

Ontogeny of Thermal and Olfactory Determinants of Huddling in the Rat

Jeffrey R. Alberts and Peter C. Brunjes
Indiana University

In standardized tests of huddling behavior, 5-, 10-, 15-, and 20-day-old rat pups spent substantial and equivalent amounts of time with an immobile rat or a heated, fur-covered tube, which suggests that the conspecific and inanimate stimuli were equally attractive to the pups. Two-choice preferences tests, however, revealed dramatic developmental differences in attraction. Younger pups preferred to huddle with the warmer, inanimate target, whereas older pups preferred the conspecific. The emergent conspecific preference appears mediated by attraction to species odors. The 5- and 10-day-olds huddled equally with an immobile rat and an immobile gerbil, stimuli with similar thermal and tactile properties, but older pups preferred the conspecific. Intranasal zinc sulfate treatment eliminated preference for the conspecific in 15- and 20-day-olds but did not disrupt huddling *per se*. Thermal cues were sufficient to elicit huddling at all ages, but olfactory cues became a more salient influence before weaning. An ontogenetic transition from "physiological" to "filial" huddling is discussed in terms of changes in sensory control of early behavior.

Contact behavior begins at birth and persists throughout adulthood in the Norway rat (*Rattus norvegicus*). Born in a litter of 6-12 pups, the altricial infants huddle in the maternal nest until weaning. The frequent social interactions of juveniles and adults almost invariably involve cutaneous contact, and colony members are typically observed to huddle and to sleep in groups (Barnett, 1963; Calhoun, 1962; Steineger, 1950; Telle, 1966). It is understandable why the rat is referred to as a "contact species."

Considering the great prominence of huddling in the rats' life history, relatively little attention has been given to the study of this lifelong behavior. Many species use huddling as a behavioral strategy for combating cold challenge (Whittow, 1971), but

Norway rats huddle even at moderate temperatures, which implies that the behavior also serves social functions (Barnett, 1963; Cairns, 1972; Telle, 1966). The ontogeny of huddling may be viewed as a central aspect of the ontogeny of social behavior in the rat.

Huddling in developing rat pups has been analyzed by Cosnier (1965) and Alberts (1978a, 1978b). Their studies demonstrated that rat pups, like adult mammals in general, derive significant thermoregulatory benefits from huddling. The metabolic consequences of huddling are, to some extent, controlled by the pups; through continuous individual adjustments, the aggregate acts as a regulative unit and responds to temperature changes in the environment (Alberts, 1978a).

Although the most apparent physiological functions of huddling relate to the effect of the behavior of body temperature regulation, both Cosnier and Alberts have shown that a variety of stimuli other than heat cues elicit, direct, and maintain huddling behavior in the rat pup. Specifically, it was found that in addition to heat, other cues such as olfactory input, two classes of tactile stimuli (surface contours and furry "comfort" cues),

This research was supported by Grant MH 28355 from the National Institute of Mental Health to Jeffrey R. Alberts. The experiments were reported at the 1977 meeting of the Midwestern Psychological Association by Peter C. Brunjes. We thank James Craig for his helpful comments on the manuscript.

Requests for reprints should be sent to J. R. Alberts, Department of Psychology, Indiana University, Bloomington, Indiana 47401.

and photic cues were involved in eliciting or mediating huddling behavior by rat pups.

It can therefore be concluded that a multitude of cues are each *sufficient* for huddling. For some of these cues, there appears to be an ontogenetic sequence of participation in behavioral control (Alberts, 1978b; Cosnier, 1965). Yet, it is not clear how, during early ontogenesis, additional sensory modalities join into the control of behavior.

The present series of experiments was designed to delineate the relative contributions of different cues to the control of huddling in the developing rat. However, it was first necessary to refine our previous methods of standardized testing (Alberts, 1978b) to permit accurate comparisons of pups of different developmental stages. Additional studies were then performed which converged on an examination of the balance between thermal and olfactory determinants of huddling during early development in the rat.

Experiment 1

The use of inanimate objects, models and immobilized animals as standardized test stimuli has been the predominant method in previous studies of the sensory control of huddling (Alberts, 1978b). This approach was adopted here but was modified to meet the requirements of the present series of experiments. In some of the earlier experiments, extensive use was made of anesthetized age-mates (5 to 20 days old) as huddling stimuli. Whereas this procedure was helpful for presenting cues representative of those found in the nest, comparisons across ages were difficult to make. Developmental changes in the subjects were confounded with parallel changes in the properties of the test stimuli. Age-related differences in huddling could be caused by changes in the subject, the age-mate stimulus, or both.

Experiment 1 sought to circumvent these problems and compare the huddling behavior of rat pups at various stages of development. A testing situation was designed to be amenable to quantitative description of the behavior across pups of different age

groups. One animate stimulus and one inanimate target stimulus were tested.

