decrement in a neophobic response to the CS which permits habituation to the CS may occur; or third, sensitization may produce the increase in responding to the CS.

It seems that one of the major problems surrounding the interpretation of the classical conditioning experiments on infant rats centers around whether behaviorally activating a rat pup in the presence of a CS is sufficient to produce a modification in the rat pup's behavior. The present series of experiments was designed to assess the following questions: 1.) Can behaviorally activating a rat pup in the presence of an odor result in a modification of a pup's behavior in the presence of that odor? 2.) Is associative learning, habituation, or sensitization responsible for the modification of the rat pup's responsiveness to that odor? 3.) Provided a pup's behavior can be modified, what are the consequences of the modification of the rat pup's behavior regarding its adaptation to the environment?

MODIFICATION OF OLFACTORY GUIDED ORIENTATION

The attraction of rat pups to natural as well as artificial odors within the nest has been well documented (Galef, 1982; and Leon, Galef & Behse, 1977). Pups also have a greater preference for odors associated with the mother and other pups than for odors associated with inanimate aspects of the environment (Galef & Kaner, 1980). This suggests that certain properties of the mother or the mother's behavior towards the pups has an effect on the pups' responses to olfactory stimuli. Since the pups have the ability to orient towards the nest on the basis of olfactory cues at 3-days of age (Altman, Brunner, Bulut & Sudarshan, 1974), it seems likely that the rat pups become familiar with and attracted to some of the nest odors soon after birth. The present paper is an investigation of how a pup, younger than 1 week old, acquires the ability to orient towards and make contact with the source of odors.

The approach to the question of how a pup begins to orient towards odors was based on a number of earlier studies. Pedersen, Williams & Blass (1982) showed that 3-day-old rats would attach to a washed lactating mother's nipples (the washing eliminates olfactory cues ordinarily required for nipple attachment) in the presence of an odor provided the pup had previously been stimulated (by physically stroking or injecting amphetamine) in the presence of that odor. Johanson & Hall (1982) and Johanson & Teicher (1980) have demonstrated that a pup will orient towards a novel odor provided the pup has been exposed to the odor while being given milk. It should be noted that the stroking used in the Pedersen et al (1982) experiment and the milk used in the Johanson & Hall (1982) and Johanson & Teicher (1980) experiments both produced behavioral activation (observable movement) in the infant rat. This raised the possibility that behavioral activation was an important element in the train of events that changes a rat pup's orientation to an odor.

The current investigation was designed to test the hypothesis that behaviors or stimuli that are associated with the mother and that behaviorally activate the pup change the way a pup responds to odors, resulting in the pup subsequently orienting to and maintaining contact with those odors. Therefore, the present studies were designed to determine whether the simultaneous presentation of an odor and one of several kinds of activating stimuli would result in the pup orienting towards the novel odor the next time the odor was encountered.

Experiment 1

The purpose of the first experiment was to determine whether a behaviorally activating stimulus, odor of maternal saliva, when presented simultaneously with a novel odor, would result in orientation towards the novel odor when it was subsequently presented. Maternal saliva was chosen as the behaviorally activating stimulus (Cornell & Pedersen, 1980) from the many stimuli encountered in the suckling situation because of its salience and relevance to the pup. Teicher & Blass (1977) have shown that maternal saliva is an effective cue for nipple attachment in 0-2-day-old pups, and maternal saliva can decrease the latency of nipple attachment in pups up to 13-days of age (Sullivan & Brake, personal observation). Thus, during treatment two odors were presented: the odor of maternal saliva and a novel orange odor. The pup's behavior towards the orange odor was then assesed in an orientation test in which the amount of time the pup spent in contact with the odor was measured.

Methods

Subjects

The subjects were 53 male and female 3 to 6-day-old rat pups from 13 litters born of Marland Farm Wistar dams in the animal care facilities of Montefiore Medical Center. Dams and litters were housed in 40 x 20 x 24 cm Plexiglass terraria, on pine shavings, in a temperature (22 C) and humidity controlled room. Dams were allowed free access to food and water, and were maintained on a 12:12 hour light-dark cycle with light onset at 7:00 a.m.

Procedure

Collection of Saliva. Maternal saliva was collected for use immediately prior to the start of treatment. A lactating dam (mean days post-partum=9) was anesthetized (urethane, 2g/kg) and placed in a warm (37 C) plastic container for 1 hr. After approximately 30 min, saliva began to drip from the dam's mouth and was collected with a pipette for the next 30 min. Approximately 2 ml of saliva were collected in this manner, enough for treatment of 2 subjects.

Deprivation conditions. All pups were separated from their dam 18 hr prior to treatment and individually housed in plastic tubs in a warm (34 C), moist incubator (Forma Scientific Diurnal Growth Chamber).

Treatment conditions. Pups were randomly assigned to one of the following five treatment conditions: 1) O & Sa - 10 min simultaneous presentation of orange odor and odor of maternal saliva; 2) O & H2O - 10 min simultaneous presentation of orange odor and tap water; 3) O - 10 min presentation of the orange odor alone; 4) Sa - 10 min presentation of the odor of maternal saliva; 5) Naive - 10 min with no presentation of orange odor or the odor of maternal saliva.

Treatment consisted of removing the pups, one at a time, from the housing incubator and placing them for 10 min in clean tubs, which were then placed into another warm (32 C), moist treatment incubator. The orange odor was presented by lining the floor of each pup's tub with 3 ml of orange scented pine shavings. The orange scented shavings were prepared 10 min prior to treatment by spraying 1.25 cc of orange extract (Ann Page; A&P) onto 500 ml of pine shavings and shaking them thoroughly in a closed container. The maternal saliva and tap water were presented on a cotton swab positioned within 5 cm of the pup's snout. The cotton swab was continually repositioned to accommodate the pup's movement. The cotton swab was resoaked (2-3 sec) every 2 min. Following treatment, all pups were placed back into their tubs and returned to the housing incubator.