Method

Subjects. A total of 64 rat pups of Sprague-Dawley descent were subjects. Male and female pups were tested at 5, 10, 15, or 20 days of age. These age groups provide four distinct stages of sensory and behavioral maturation, spanning the neonatal to weaning periods of early ontogenesis.

All pups were born in the Indiana University colony, outbred from stock purchased from Laboratory Supply (Indianapolis, Indiana), and reared in standard plastic maternity cages (48 × 20 × 26 cm). The colony room was maintained at 22–24 °C on a 16:8 hr light/dark schedule. Purina Rat Chow and water were continuously available. Three days after birth (Day 0) litters were culled to eight pups each and otherwise left undisturbed until testing.

Testing apparatus. Pups were tested individually in boxes roughly scaled to the subjects' size and locomotor ability. These open-topped boxes were constructed from plywood and galvanized metal. The dimensions of the chamber floors were 10 × 8 cm for the 5-day-olds, 30 × 13 cm for the 10-day-olds, and 30 × 25 cm for the 15- and 20-day-old subjects. The walls were sufficiently high to contain the subjects and were sloped outward at an angle of approximately 70° to provide a clear view from above. At one end of each chamber, a rectangular opening was cut at floor level near one corner. The test stimuli protruded into the chamber through this opening. These windows, like the test chambers, were scaled to the subjects and measured approximately 3 × 8 cm for the 5-day-olds, 3 × 10 cm for the 10-day-olds, and 4 × 11 cm for the older pups.

Test stimuli. Two different test stimuli were used in this experiment. For half of the pups, the test stimulus was a sexually immature rat (both males and females were used) at least 40 days of age. The stimulus animal was injected with a long-lasting anesthetic (ethyl carbamate, 250 mg/kg, ip) and placed against the outside of the test chamber so that its lateral surface was pressed against the rectangular window of the cage. Thus, in this condition all the subjects were presented with a portion of the flank of a living but immobile rat.

The second condition of the present experiment was identical to the first except that the target stimulus was a warm, fur-covered cylinder similar in size to the rat stimulus. The inanimate stimulus was an aluminum tube, covered with cotton gauze and wrapped with commercial heating tape and then with the tanned pelt of a rabbit. A voltage regulator maintained the surface temperature of this furry tube at approximately 34 °C. The overall diameter of the cylinder was approximately the same as the conspecific stimulus, and the cylinder could be positioned against the windows of the test cage in the same way described for the anesthetized rat.

Observational method. The methods used to observe and quantify huddling behavior were identical to those described previously (Alberts, 1978b). Briefly, huddling tests were each 4 hr long and were recorded

with a time-lapse video recorder. Test sessions were taped at a slow recording speed (.2 sec/field) and scored later at a rapid playback speed (60 fields/sec) that yielded a record:playback ratio of 1:24. Because huddling is a long-duration behavior, the reliability of quantifying the behavior even at such rapid playback speeds is excellent, usually varying less than 5% between independent measurements.

The tapes were scored by an observer who counted with electromechanical timers, the duration of huddling exhibited to the target stimulus for each hour of the 4-hr test; video tapes were scored with rapid playback (Alberts, 1978b). As in previous studies (Alberts, 1978b), huddling was defined as any body contact between the subject and the target stimulus, tail excepted.

Results and Discussion

The results of this experiment indicated that both an anesthetized rat and a heated, fur-covered tube are sufficient to elicit and maintain huddling in rat pups from 5 to 20 days of age. Figure 1 shows the total time spent huddling for each age group. Pups spent between 79.0% and 94.5% of the test huddling with the exposed flank of an anesthetized prepuberal rat (average across ages = 87.4%). The heated furry tube similarly attracted the pups, which huddled with the inanimate target stimulus for 82.8%–97.7% of the test session, together averaging 92.5%.

Performance differences, related to the

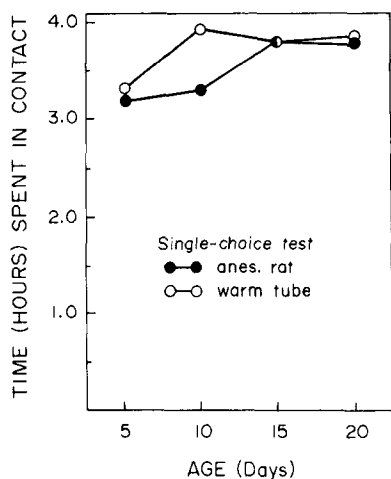


Figure 1. Median time spent in contact with either an anesthetized prepuberal rat or a warm, fur-covered cylinder in a standardized single-choice test of huddling ($n = 8$ for each age group).

contrasting size and locomotor ability in the age groups, were minimized with the present methods. Although Kruskal-Wallis one-way analysis of variance showed age effects for huddling with the conspecific stimulus ($H = 15.0, p < .005$) and with the inanimate stimulus ($H = 8.3, p < .05$), Mann-Whitney U tests indicated that the time spent huddling with these two stimuli did not differ for any of the age groups ($U_s = 37, 48, 35$, and $39, p_s > .10$). The results indicate that both target stimuli tested in this experiment are highly attractive for huddling in the 5–20-day-old rat. The equivalence of the values obtained in the two test conditions suggests that the warm furry tube and anesthetized rat may be about equally attractive to the pups. This is a rather remarkable result, implying that rat pups display no special attraction to conspecific stimuli. The possibility exists, however, that pups possess preferences for huddling but that the testing conditions of Experiment 1 were not sensitive to these differences. The huddling response may be so prepotent in pups, that any sufficient target will elicit vigorous huddling responses. Thus, in single-choice tests, such as those employed in Experiment 1, the pups' strong tendency to huddle may produce a ceiling effect, which precludes finding preferences reflected in quantitative differences in time spent huddling. Indeed, as can be seen in Figure 1, pups huddled for nearly the entire duration of the 4-hr test session.

There are many cues that may have attracted and maintained huddling in these situations. Both target stimuli were warm, which makes temperature cues likely candidates in these tests. It is possible that in addition to heat, the tactile characteristics of both stimuli were attractive (Alberts, 1978b). The two target stimuli used here undoubtedly differed in their olfactory properties, but again, this did not significantly attenuate the degree to which the two stimuli elicited huddling.

One cannot help but be impressed with the vigor and persistence of the huddling response found under such atypical testing conditions. The reliability of the pups' response in the test conditions employed here provide a standardized testing situation in

which the behavior of 5–20-day-old rats can be compared.

Experiment 2

The present experiment employed a simultaneous two-choice procedure in which rat pups had available two distinct target stimuli with which to huddle. Each target stimulus was known to be a sufficient stimulus for huddling; in the previous experiment these stimuli had elicited vigorous and equivalent amounts of huddling. By simultaneously presenting a warm furry tube and an immobile conspecific, it was hoped that the relative attractiveness of the stimuli could be assessed.

Method

Subjects. A total of 32 Sprague-Dawley rats, reared in the manner described earlier, served as subjects. In this experiment, as in the experiments to follow, the number of subjects reported refers to the number of pups that satisfied the criteria for inclusion in the analysis, as described below. Pups were tested at 5, 10, 15, or 20 days of age ($n = 8$ for each age). No more than two pups from any litter were used in each age group.

Procedure. The testing chambers were the same as those described in Experiment 1 except that both ends of the rectangular cage contained a window allowing access to a target stimulus. The heated, fur-covered tube protruded into the test chamber on one end, and a similar area of the flank of an anesthetized prepupal rat was presented at the opposite end of the cage.

The observational methods employed in the previous experiment were again used. Four-hour trials were observed by time-lapse video tape playback and were scored, with electromechanical timers, for the time spent in contact with each of the two stimuli.

Sessions began by placing the subject in the center of the test cage, equidistant from the two target stimuli and oriented 90° away from both of them. Because a preference measure was employed in this experiment, it was essential that the two available target stimuli be sampled by the subject so that a valid estimate of relative preference could be obtained. Only data from tests in which the subjects sampled (i.e., contacted) both stimuli during the first hour of the test were used. Sessions in which early sampling did not occur were discarded. With this criteria and the present testing conditions, it was necessary to discard about 20% of sessions with the 5-day-olds and 40% of the sessions with 10-day-olds. Older pups sampled more reliably, and all but 10% of the sessions were used. For each subject, the huddling preference for the rat was calculated by the following formula: Huddling preference for the conspecific stimulus = time spent huddling with the rat / (time spent huddling with the rat + time spent huddling with the tube) × 100.

Results and Discussion

Figure 2 presents a summary of the results of the present experiment, showing the proportion of the test session spent huddling by the pups with each of the two available target stimuli. It is clear from the figure that the two-choice method employed in this experiment revealed dramatic developmental trends in stimulus preferences for huddling. It can be seen that the warm furry tube was the stronger target stimulus for huddling in the 5-day-olds. During early life, certain stimulus complexes can be more attractive than those characteristic of the species. There was, however, an age-related decrease in the time spent huddling with the inanimate target and a concomitant increase in huddling with the conspecific stimulus.