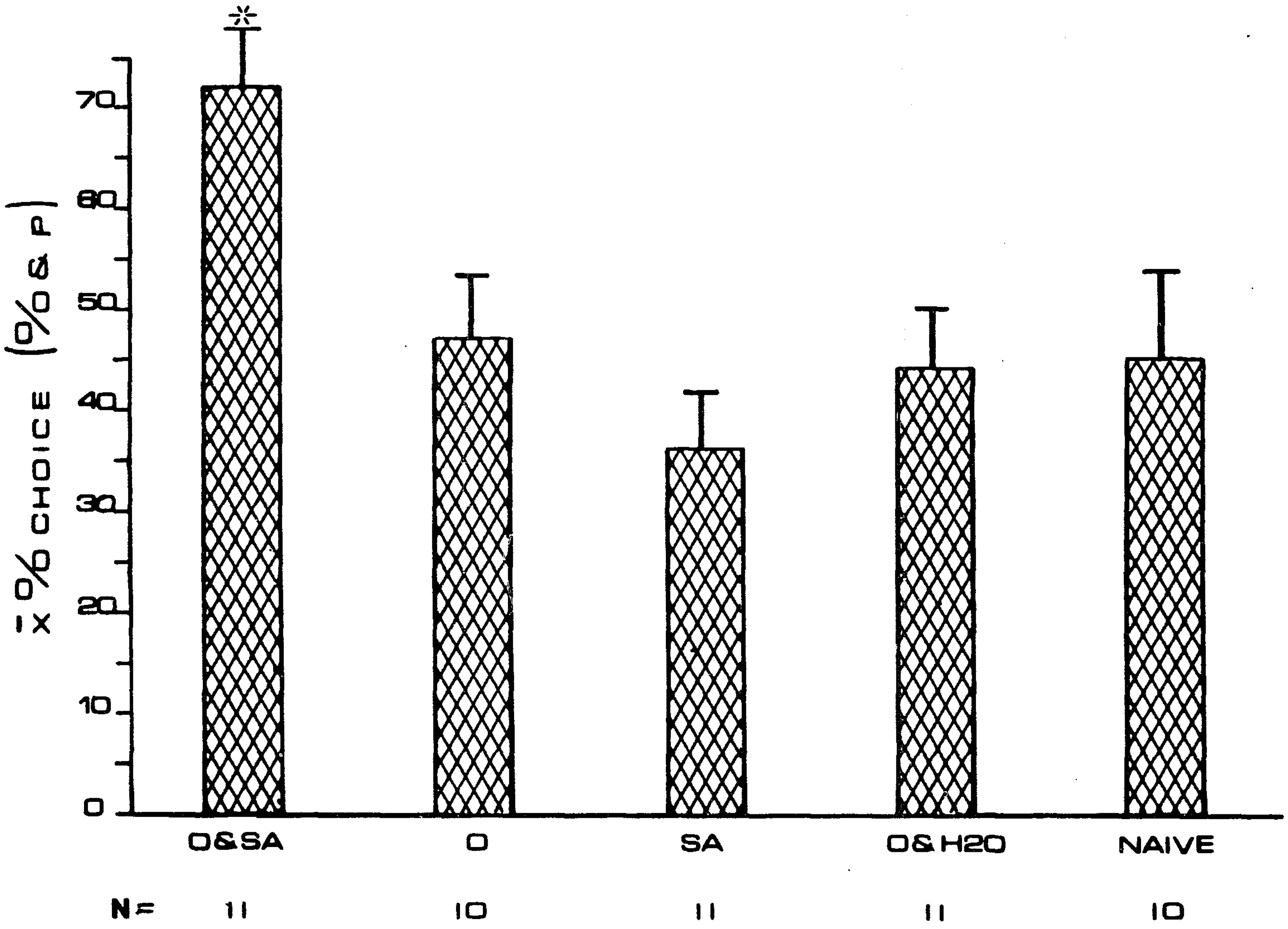
Choice test. All pups were given an olfactory choice test one hr following treatment. This type of test has been used in a number of other rat pup learning experiments (Cornwell-Jones, 1979; Johanson & Hall, 1982; Rudy & Cheatle, 1977 and others). The test apparatus consisted of a 33 x 21 x 14 cm Plexiglass box with a small grid wire mesh (.4 x .4 cm) floor divided in half by a 4 cm wide "neutral zone" which ran the length of the box. A 33 X 8.5 cm container of shavings was placed beneath each half of the wire mesh floor. One container was filled with 200 ml of orange-scented pine shavings (prepared as described above), and the other was filled with 200 ml of unscented pine shavings. Pups received 3 consecutive 2 min trials in the apparatus. A trial was begun by placing the pup on the neutral zone parallel to the long side of the test box between the two odors. When the pup's head and forepaws crossed from the neutral zone into either half of the test box, a timer was started. At the end of the 2 min trial, the total amount of time the pup spent over the orange scented pine shavings and the total amount of time it spent over the unscented pine shavings were recorded. Between each 2 min trial, the pup was taken out of the test box, and the wire mesh floor was wiped clean with water. The direction in which the pup was placed back into the box was reversed for each trial to prevent confounds resulting from turning preferences.

For all groups, the statistical analyses were done on a ratio of the amount of time spent over the orange odor divided by the sum of the time spent over the orange and pine shavings (i.e., O/O+P). The use of the ratio score has the advantage of eliminating the no-choice time spent over the neutral zone and thus minimizes differences in absolute locomotion within groups.

Results and Discussion

There was a significant difference in the amount of time spent over the orange odor for different treatment groups (ANOVA, $F(4,48)=4.21, p<.01$). As illustrated in Figure 1, only pups simultaneously exposed to the orange odor and the odor of saliva (O & Sa) spent a greater percentage of time over the orange odor than did the untreated pups (Post hoc Newman-Keuls revealed that the means of O, Sa, O & H2O, and Naive groups each differed from the mean of the O & Sa group at the $p<.05$ level; all other differences were non-significant). This suggests that simple exposure to the orange odor was insufficient to produce a change in the response to the orange odor as assessd by the choice test. Thus, only the simultaneous exposure to orange odor and the odor of maternal sahiva was sufficient to produce a change in orientation towards the orange

Figure 1 Mean percentage of time spent over the orange odor (O/ O&P) in the two-odor choice test by the different treatment groups.


x̄ % O CHOICE (O / O & P)
0
10
20
30
40
50
60
70
*
O&SA
O
SA
O&H2O
NAIVE
N=
11
10
11
11
10

odor.

The fact that the pup's response to the orange odor is changed only by simultaneous presentation of orange odor and maternal saliva suggests that the pups may have associated the two stimuli. If so, maternal saliva may have been functioning as a primary or secondary reinforcer in a classical conditioning paradigm.

Experiment 2

The odor of maternal saliva produces behavioral activation in the pups, but it is unclear whether the behavioral activation or other properties of the odor of maternal saliva (eg, secondary reinforcing value due to its association with the reinforcing properties of the mother) produced the modification of the pup's behavior. The first purpose of the present experiment was to determine the generality of the effects of behaviorally activating stimuli. Therefore, two additional manipulations were employed to produce behavioral activations: stroking the pup with a sable-hair brush (Pedersen et al, 1982) which simulates licking and nudging of the pups by the mother, and a forceful tailpinch (Szechtman & Hall, 1980) which simulates the mother's stepping on the pups. The second purpose of this experiment was to determine whether the pups in Experiment 1 were exhibiting an orientation as the result of classical conditioning. Therefore, we included control manipulations designed to assess whether associative or non-associative processes were responsible for the effects we observed in the first experiment.

Methods

Subjects

The subjects were 72 male and female 3-6-day-old Wistar rat pups from 14 litters born and raised as described in Experiment 1.

Deprivation conditions were identical to those in Experiment 1.

Procedure

Collection of saliva. In this experiment the odor of maternal saliva was presented as a homogenized salivary gland of a donor dam. The gland was excised from an ether anesthetized lactating female (mean days post-partum=12). The gland was mashed, mixed with 1 cc of distilled water, and magnetically spun (Corning PC-351) for 5 min. Each gland was used for 1 or 2 pups.

Treatment conditions. Pups were randomly assigned to one of the following treatment conditions: 1) O & SG - 10 min simultaneous exposure to orange odor and salivary gland extract; 2) SG/O - a "backwards" presentation of 10 min exposure to salivary gland extract followed by 10 min exposure to orange odor; 3) O & ST - 10 min simultaneous exposure to orange odor and vigorous stroking with a sable-hair brush; 4) ST/O - 10 min "backward" presentation of vigorous stroking with a sable-hair brush followed by 10 min exposure to orange odor; 5) O & TP - 10 min simultaneous exposure to orange odor and a forceful tailpinch delivered every 30 sec; 6) O - 10 min exposure to orange odor alone; and 7) Naive - no treatment.

The orange odor and the odor of maternal saliva were presented as described in Experiment 1. Stroking consisted of vigorous brushing of the pup's back with a soft, sable-hair brush. The pup was stroked continuously during the 10 min treatment. Stroking invariably elicited body movement. Tail-pinching consisted of a forceful pinch of the pup's tail for 1 sec every 30 sec for the duration of the 10 min treatment. Pinches were forceful enough to elicit body movement and squealing.