Expressed in terms of the preference ratio for the conspecific (rat) stimulus, formulated above, these data show that a preference for conspecific stimuli develops gradually in the rat pup. The median preference score for the conspecific stimulus in this study was only 25% in the 5-day-olds, indicating that these pups preferred contact with the warm furry tube to contact with an anesthetized rat. By 10 days of age, however, the inani-

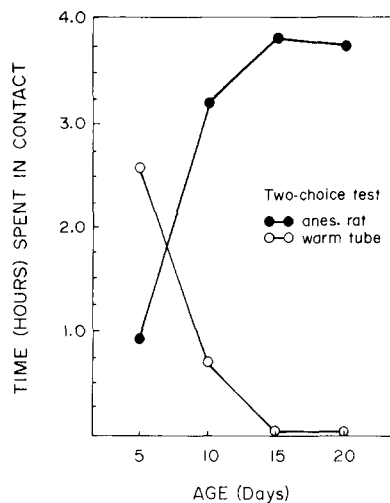


Figure 2. Median time spent in contact with an anesthetized rat and a warm, fur-covered tube in a standardized two-choice test of huddling preference ($n = 8$ for each age group).

mate target decreased considerably in potency, and the rat was the preferred huddling target (see Figure 2). The preference for the conspecific stimulus over the inanimate target appeared to reach its maximum value in these tests, with preference scores of 98.8% and 98.5% for the 15- and 20-day-olds, respectively. The change in preference for conspecific stimuli was tested with the Kruskal-Wallis one-way analysis of variance and was significant ($H = 17.8, p < .005$).

The developmental shift from one stimulus complex to another suggests that the cue(s) presented by the test stimuli change in the degree to which they control the behavior of the pups. Visual cues probably play an unimportant role in the determination of the huddling preferences studied here since the 10-day-olds, whose eyes had not yet opened, had already left the inanimate tube in preference for the conspecific stimulus. It also seems unlikely that tactile differences between the target stimuli are of major consequence because results identical to those reported in the present experiment were obtained in a pilot study in which huddling preferences were measured between an anesthetized rat and a warm but smooth (vinyl) surfaced cylinder.

Olfactory and temperature differences between the two target stimuli may account for the ontogenetic shift in huddling preference observed in this experiment. Direct measurement of the surface temperatures of the heated cylinder and the anesthetized rat indicated that although the two stimuli had equivalent surface temperatures at the beginning of the test, the body surface temperature of the anesthetized animal decreased by about 4 °C over the course of the 4-hr observation whereas the surface temperature of the electrically heated tube remained constant and warm. Thus, although previous data indicate that heat cues are generally sufficient to elicit and maintain huddling in rat pups (Experiment 1; Alberts, 1978b; Cosnier, 1965), it may be that these cues decrease in their attractiveness as the pups' thermogenic capabilities improve (Conklin & Heggenes, 1971). Details on the development of the rats' olfactory sense are lacking, although there is some evidence that during the early postnatal period the pups

behavior may come under increasingly wide olfactory influence (see Alberts, 1976, for a review and discussion of this problem). Experiments 3 and 4 were designed to examine the relative salience of olfactory and temperature cues in determining the huddling preferences of developing rat pups.

Experiment 3

The purpose of Experiment 3 was to examine further the development of huddling preferences in rat pups. We were concerned with the interpretive problems posed by the temperature differences between the huddling stimuli in the previous experiment and sought to test the pups with two huddling targets that had similar thermal properties. If temperature cues determined the 5-day-olds' huddling bias towards the heated cylinder in Experiment 2, then thermally guided pups should not manifest a preference between two stimuli that differ in their nonthermal properties but have the same surface temperature. Furthermore, it was tentatively suggested in Experiment 2 that the conspecific preference displayed by the older pups may have been mediated by the species smells of the anesthetized rat or by the odors of the inanimate, heated tube.

The present experiment was designed to address both of these issues. Pups were again tested in a two-choice preference situation. The huddling stimuli consisted of an anesthetized prepuberal rat and an anesthetized adult Mongolian gerbil. The body surface temperature of anesthetized like-size rats and gerbils are similar; rate of heat loss was also found to be approximately equivalent. Thus, the rat-versus-gerbil test provided two huddling stimuli that did not differ in temperature, possessed similar tactile (furry) qualities, but had different olfactory characteristics. There was no way to ascertain whether the smell of the heated cylinder or the gerbil differed more in comparison with the odor of the anesthetized rat. Nevertheless, the gerbil-versus-rat preference test involved two biological odors in normal concentration and thus represented a novel and useful preparation in this study.

Method

Subjects. A total of 64 Sprague-Dawley rat pups were used as subjects in this experiment. Sixteen pups, no more than two from one litter, were tested at 5, 10, 15, and 20 days of age. Rearing conditions were as described in the earlier experiments.

Procedure. The procedure was the identical simultaneous two-choice test used in Experiment 2 except that the huddling stimuli in the present study consisted of a prepuberal rat (as in the previous experiment) and a like-size adult Mongolian gerbil (*Meriones unguiculatus*). The target animals were both anesthetized with urethane (250 mg/kg, ip) and were presented through calibrated openings in the test cages as described in the previous experiment. Observational methods, test length, and scoring procedures were identical to those described above. Again, approximately 20% of the tests with 5-day-old subjects were discarded prior to analysis, and (in these tests) 50% of the 10-day-olds also failed to sample and were therefore not used. Sampling was more reliable in the older groups, and 90% of the animals used were included in the sample.