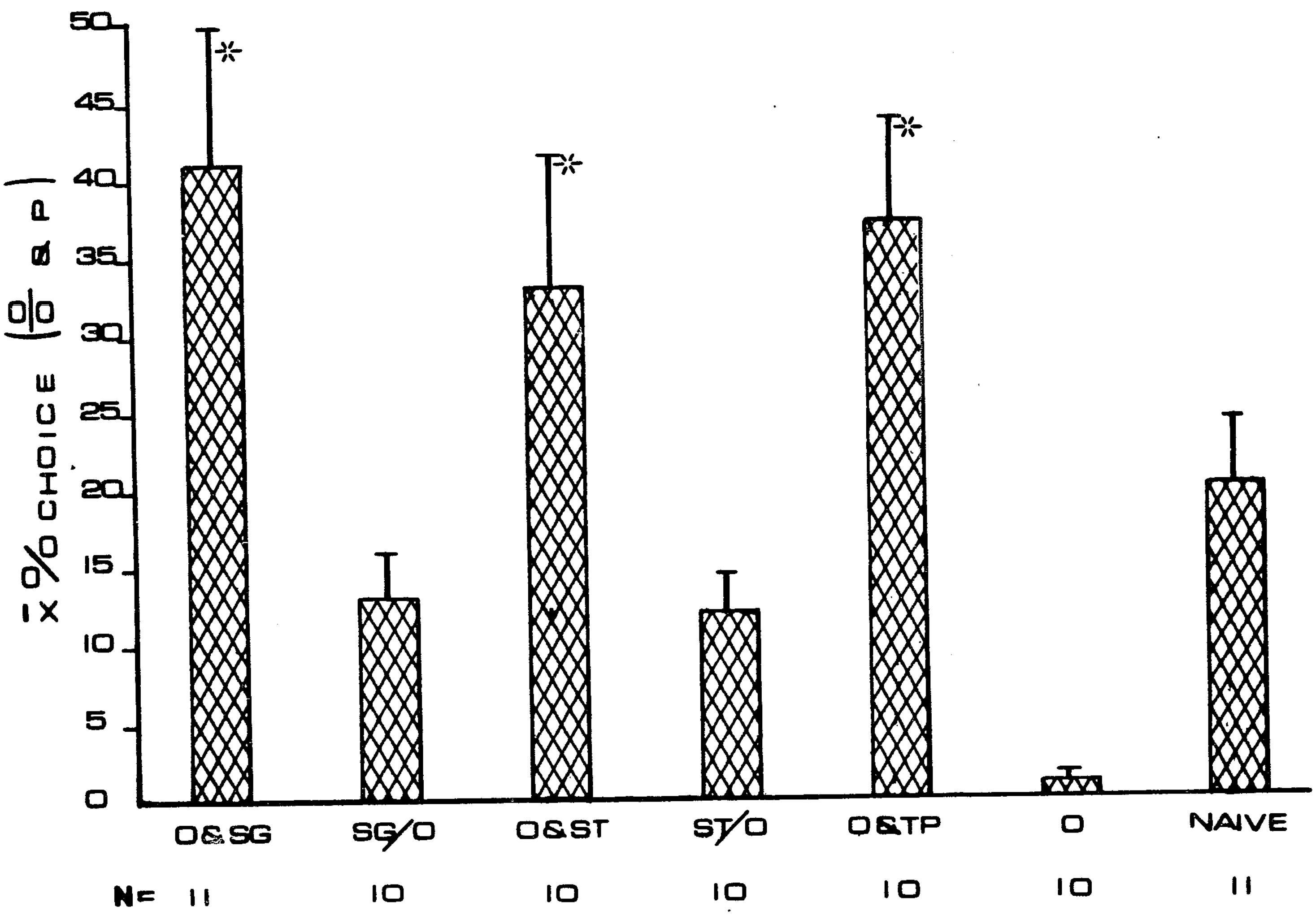
Results and Discussion

There were significant differences among groups (ANOVA F(6,65)=5.92,p<.01). As illustrated in Figure 2, those pups simultaneously exposed to orange odor and either saliva (O & SG), stroking (O & ST), or tailpinch (O & TP) spent a greater amount of time over the orange odor relative to all other pups (Post hoc Newman-Keuls revealed that the means of the Naive, O, ST/O, and SG/O groups were each significantly different from the mean of the O & ST group at the p<.05 level). The O & SG , O & TP, and O & ST groups did not differ significantly from one another. Nor did the O, N, SG/O, and ST/O groups differ significantly from one other. Thus, exposure to the orange odor alone or even exposure to the orange odor immediately after exposure to the activating stimulus was insufficient to increase the relative value of the odor. The necessary conditions to modify an orientation to the orange odor in the 2-odor choice test were the simultaneous exposure to orange odor and either tailpinching, stroking, or the odor of maternal saliva.

The results suggest that the rat pups were exhibiting a modification of an orientation to the orange odor as a result of an acquired association. Therefore, it appears that the tailpinching, stroking, and the odor of maternal saliva may have reinforcing value to infant rats. They may have primary reinforcing value, as was suggested by Johanson & Hall (1982) and Johanson & Teicher (1980), or secondary reinforcing value as was suggested by Pedersen et al, (1982). The view that associative learning had occurred is supported by the failure of the backward pairing groups to spend more time over the orange odor in the choice test.

However, there is an alternative explanation, which holds that the orange odor is, initially, mildly aversive to rats of this age.

Figure 2 Mean percentage of time spent over the orange odor (O/ O&P) in the two-odor choice test by the different treatment groups.



$\bar{X}$ % O CHOICE $\left(\frac{O}{O\ \&\ P}\right)$
50
45
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35
30
25
20
15
10
5
0
*
*
*
O&SG
SG/O
O&ST
ST/O
O&TP
O
NAIVE
N= 11
10
10
10
10
10
11

Since tailpinching, stroking, and odor of maternal saliva all produce behavioral activation in infant rats, it is possible that this activation resulted in differential habituation or attention to the orange odor in the experimental groups during treatment, resulting in a reduction in the aversion to the orange odor during testing. Even the fact that the backwards pairing groups did not spend more time over the orange odor than did the naive group is consistent with the habituation/attention explanation. Our observations indicated that the behavioral activation accompanying stimulation only lasted during the presentation of the stimuli. Therefore, during the presentation of the orange odor, which occurred after the behaviorally activating stimulus had terminated, the pups were in the same behaviorally non-active state as were pups exposed only to the orange odor. In fact, it is possible that the control pups may have spent as much as 80% of the treatment time asleep (Gransbergen, Schwartze & Prechtl, 1970; Jouvet-Mounier, Astic & Lacote, 1970; Shair, Brake & Hofer, 1981). Thus, although our results appear to represent associative learning, this can not conclusively be determined from this study.

Regardless of the specific mechanism involved, however, the results demonstrate that behaviorally activating events that occur simultaneously with the presentation of an odor are capable of increasing the pup's subsequent orientation towards that specific odor.

General Discussion

The present results indicate that in 3-6-day-old rat pups, the brief simultaneous presentation of a novel odor with either tailpinching, stroking, or the odor of maternal saliva is capable of modifying an orientation to the odor when it is later encountered.

This suggests that certain olfactory and tactile stimulation provided by the mother in the presence of a novel odor can result in an orientation to the odor when that odor is later encountered. These results may illustrate a basic mechanism contributing to the development of olfactory based social behaviors. This conclusion has considerable support from other laboratories. Pedersen et al (1982) were able to elicit nipple attachment on a chemically lavaged lactating female in the presence of an artificial odor, provided the pup had previously been stimulated in the presence of that odor. In addition, prenatal and postnatal stimulation in the presence of an odor can elicit the pup's first nipple attachment (Pedersen & Blass, 1982), and the simultaneous exposure to milk (which behaviorally activates infant rats) and a novel odor is capable of modifying the subsequent orientation to that odor (Johanson & Hall, 1982; Johanson & Teicher, 1980). Furthermore, under conditions when milk is not behaviorally activating, such as in non-deprived pups, dehydrated pups, or pups fed at room temperature, orientation to an odor can not be modified (Polefrone, Johanson & Bruno, unpublished observations).

Others have demonstrated that properties of the mother, as well as other pups, can produce a preference for an artificial odor (Galef 1980; Marr & Lilliston, 1968), although the exposure to the odor usually lasted many days. The preference for an artificial odor may be as strong as the preference exhibited by pups to naturally occuring odors (Galef & Kaner, 1980).

The significance of the present studies is in demonstrating that very brief exposures to simultaneously occurring novel odors and either tactile or olfactory stimulation is sufficient to produce a subsequent modification in the orientation to the novel odors in very young pups. Several possible mechanisms may explain these findings. Cairns (1966) has suggested that a rat pup learns about the odors in its environment by their association with primary reinforcement provided by the other rats. The presentation of the stimuli during the treatment phase of our studies conforms to a classical conditioning procedure if one views tailpinching, stroking, and the odor of maternal saliva as primary reinforcers, or as secondary positive reinforcers. In either case, classical conditioning requires that a positive contingency exist between 2 stimuli (Rescorla, 1967), and this positive contingency did exist between the 2 stimuli in our experiment. The failure of the backwards pairing groups, which received a negative contingency between the two stimuli, to modify their orientation supports this view.

Our results may also be due to differential habituation or attention. Behavioral activation, which is the common factor underlying tailpinching, stroking, and odor of maternal saliva, may be an important characteristic of the stimulus-response events necessary to determine olfactory orientation. It should be noted that many of the stimuli that have been used as reinforcers in infant rat learning studies are behaviorally activating, although behavioral activation was not directly manipulated. These stimuli are milk (Brake, 1981; and Hall, 1979), temperature (Guenaire, Costa & Delacour, 1982; and Johanson 1979), electrical brain stimulation (Lithgow & Barr, 1981; and Moran, Lew & Blass, 1982), and suckling (Amsel, Burdette & Letz, 1976; Kenny, Stoloff, Bruno & Blass, 1979). Although most of these researchers have viewed the stimuli used in their experiments as a primary reinforcers, it is possible that behavioral activation resulted in the pups attending or habituating to other cues in the environment more than they ordinarily would. If habituation is the mechanism responsible for our results, then one would have to assume that the odor is initially aversive to the pups, and the aversiveness inhibits orientation. Furthermore, one would have to assume that behavioral activation is capable of enabling habituation to occur in the experimental groups.

In conclusion, behavioral activation, however it operates, appears to be sufficient to produce a behavioral change in infant rats and may represent the basis for the development of olfactory based social behaviors.

OLFACTORY INFLUENCE OF HUDDLING BEHAVIOR

The behavioral responsiveness of a pup to specific stimuli is not static. Nor do the behavioral responses to specific stimuli occur in isolation of other stimuli that are contained in the pups' environment. The present series of experiments was designed to determine how stimuli other than those eliciting a response may influence a pup's responsiveness to the eliciting stimulus.

The method used to examine the influence of such background stimuli was based on recent research that suggests that simultaneous presentation of a behaviorally activating stimulus and a novel odor may result in that odor acquiring "arousing" properties (Pedersen & Blass, 1982; Pedersen, Williams & Blass, 1982; Sullivan, Hofer & Brake, 1983). We therefore hypothesized that "arousing" properties of an odor may be capable of producing a general enhancement of responsiveness to a stimulus that elicits a specific behavior.

In the present experiments, a novel odor was paired with a behaviorally activating stimulus, which resulted in the odor's acquiring "arousing" properties. The influence of the "arousing odor" was assessed on two behaviors: huddling and feeding.

Experiment 1

The altricial rat pup is not capable of physiological thermoregulation and must rely on the behavioral thermoregulatory response of huddling with its siblings to maintain an optimal body temperature (Alberts, 1978a). Alberts (1978b) has shown that during the first week of life the controlling stimulus of huddling are thermal gradients. Our first experiment seeks to determine whether an olfactory stimulus that has "arousing" properties can influence the amount of time a pup spends huddling.

Methods

Subjects

The subjects were 48 male and female 4 to 5-day-old Marland Farm Wistar rats from 6 different litters born and bred at the Montefiore Medical Center animal care facilities. Pups were housed with their dam in a plexiglass terraria (40 X 20 X 24 cm), on pine shavings, in a temperature (22 C) and humidity controlled room. Dams were allowed free access to food and water, and were maintained on a 12:12 hour light-dark cycle with light onset at 7:00 am.

Deprivation Conditions

Pups were separated from the dam 3-4 hours before the start of the experiment. During the deprivation period, pups were housed in a warm (33 C) moist incubator.

Treatment

Pups were randomly assigned to 1 of the following 4 treatment conditions: 1.) O & ST - 10 min simultaneous presentation of orange odor and stroking; 2.) 0 - 10 min presentation of orange odor; 3.) ST - 10 min of stroking; 4.) Naive - 10 min with no stroking and no orange odor.

Treatment consisted of removing a pup from the housing incubator and placing it in another tub, which was then placed in a warm (30 C) moist treatment incubator for 10 min. The orange odor was presented by suspending pine shavings soaked with .02 cc of orange extract (Ann Page; A & P) 5 cm above the treatment tub. Stroking consisted of vigorously stroking the pup's back with a soft, sable-hair brush. The pup was stroked continuously during the 10 min treatment.

Testing

Pups were tested for huddling behavior 1 hr after treatment. The apparatus and testing conditions for huddling behavior were similiar to those used by Alberts (1978b). Animals were tested at room temperature (25 C) in a plexiglass box (12 X 17 X 4 cm) with a small grid (.4 x .4 cm) wire mesh floor. A sacrificed stimulus pup, which was the huddling target and littermate of the treated pup, was placed ventral side down against the long side of the testing apparatus. The orange odor was presented during the test by suspending pine shavings soaked with orange extract (.02 cc) 10 cm above the center of the test box. At the start of the test, the test pup was placed into the test box with its nose 2 cm from the stimulus pup.

Data Collection

The test consisted of a 6-min observation period. A time sampling technique (Altmann, 1974) was used, so that the test pup's behavior was observed every 20 sec for 2-3 sec, resulting in a total of 19 observations for each test pup. A Casio electromechanical timer was used to time the observation intervals. At each observation, it was noted whether the test pup was in contact with the stimulus pup and which of the following behaviors the pup was engaged in: 1.) the test pup's paw was in contact with the stimulus pup; 2.) the test pup's nose was in contact with the stimulus pup; 3.) less than 1/2 of one surface of the test pup was in contact with the stimulus pup; 4.) greater than 1/2 of one surface of the test pup was in contact with the stimulus pup; 5.) the test pup probed the stimulus pup. The experimenter observed only one pup during each obervation.

Observations were done by 2 independent raters with an inter-rater reliability correlation of r=.94. One third of the litters were tested blind.

Results and Discussion

There was a significant difference between treatment groups in the amount of time spent in contact with the stimulus pup during the huddling test (ANOVA, F(3,44)=19.2525, p<.01), indicating that huddling behavior can be influenced by prior olfactory stimulion. As illustrated in Figure 3, those pups that received simultaneous presentation of the odor and stroking (O & ST) during the treatment subsequently spent more time in contact with the stimulus pup than each of the other treatment groups (Post hoc Newman-Keuls showed that the means of the O & ST group differed at the p<.01 level from each of the means of the O, ST, and Naive groups and none of these groups differed from each other). This seems to be partly due to the O & ST group having a significantly shorter latency to make initial contact with the stimulus pup (ANOVA,F(3,44)=4.307,p<.05; Post hoc Newman-Keuls showed hat the O & ST showed that the O & ST group differed fom each of the other treatment groups at p.<.05).

Pups in the O & ST group exhibited some huddling behaviors significantly more often than did those in the O, ST, and Naive groups (probe, ANOVA,F(3,44)=4.372,p<.05; and paw, ANOVA,F(3,44)=8.496,p<.01: see Figure 4), indicating that specific behaviors associated with huddling behavior can be enhanced. However, analyses of the total # of observations in which a specific response occurred divided by the total number of times in contact with the stimulus pup did not reveal a significantly different probability of exhibiting any huddling

Figure 3 The mean number of times in contact with the stimulus pup during the huddling test for the various treatment groups.