Results and Discussion

The 5- and 10-day-old rats failed to show a huddling preference between the anesthetized conspecific and the anesthetized gerbil, whereas older pups displayed a strong preference for huddling with the conspecific. Figure 3 shows the amount of time spent in contact with each of the two huddling stimuli in this experiment. As can be seen in the

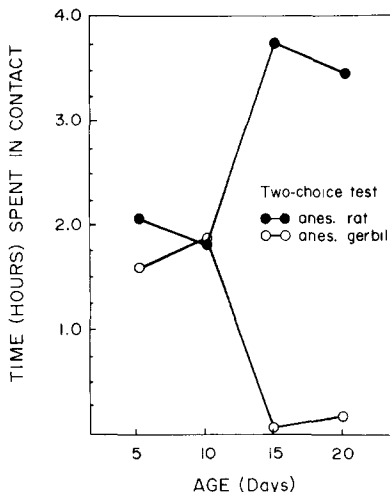


Figure 3. Median percentage of time spent in contact with an anesthetized prepuberal rat and a like-size anesthetized adult gerbil in a two-choice test of huddling preference ($n = 16$ for each age group).

figure, 5- and 10-day-olds huddled for more than 90% of the 4-hr test but were indifferent in their selection of targets. Both the rat and the gerbil were suitable for huddling, but neither was preferred. In striking contrast to the juveniles' indiscriminate affiliations, the 15- and 20-day-olds were highly selective, each producing conspecific preferences above 90%.

The age effect for conspecific preference was significant ($H = 13.72, p < .005$), which indicates that the rat displays an age-related progression in selectivity of huddling behavior. Inspection of Figure 3 strongly suggests that the change occurs relatively abruptly, between Day 10 and Day 15.

In contrast to the results of Experiment 2, the 5- and 10-day-olds in the present experiment showed no preference between the two huddling stimuli. With temperature differences eliminated, the preferences of the younger groups disappeared (compare Figures 2 and 3), which suggests that the neonates' behavior may, in fact, be dominated by thermal cues. By Day 15, a strong conspecific preference appeared, which lends support to the suggestion made previously that with age, the role of olfactory cues may come to guide, if not dominate, the rats' huddling preferences. However, the emergence of olfactory control of huddling is, at best, only inferential from these data. The following experiment directly examines this possibility.

Experiment 4

Weanling rat pups (15–20 days) prefer to huddle with an immobile conspecific rather than other suitable stimuli (Experiment 2 and 3). It was suggested that species odors mediate the weanling pups' preference. Huddling preferences of anosmic (zinc sulfate treated) and control pups were therefore studied with the two-choice test of Experiment 2.

Method

Subjects. Sixteen 15-day-old and sixteen 20-day-old pups were used as subjects in this experiment. The standard rearing conditions were maintained. Pups from more than 10 litters were used; experimental and control pups were taken from the same litters.

Procedure. Olfactory disruption was induced by a single intranasal treatment with zinc sulfate. Several laboratories have used intranasal zinc sulfate as a means of inducing an olfactory deficit in rat pups (Alberts, 1976, 1978b; Hofer, 1976; Singh, Tucker, & Hofer, 1976).

Experimental animals received an intranasal infusion of isotonic zinc sulfate (7.65%) under ether anesthesia. The pup's mouth was thoroughly aspirated before and after injection of the solution. Solutions were administered via the posterior choanae, as described by Alberts and Galef (1971). The hooked catheter (25 × 35 mm) used for the rat pups was fashioned from a 23-ga. syringe needle. Control animals received identical treatments with normal saline. The solutions were dyed with food coloring to facilitate visibility.

Intranasal treatments were administered when the pups were either 14 or 19 days of age, the day before testing. After treatment, experimental and control rats were housed with a nonlactating but maternally responsive female rat (Rosenblatt, 1967). Thus all subjects received maternal warmth and tactile stimulation during the interval between treatment and testing (13–18 hr), and nutritional differences related to nursing deficits for the experimental pups (Alberts, 1976; Hofer, 1976) were eliminated.

After olfactory testing, anosmic pups and littermate controls were tested simultaneously in a huddling preference test. The test regimen used in this experiment was identical to that of Experiment 2. Subjects were given a 4-hr huddling preference test in a standardized compartment that contained two target stimuli: the flank of an anesthetized rat at one end and a similar-size portion of a heated, fur-covered cylinder at the other. Each session was videotaped and scored in the manner described above. Data are again expressed in terms of the ratio score of preference for the conspecific huddling stimulus.

Test for anosmia. Each subject was given a test of olfactory-guided orientation immediately prior to the experimental session proper. Gregory and Pfaff (1971) and others (Altman, Brunner, Bulut, & Sudarshan, 1974) have reported that rat pups reliably orient and approach the soiled nesting material of the home cage. Elimination of the pup's normal preference for the soiled nest shavings of the home cage, over clean woodshavings, was taken as the criterion for defining the olfactory deficit. Animals lacking olfactory-guided home orientation are termed "anosmic," although this term refers only to their tested indifference to nest odors.

The home-orientation test consisted of placing each pup individually in the center of a 30 × 18 × 15 cm plastic box, the floor of which was covered on one side with soiled woodshavings taken from the pup's home cage and on the other side with clean pine shavings. A 2-cm boundary between these two sides was devoid of shavings. After an initial 30-sec period for habituation, a 1-min trial began. Time spent entirely on each side of the cage (soiled and fresh) was recorded. Each pup was given four such trials for a home-orientation test.

Pups defined as anosmic in this experiment spent more than 30% of the orientation test on the side of the test cage blanketed with clean shavings. Animals that displayed labored breathing and congestion of the air-

way passages or appeared debilitated after intranasal treatment were not used. Pups spending less than 85% of the orientation test on the side of the cage with the home shavings were discarded from the control group. Approximately 30% of the pups were disqualified from the analyses.

Results and Discussion

Rat pups rendered anosmic by intranasal zinc sulfate treatment did not display the strong huddling preference for a conspecific shown by their littermate controls. The histograms in Figure 4 depict the data in terms of the conspecific preference score. As can be seen, the control pups in both age groups strongly preferred to huddle with the flank of the conspecific, which indicates that only a small fraction of the test sessions was spent in contact with the heated cylinder. In contrast, the experimental animals were relatively indiscriminate in their huddling behavior and spent similar amounts of time with each of the two stimuli.

The 15-day-old control animals spent a total of 3.1 hr in contact with the anesthetized rat and .28 hr huddled with the warm tube, which yields a conspecific preference score of 91.4%, as shown in Figure 4. Their anosmic littermates, however, spent 2.0 and 1.8 hr huddling with the rat and tube, re-

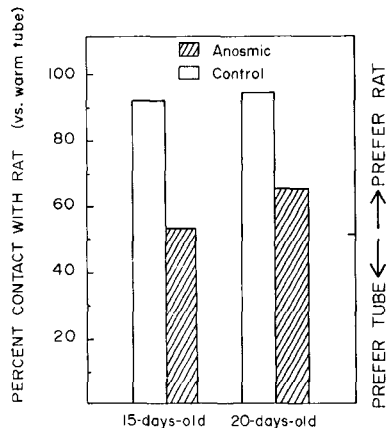


Figure 4. Median percentage of time spent in contact with an anesthetized rat in a two-choice test of huddling preference. (The hatched bars show the preference scores of zinc sulfate treated [anosmic] pups. The open bars show the huddling preference of the saline-treated littermate controls [ns = 8 for each group at each age after test for anosmia].)

spectively, which yields a 52.0% preference score and is indicative of no preference. The 20-day-old group presented a similar picture. The difference in conspecific preference between the experimental and the control animals was significant ($U_s = 52$ and 51 , $p < .05$) for each age group.

Although intranasal zinc sulfate treatment clearly disrupted the normal huddling preferences by weanling rat pups, it did not diminish their huddling behavior per se. Fifteen-day-old control pups spent nearly all, 96.7%, of the test session in contact with the available stimuli, and the anosmic littermates performed similarly, spending 94.1% of the test session engaged in huddling. The 20-day-old experimental and control pups exhibited the same correspondence in total huddling time, spending 98.9% and 88.3% of the 4-hr test engaged in contact behavior, respectively. Thus, the effects of anosmia on huddling appear to be on the specificity of its direction, not on the expression of the behavior.

The reader may note an apparent discrepancy between the maintenance of normal levels of huddling by anosmic pups described in the present experiment and the finding in an earlier study that intranasal zinc sulfate treatment disrupts huddling by rat pups (see Alberts, 1978b, Figure 3). The location of the huddling stimuli in the test apparatus accounts for the difference in the results. When an anesthetized age-mate is placed in the center of a test arena, away from the walls and corners of the cage, anosmia can drastically reduce huddling. When attractive huddling stimuli are located along a wall, however, the addition of thigmotactic and contextual cues (Alberts, 1978b) obviates the disruptive effects of anosmia on the maintenance of huddling. In the present experiment, two huddling targets were present along the walls of the test cage, and the abundant nonolfactory cues were sufficient to maintain robust levels of huddling.