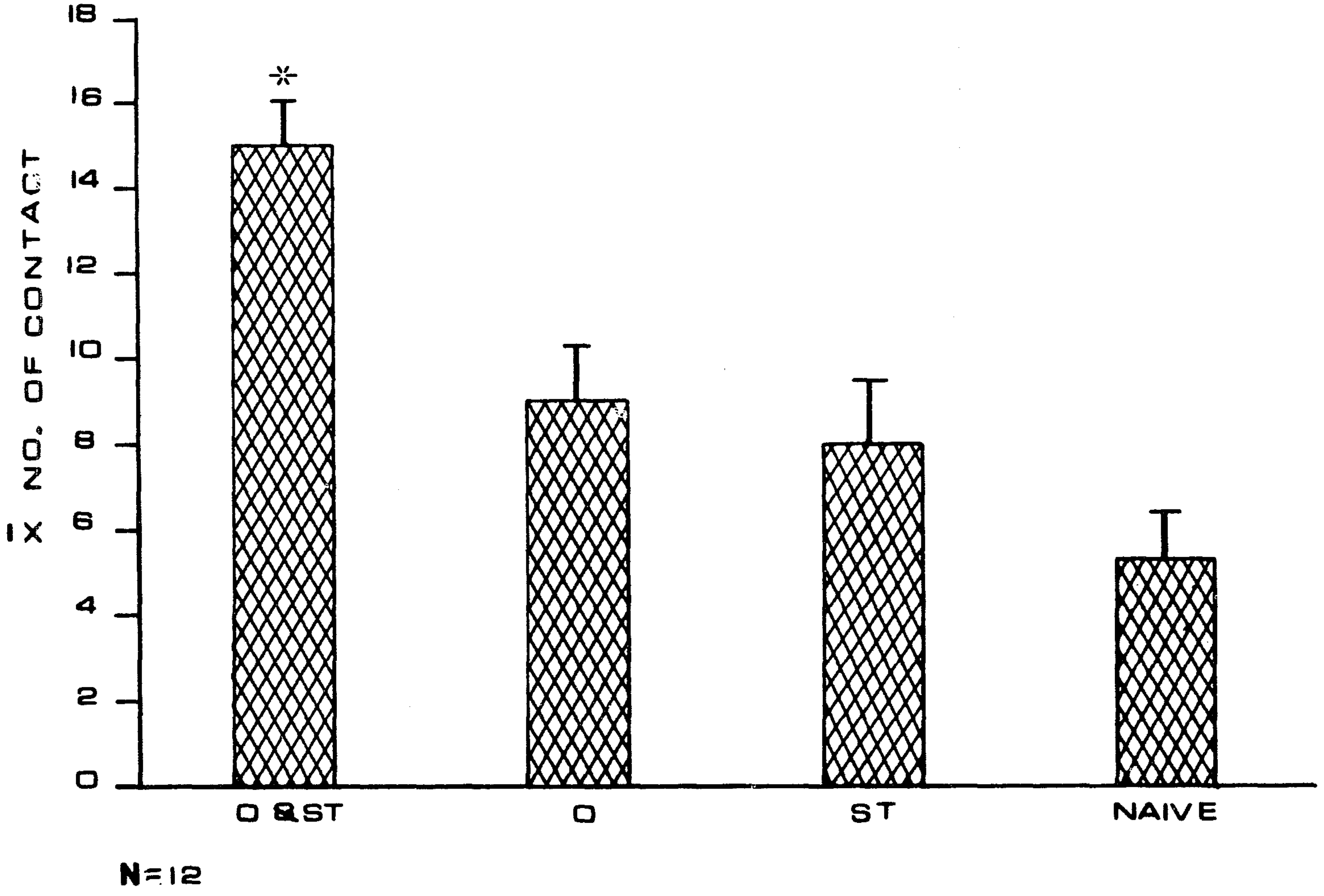
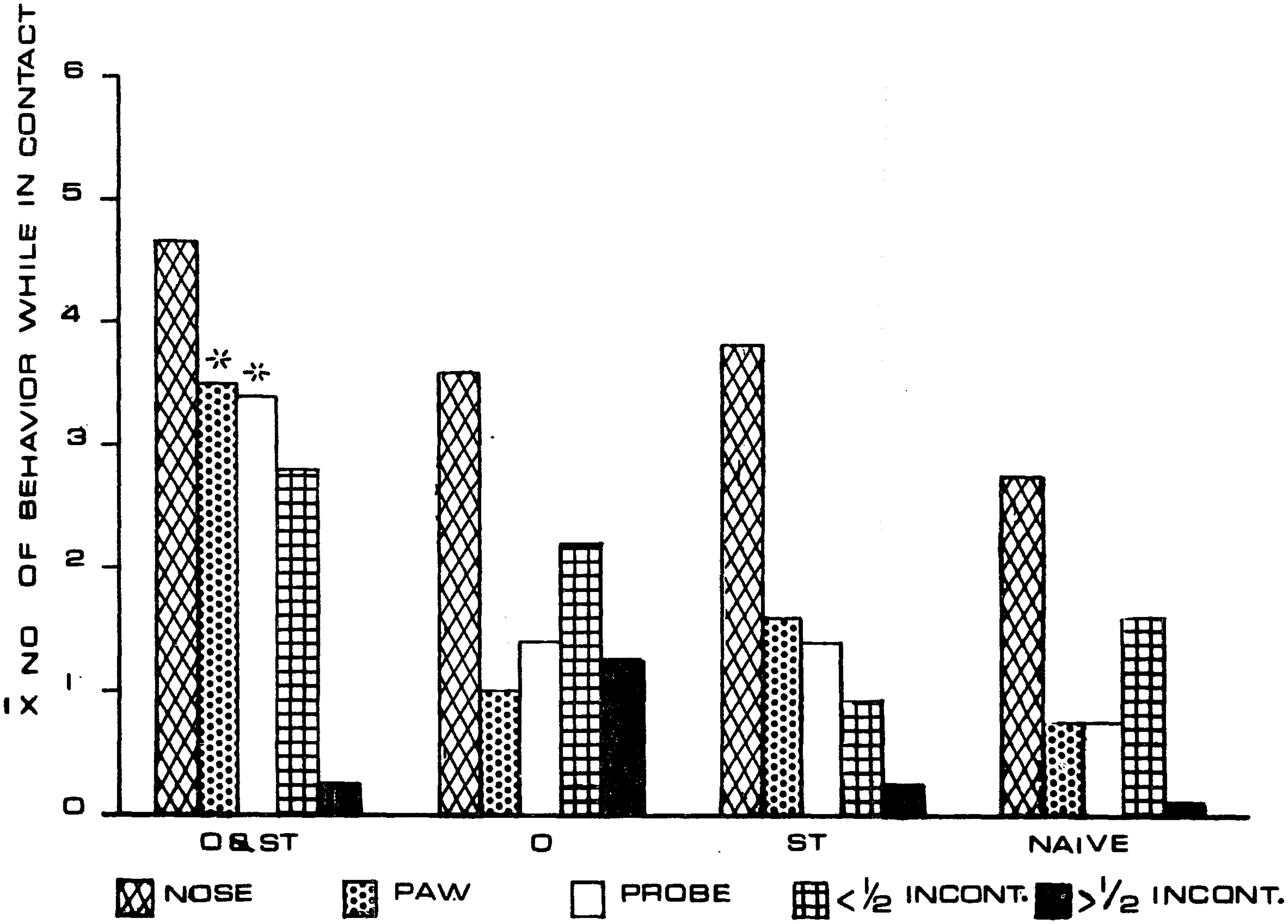

$\bar{X}$ NO. OF CONTACT
0
2
4
6
8
10
12
14
16
18
*
O & ST
O
ST
NAIVE
N=12

Figure 4 The mean number of specific behaviors exhibited during the huddling test while the pups were in contact with the stimulus pup by the pups in the various treatment groups.