The major implication of the present experiment is that anosmia, or at least an olfactory disruption sufficiently severe to eliminate attraction to the smells of the home nest, also eliminates the weanlings' attraction to conspecific stimuli. This

finding strongly suggests that the normal attraction is mediated by olfaction and that the ontogenetic emergence of huddling preferences for rats, compared with other warm furry objects, is linked to the development of olfactory domination of behavior. In the absence of normal olfactory input, the rat pup loses specificity in its huddling choices.

General Discussion

The present series of experiments examined the ontogeny of the sensory control of huddling in rat pups during the first 3 wk of development. Of the multitude of cues that affect the huddling behavior of rat pups, heat and olfactory stimuli were specifically investigated. Standardized huddling targets that elicited equivalent amounts of contact behavior from 5- to 20-day-old rats when presented singly differed dramatically in attractiveness when a simultaneous two-choice preference test was used. A consistent pattern of results emerged. Younger pups primarily respond to thermal cues, whereas by Day 15 the salience of olfactory input surpasses thermal properties of a potential huddling stimuli. Weanling rat pups huddle with a conspecific in preference to a stimulus lacking rat odors and in preference to a similar, living target possessing the biological odors of a different species (Experiments 2 and 3). Zinc sulfate induced anosmia disrupted the typical age-related changes in huddling preference (Experiment 4). These experiments characterize the early ontogeny of huddling in the rat as consisting, in part, of a sequential transition in the cues that most powerfully govern the behavior. The transition is from thermal to olfactory dominance.

These findings support and extend Cosnier's (1965) initial appraisal of the ontogenesis of huddling, as well as similar interpretations offered tentatively from this laboratory (Alberts, 1978b). Cosnier suggested that thermal control of huddling begins to diminish by Days 8–10. His analysis, though recognizing the waning of thermal controls, did not reveal the olfactory components of huddling that were found in the present work.

Several experimenters have suggested that during infancy, huddling by the ectothermic pups is primarily a physiological response but later in life, as body temperature regulation becomes a more independent process, huddling becomes primarily a social activity (Cairns, 1972; Cosnier, 1965; Jeddi, 1971; Rosenblatt, 1976). The results of the present series of experiments are in concordance with this view. We suggest that the early postnatal ontogenesis of contact behavior in the rat can be characterized as a change from "physiological" to "filial" huddling. The transition from physiological huddling to filial huddling can be defined in terms of changes in the sensory control of the behavior. Physiological huddling is performed as a biological adjustment, activated by an immediate homeostatic need and directed at immediately relevant proximate stimuli, namely, sources of heat. In contrast, filial huddling is directed by non-physiological cues; the stimuli at which it is directed are representative of social affiliation. For the rat, species-typical olfactory cues assume an important eliciting or directing function for the more ontogenetically advanced huddling response.

The distinction between physiological and filial huddling is not meant to imply that with age the rat becomes emancipated from either the attractive qualities of heat cues or the thermoregulatory consequences of clumping. Nor does this perspective ignore the fact that under normal conditions thermal cues arise from social stimuli. Our interpretation emphasizes that the infant rat is indifferent to nonthermal qualities of warm stimuli whereas the older pup is not. By Day 15 the rat pup is highly influenced by the nonthermal characteristics of warm huddling cues.

Gottlieb (1971) recognized basic sequential patterns in the development of physiological activity in sensory systems. It is tempting to postulate that the thermal-to-olfactory transition in the sensory control of huddling simply reflects a sequence of functional maturation of the afferent systems carrying the relevant signals. From this perspective, one might predict that olfaction first becomes functional during the second postnatal week, paralleling the ap-

pearance of olfactory-guided huddling. Recent research, however, does not support this parsimonious view. Thermal sensitivity is present in the rat at birth, and there are now several lines of evidence indicating that olfaction may also be operable in the neonate (see Alberts, 1976). Perhaps the most forceful example of this is Teicher and Blass's (1977) recent work which indicates that olfactory input plays a crucial role in suckling within hours of birth. It is clear that the beginning of measurable olfactory involvement in huddling is not coincident with the onset of olfactory function *per se*.

The present results fit closely to a general ontogenetic pattern recently proposed by Rosenblatt (1976). According to his descriptive scheme, based on a survey of a variety of altricial species, there are sequential stages of behavioral development which can be characterized by the stimuli that activate and orchestrate behavior. Rosenblatt postulated that the first two stages are marked by thermotactile and olfactory controls, respectively, and the present findings serve as an illustrative example of his suggested principle. It should be noted, however, that our results do not suggest that the role of heat cues diminishes in the control of huddling. Rather, there appears to be an ontogenetic reorganization of sensory control in which olfactory cues gain in relative salience while thermal stimuli remain sufficient and highly attractive.