X̄ NO OF BEHAVIOR WHILE IN CONTACT
0
1
2
3
4
5
6
*
*
O&ST
O
ST
NAIVE
NOSE
PAW
PROBE
<½ INCONT.
>½ INCONT.

behaviors while a pup was in contact with the stimulus pup. Thus, although the quantity of huddling behavior was enhanced by the O & ST treatment procedure, it does not seem to influence the quality of huddlng behavior. Together, these results seem to suggest that the orange odor was "arousing" to the pups in the O & ST treatment condition, which resulted in pups in this group coming in contact with the stimulus pup sooner than pups in the control groups. Furthermore, the arousing properties of the odor did enhance the quantity of huddling behavior but it did not affect "how" a pup would respond to the stimulus pup once contact was made. However, the differences in the various treatment groups' performance in the huddling test does not necessarily reflect an increased "arousal" in the O & ST group; rather, it may reflect an inhibition or disruption of huddling behavior in the control group due to the presence of the orange odor. That is, stroking a pup in the presence of the odor may produce a decrease in neophobia or hasten habituation to the aversiveness of the odor, resulting in a normal baseline huddling behavior. Those pups that were presented the odor alone or were not presented the odor may still have a neophobic response to the odor or may not have habituated to the aversiveness of the odor.

Experiment 2

This experiment was designed to determine whether the O & ST pups were being "aroused" by the presence of the orange odor and/or whether the control groups were being inhibited by the presence of the orange odor. The treatment procedure was therefore repeated but 1/2 of the pups from each treatment group were tested in the presence of orange odor and the remaining 1/2 of the pups were tested without orange odor. The reasoning was that if the O & ST pups tested in the presence of the odor were actually being "aroused" by the presence of the odor during testing, then these pups would have a significantly greater amount of time in contact with the stimulus pup than pups in other treatment and testing conditions. Furthermore, if the O, ST, and Naive pups were not being inhibited by the presence of the odor during testing, then they should not differ significantly from those pups tested without the odor. The second objective was to determine if certain specific behaviors accounted for the difference in contact time with the stimulus pup among the various treatment groups during the huddling test. Therefore, two additional behavioral categories were included during the observation period: 1) an activity rating scale (Hall, 1979). 2) movement to a new area of the test box.

Methods

Subjects.

The subjects were 48 male and female rat pups from 6 different litters born and bred under the same conditions as described in Experiment 1.

Procedure.

The deprivation and treatment conditions were similar to those of Experiment 1. The testing procedure differed only in the presence or absence of the orange odor. For each treatment condition, 1/2 of the pups were tested in the presence of orange odor and 1/2 of the pups were treated without the presence of orange odor. The 2 additional behaviors noted during the observation period were: 1) an activity rating score (0-not active; 1-movement of part of the body, e.g., face washing, head raising; 2-movement of the entire body, e.g., locomotion; 3-extreme behaviors, e.g., rolling over, wall climbing), and 2) at least 2 cm of body movement by the pup which resulted in the pup being placed in a new area of the test box.

Results and Discussion

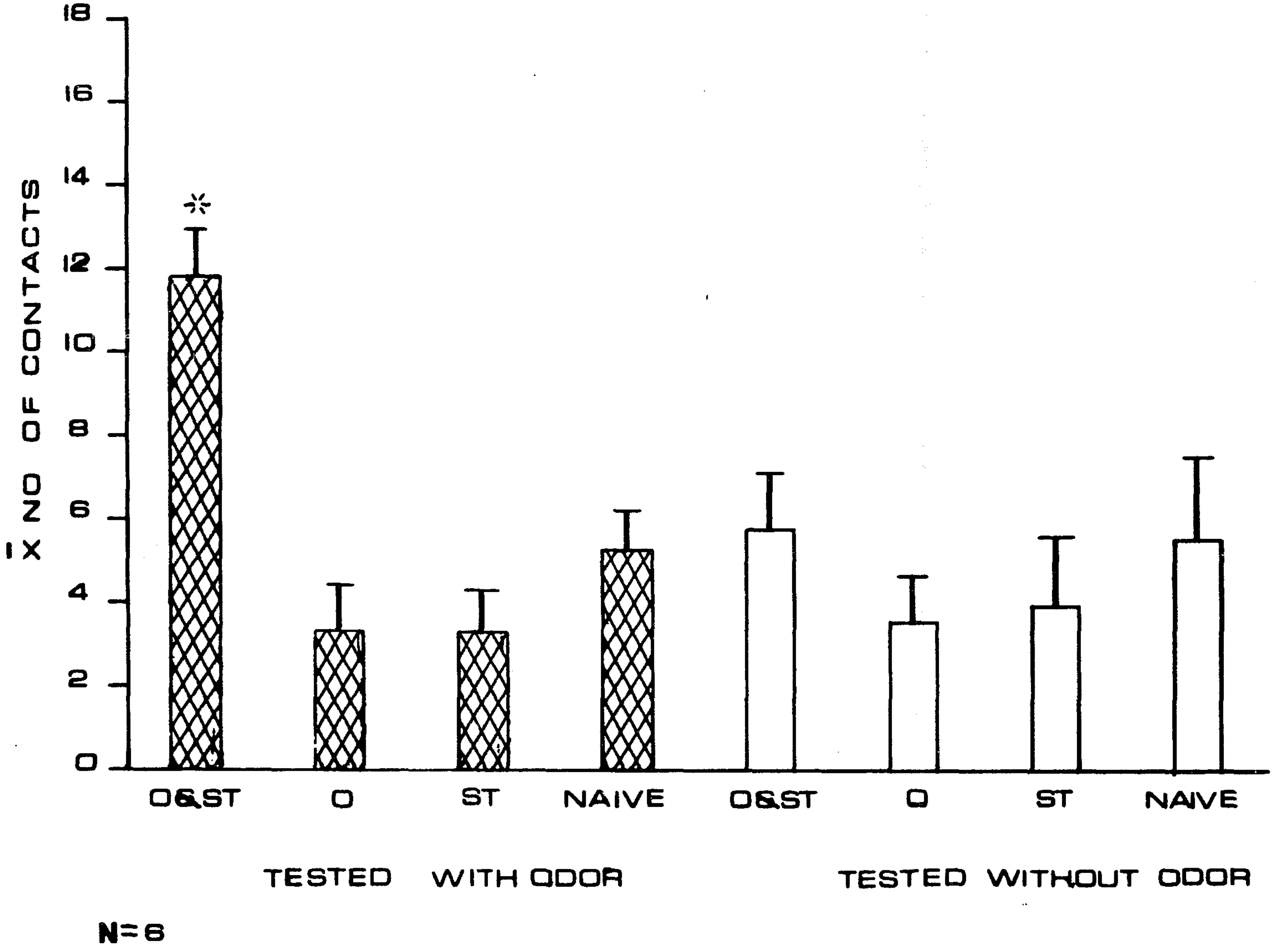
There was a significant difference among treatment conditions in the time spent in contact with the stimulus pup (2 way ANOVA,$F(7,40)=4.723, p<.05$). As illustrated in Figure 7, the O & ST treatment group tested in orange odor spent more time in contact with the stimulus pup than all other treatment groups tested with and without orange odor (Post hoc Newman-Keuls revealed that the means of the O & ST tested with orange odor differed from all other treatment groups tested with and without orange odor at the $p<.01$ level and none of the other groups differed from one another). These results suggest that the O & ST treatment group tested in orange odor was being "aroused" by the presence of orange odor during the huddling test and that the control groups were not being inhibited by the presence of the orange odor.

The mean activity scores of the treatment groups, whether in contact with the stimulus pup or not, did not significantly differ from one another (non-significant 2-way ANOVAs). Furthermore, there were no differences among the various treatment groups in terms of the pups' movement within the test box (non-significant 2-way ANOVA). These behaviors, therefore, probably do not account for the difference in the amount of contact exhibited by the O & ST pups tested in orange odor.