Many fundamental questions, crucial to understanding the ontogeny of huddling, are raised by the present investigation. Species odors gradually emerge as a determinant of huddling and by Day 15 appear to surpass heat cues in relative salience for the pup. It is not known what odor or odors are attractive to the pups, although neither smells of the home nest and litter nor a maternal odor is necessary. The specificity of the filial response is also unknown, except that pups can discriminate biological odors of different rodent species. Future analyses should examine the developmental process that produces huddling preferences for conspecifics. This process may be simply maturational, dependent on the emergence of predetermined peripheral or central detectors. As an alternative, conspecific preference could

be acquired during early life. Experience-dependent mechanisms might be governed by rules of associative learning or some other process of exposure familiarity, such as that recently demonstrated for odor-approach cues by Leon, Galef, and Behse (1977) in which mere exposure to the smell of peppermint, in the absence of conventional reinforcers, rendered that odor attractive to rat pups.

References

- Alberts, J. R. Olfactory contributions to behavioral development in rodents. In R. L. Doty (Ed.), *Mammalian olfaction, reproductive processes, and behavior*. New York: Academic Press, 1976.
- Alberts, J. R. Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology*, 1978, 92, 231-245. (a)
- Alberts, J. R. Huddling by rat pups: Multisensory control of contact behavior. *Journal of Comparative and Physiological Psychology*, 1978, 92, 220-230. (b)
- Alberts, J. R., & Galef, B. G., Jr. Acute anosmia in the rat: A behavioral test of a peripherally-induced olfactory deficit. *Physiology and Behavior*, 1971, 6, 619-621.
- Altman, J., Brunner, R. L., Bulut, F. G., & Sudarshan, K. The development of behavior in normal and brain-damaged infant rats, studied with homing (nest-seeking) as motivation. In A. Vernadakis & N. Weiner (Eds.), *Drugs and the developing brain*. New York: Plenum Press, 1974.
- Barnett, S. A. *A study in behaviour*. London: Methuen, 1963.
- Cairns, R. B. Fighting and punishment from a developmental perspective. J. K. Cole & D. D. Jensen (Eds.), In *Nebraska Symposium on Motivation* (Vol. 20). Lincoln: University of Nebraska Press, 1972.
- Calhoun, J. B. *The ecology and sociology of the Norway rat*. Bethesda, Md.: Department of Health, Education, and Welfare, 1962.
- Conklin, P. & Heggenes, F. W. Maturation of temperature homeostasis in the rat. *American Journal of Physiology*, 1971, 220, 333-336.
- Cosnier, J. *Le comportement du rat d'élevage*. Unpublished doctoral dissertation, University of Lyon, France 1965.
- Gottlieb, G. Ontogenesis of sensory function in birds and mammals. In E. Tobach, L. R. Aronson, & E. Shaw (Eds.), *The biopsychology of development*. New York: Academic Press, 1971.
- Gregory, E., & Pfaff, D. W. Development of olfactory-guided behavior in infant rats. *Physiology and Behavior*, 1971, 6, 573-576.
- Hofer, M. A. Olfactory denervation: Its biological and behavioral effects in infant rats. *Journal of Comparative and Physiological Psychology*, 1976, 90, 829-838.
- Jeddi, E. Confort du contact et thermoregulation comportementale. *Physiology and Behavior*, 1970, 5, 1487-1493.
- Leon, M., Galef, B. G., Jr., & Behse, J. Establishment of pheromonal bonds and diet preference in young rats by odor pre-exposure. *Physiology and Behavior*, 1977, 18, 387-392.
- Rosenblatt, J. S. Nonhormonal basis of maternal behavior in the rat. *Science*, 1967, 156, 1512-1514.
- Rosenblatt, J. S. Stages in the early behavioural development of altricial young of selected species of non-primate mammals. In P. P. G. Bateson & R. A. Hinde (Eds.), *Growing points in ethology*. London: Cambridge University Press, 1976.
- Singh, P. J., Tucker, M., & Hofer, M. Effects of nasal ZnSo₄ irrigation and olfactory bulbectomy on rat pups. *Physiology and Behavior*, 1976, 17, 373-382.
- Steiniger, F. von Beiträge zur Soziologie und sonstigen Biologie der Wanderratte. *Zeitschrift für Tierpsychologie*, 1950, 7, 356-379.
- Teicher, M. H., & Blass, E. M. The role of olfaction and amniotic fluid in the first suckling response of newborn albino rats. *Science*, 1977, 198, 635-636.
- Telle, H. J. Beitrag zur Kenntnis der Verhaltensweise von Ratten, vergleichend dargestellt bei *Rattus norvegicus* und *Rattus rattus*. *Zeitschrift für angewandte Zoologie*, 1966, 53, 129-196.
- Whittow, G. C. (Ed.) *Comparative physiology of thermoregulation* (Vol. 1). New York: Academic Press, 1971.

Received October 4, 1977 ■