Experiment 3

Hall (1979) has shown that rat pups as young as 3 days of age are

Figure 5 The mean number of times in contact with the stimulus pup during the huddling test for the various treatment and testing conditions.


X̄ NO OF CONTACTS
18
16
14
12
10
8
6
4
2
0
*
O&ST
O
ST
NAIVE
O&ST
O
ST
NAIVE
TESTED WITH ODOR
TESTED WITHOUT ODOR
N=6

capable of feeding from a puddle of milk and that the amount of milk consumed is dependent on the pup's level of deprivation. Since the consumption of milk can be altered by deprivation, it seems likely that milk consumption might also be altered by other "arousing" procedures, such as those which resulted in altered huddling behavior. In the present experiment we sought to assess whether a novel odor previously paired with stroking would be capable of modifying the amount of milk consumed by a pup.

Methods

Subjects

The subjects were 90 male and female 4 to 5-day-old Sprague Dawley rat pups from 10 different litters born and bred at Barnard College animal care facilities. Pups were housed with their dam on shavings in a temperature and humidity controlled room. Dams were allowed free access to food and water and were maintained on a 12:12 hr light-dark cycle with light onset at 7:00 am.

Deprivation Conditions

Pups were separated from the dam 5-6 hr before the start of the experiment. During the deprivation period pups were housed in a warm (33 C) moist incubator.

Treatment

Pups were randomly assigned to 1 of the following 4 treatment procedures: 1.) O & ST - 30 min exposure to orange odor and stroking; 2.) O - 30 min exposure to orange odor; 3.) ST - 30 min of stroking; 4.) Naive - 10 min with no stroking and no exposure to orange odor.

Treatment consisted of placing the pups in a 28 C tub for 30 min. A tissue with .04cc of orange odor was placed on the bottom of the tub. Stroking consisted of vigorous, continuous stroking of the pup's back with a soft sable-hair brush.

Testing

The 30-min feeding test began 1 hr after treatment. The testing procedure was based on those described by Hall (1979). The pups' body weights were taken immediately before and after the feeding test, and the percentage of body weight gain was used as the dependent variable. At the start of testing, pups were placed in individual clear plastic tubs in a warm (33 C) incubator. The bottom of the tubs contained a paper towel soaked with 5 cc of warm milk. For 1/2 of the pups in each treatment condition, .2 cc of orange extract in 1 pt of water was placed in the incubator during testing. No odor was present during the feeding test for the remaining pups in each treatment condition.

Results and Discussion

Although the pups did consume milk (X=2% body weight gain), no significant differences were found between the various treatment groups with respect to the percentage of body weight gain (non-significant 2-way ANOVA). It is possible that the stimulus parameters of this experiment were simply not appropriate to produce a modification of feeding behavior, although this seems unlikely for 2 reasons. First, our numerous additional attempts to modify feeding behavior, which included increasing and decreasing odor concentration, decreasing testing temperature, and using an anterior cannula implant for milk consumption (Hall, 1979), have all been equally unsuccessful in modifying feeding behavior. Second, Pedersen et al (1982) have shown an inverted U-shaped curve in performance during a nipple attachment test as a function of increases and decreases in the stimulus intensity during treatment. Our results with varying stimuli intensities show no indication of an inverted U-shaped performance curve. Our failure to modify feeding behavior is in sharp contrast to successful attempts using similar treatment procedures to modify other behaviors, such as orientation (Sullivan, Hofer & Brake, 1983), nipple attachment (Pedersen & Blass, 1982; Pedersen, Williams & Blass, 1982), and huddling behavior (present paper). Although the reason for this failure to modify feeding behavior is unclear, it may be due to the powerful stimulus properties of milk, which enable milk to function as a reinforcer (Johanson & Hall, 1982; Johanson & Teicher,1980). If this is true, then it is likely that the level of milk consumption in pups of the present experiment has reached a ceiling.

General Discussion

The present series of experiments illustrate that the huddling behavior of 4 to 5-day-old rat pups can be enhanced by the presence of an odor that had previously been paired with a behaviorally activating stimulus. The modification of huddling does not seem to alter the specific behaviors defining huddling behavior but rather is simply the result of enhancing the frequency of these behaviors. Further, the increase in huddling did not seem to be due to a general increase in activity or locomotion during testing.

A treatment procedure capable of enhancing huddling behavior, however, was not capable of modifying feeding behavior. There are 2 possible reasons for this. First, the powerful stimulus properties of the milk may have resulted in arousal, which obscured the arousal effects produced by the odor. Second, the pups in the feeding test were in almost constant contact with the milk, and the results of the huddling test showed that huddling was modified largely because of a shorter latency to make contact with the stimulus pups. It is possible that this difference in contact with the stimulus (pup or milk) in the two tests can account for the ability to modify huddling behavior but an inability to modify feeding behavior. The behaviors of the pups in the various treatment and testing conditions did not differ in their behaviors while they were in contact with the stimulus pup. Also, unpublished results (Williams & Sullivan) on feeding behavior using an anterior tongue cannula (Hall, 1979) showed that there were no differences in the behavioral responses (mouthing, probing etc.) to milk of pups in the presence of an odor that had previously been paired with a behaviorally activating stimulus and the control groups.

There are at least two possible mechanisms for the enhancement of huddling behavior. They are associative learning and sensitization. Classicial conditioning requires that an association exist between 2 positively contingent stimuli for learning to occur. Since only those animals that received the simultaneous pairing of the odor and the behaviorally activating stimulus increased the amount of time spent in contact with the stimulus pup, our results are consistent with the associative learning explanation. However, this leaves unanswered the question of what is learned during treatment which could result in increased huddling.

Sensitization also seems to be able to accomodate the results of the present studies, as well as related studies from other laboratories. According to Kandel (1976), sensitization poduces a general state of arousal which can be expressed in two ways:

orientation and an enhancement of responding to another stimulus in the environment. In the suckling context it might produce orientation (Sullivan, Hofer & Brake, 1983), a general enhancement of nipple attachment (Pederson, Williams & Blass, 1982; Pederson & Blass, 1982), and enhancement of huddling (present paper). However, the question is raised as to why the sensitization was only expressed in the presence of the orange odor. It would seem that the orange odor acquired sensitizing properties during treatment. There is some suggestion that the stimuli used during treatment have additive effects (Pedersen et al, 1982) and perhaps the stroking alone was not a strong enough stimulus to produce sensitization but the odor and the stroking presented at the same time was. Thus, only the pups that received the simultaneous presentation of odor and stroking were sensitized and the sensitization to the orange odor was robust enough to produce the behavioral effects seen during testing.

Although the results presented in this paper cannot conclusively determine whether associative learning or sensitization was responsible for the modification of the experimental group's behavior, they seem to demonstrate that stimulation in the presence of a novel odor may represent one of the basic mechanisms of the formation of olfactory based social behaviors. In fact, Rosenblatt (1979) has developed a theoretical framework of behavioral development of the rat pup which accomodates our results. He suggests that certain stimuli produce a non-specific arousal (in the present case, behaviorally activating stimuli). The non-specific arousal is sufficient to produce an association between the arousal producing stimulus and odors, resulting in the odors acquiring "incentive." The "incentive" properties of the odor then becomes capable of producing two behaviors in the rat pup: first, an orientation towards the source of the odor, and second, a general behavioral enhancement. Clearly, our results indicate that our treatment procedure is capable of producing both orientation and behavioral enhancement.

DISCUSSION

The discussion of the results of the orientation, huddling, and feeding experiments will focus on the 3 experimental questions outlined in the introduction.

I.) Can behaviorally activating a pup in the presence of an odor result in a change in the pups responsiveness to that odor?

The orientation experiments clearly illustrate that behaviorally activating a pup in the presence of an odor is sufficient to modify a pup's behavior towards that odor. The huddling experiments illustrate that behaviorally activating a pup in the presence of an odor is not only capable of modifying a pup's response to the odor, but also is capable of modifying a pup's response to another stimulus in the presence of that odor. The feeding experiments, however, illustrate that behaviorally activating a pup in the presence of a novel odor is not capable of modifying a pup's responsiveness to all stimuli in the environment.

These findings suggest that behaviorally activating a pup in the presence of an odor may be either sufficient or necessary to alter the pup's subsequent behavior toward the odor. Based on the literature on learning in the infant rat discussed earlier, an additional question is raised as to whether the behavioral modification produced in the rat pups was due to a specific property of the reinforcer used or the behavioral activation produced by the reinforcer. This question has also not been answered by the present experiments. However, there is some evidence which suggests that in situations when a reinforcer is not behaviorally activating pups do not seem to learn (Johanson, Polefrone & Bruno, unpublished observations). Thus, there is some suggestion that behavioral activation may be a necessary characteristic of the stimulus-response sequence necessary to produce a behavioral modification in rat pups less than 1-week-old.

II.) Is associative learning, habituation, or sensitization responsible for the observed modifications of the pup's behavior?

The modification of a pup's behavior in the presence of an odor which had previously been paired with a behaviorally activating stimulus can be due to a number of factors.

1.) Associative learning - The pups that received the behaviorally activating stimulus in the presence of the odor may have associated the behaviorally activating stimulus with the odor during treatment. All experimental and control groups' performance in both the orientation and huddling studies are consistent with this interpretation. Only those pups that received the simultaneous presentation of an activating stimulus and the odor during treatment showed a modification of behavior during testing with the odor present. All other groups, including odor alone, activating stimulus alone, and "backwards" presentation of the odor and activating stimulus, whether tested with the odor or without the odor, did not exhibit a behavioral modification during testing. Thus, associative learning is a possible mechanism responsible for the behavioral modification exhibited in the orientation and huddling studies.

2.) Habituation - A decrease in responding as a result of repeated presentations of a stimulus may be the result of habituation. In the present discussion, the habituated response would be an aversive response or a neophobic response. The following explanation requires two assumptions. First, the orange odor has aversive properties or rat pups exhibit neophobia to the odor used in these experiments. Second, a rat pup that is placed in isolation and receiving low levels of stimulation is likely to be asleep or unattentive. Based on these two assumptions, one could speculate that only those pups that were kept awake or attentive (by being behaviorally activated) could attend to the odor and thus habituate to the odors aversiveness, or become familiar to the odor to reduce the neophobic response. Thus, those pups that were not active during the presentation of the odor (odor only and backward pairing groups) would be experiencing the odor during testing as though it were being presented for the first time, resulting in testing performance that was not significantly different from those pups actually experiencing the odor for the first time (behaviorally activating stimulus alone and naive).

Thus, based on the results of the orientation experiment, habituation is a possible mechanism responsible for the behavioral modification of the rat pups. However, the results of the huddling experiment seem to indicate that habituation is not responsible for the behavioral modification of the pups. In the huddling experiment, only those pups that received the simultaneous exposure to the odor and the behaviorally activating stimulus during treatment, and which were tested with orange odor, spent more time in contact with the stimulus pup. Furthermore, the habituation explanation requires that the orange odor be initially aversive to the pups or the pups have a neophobic reaction to the odor, which results in the inhibition of a behavior. Since the performance of the control groups did not significantly differ from the performance of those pups tested without orange odor, it is unlikely that the orange odor was aversive and inhibiting behavior. Thus, based on the results of the huddling experiments, habituation can be ruled out as a possible mechanism for the modification of behavior.

3.) Sensitization - An increase in responding to a stimulus as a result of the presentation of another (usually strong or noxious) stimulus does not depend on the pairing of the two stimuli (Kandel, 1976). Kandel also suggests that sensitization may be related to a general arousal in behavior, which has two components: 1.)orientation toward the stimulus, and 2.) heightened responsiveness in some behavioral systems. Clearly, Kandel's use of sensitization fits the results of both the orientation and huddling experiments well, if one assumes that the pairing of the odor and activating stimulus sensitizes the pups such that they show enhanced responding during the test in the presence of the odor. That is, the pups which received the simultaneous presentation of an odor and a behaviorally activating stimulus may have been sensitized by the combined presentation of the two.

Based on the above analysis of the possible mechanisms responsible for the behavioral modification of orientation and huddling, we are left with 2 possibilities: associative learning and sensitization. However, there are additional difficulties in the use of the learning mechanism which do not exist in the sensitization mechanism. For instance, if the pups in the experimental groups did learn, it is difficult to determine exactly what was learned. The pups were never trained to orient towards the odor or huddle, yet, those were the behaviors that were exhibited during testing. On the other hand, according to Kandel (1976), sensitization can account for the enhancement of both orientation and huddling behavior. Thus, although it is a possibility that associative learning is responsible for the behavioral modification of the experimental animals, it seems as though sensitization can also account for the results.

III.) What are the consequences of the modification of the rat pups behavior with regards to its adaptation to the environment?

The results of the series of experiments presented in this paper may be the basis for olfactory based social behaviors. The present studies illustrate that in the presence of an odor, which had previously been paired with a behaviorally activating stimulus, two social behaviors, orientation and huddling, were enhanced. This conclusion has considerable support from other laboratories. Pedersen et al (1982) and Pedersen & Blass (1982) were able to elicit nipple attachment on a chemically lavaged post-partum rat, provided the odor had previously been paired with a behaviorally activating stimulus. Johanson and colleagues have shown that when milk produces behavioral activation in the presence of an odor, a pup will orient to that odor when it is later encountered (Johanson & Hall, 1982; Johanson & Teicher, 1980). Johanson has also shown that when milk is not behaviorally activating, the pup will not orient to the odor when it is later encountered (Johanson, Polefrome & Bruno, unpublished observations). Consequently, behavioral activation may be one of the important mechanisms responsible for the development of olfactory based social behaviors.

Rosenblatt (1979) has formulated a theoretical framework of behavioral development in altricial mammals which seems to accomodate the results of the orientation and hu`dling studies. He suggests that certain stimuli produce a non-specific arousal (in the present case, behaviorally activating stimuli). The non-specific arousal may be sufficient to produce an association between the arousing stimulus and a novel odor, resulting in the odor acquiring "incentive." Once the odor has acquired "incentive," it then becomes capable of eliciting orientation to the source of the odor and a general behavioral arousal. Rosenblatt suggests that the acquired "incentive" of the odor may serve to organize behavioral development. Interestingly, the odor, which was previously paired with a behaviorally activating stimulus, seems to acquire the two properties that Rosenblatt has suggested, orientation and arousal (as defined by a general enhancement in the responsiveness to stimuli). Thus, the conclusion that our treatment procedure results in the development of olfactory based social behaviors seems to have both experimental and theoretical support.

APPENDIX

The following is a description of the additional experiments and pilot work done in an attempt to modify feeding behavior. All were unsucessful.

All subjects, deprivational, treatment, and testing conditions were identical to those used in the feeding experiment described in the paper except where noted. There were at least 9 pups in each treatment-test condition and no more than 2 pups from the same litter were used in the same treatment-test condition.

Experiment 1

Treatment-The odor intensity was increased by .01cc.

Experiment 2

Treatment-The odor intensity was decreased by .01cc.

Test-Pups were only tested in orange odor.

Experiment 3

Test-An anterior tongue cannula, which was implanted at the beginning of the deprivation period, was used to deliver milk rather than on the floor (Hall, 1979). Also, additional behavioral dependent variables were measured using a time sampling technique. Every 30 sec the pups behavior was observed for 2 sec. For each observation a 0-3 activity rating score was given (same as described for the huddling experiment) and the occurance of the following behaviors were noted; mouthing, head-up, face wipe, probing, rolling over, gape, lick and climb.

Pilot work

In pilot work the following variables were manipulated.

1) length of feeding tests

2) length of deprivation period

3) concentration of odor during treatment and test

4) length of time between treatment and test

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