

Huddling by Rat Pups: Group Behavioral Mechanisms of Temperature Regulation and Energy Conservation

Jeffrey R. Alberts
Indiana University

Body heat loss was attenuated and oxygen consumption was reduced by huddling in litters of developing rats. Rat pups derive physiological benefits from huddling similar to those enjoyed by adult mammals; these findings contrast with previous characterizations of the altricial rat as poikilothermic. Huddling insulates by lessening the exposed body surface area of the participants, thus retarding heat loss and enhancing the efficiency of thermogenesis. These physical mechanisms of the clump are actively regulated by the pups. A novel quantitative measure of huddle size revealed a form of group regulatory behavior in rat pups whereby the surface expanded and contracted with increases and decreases in ambient temperature. The individual basis of this group regulatory activity was investigated by marking individual pups and observing them in huddles by means of time-lapse videography. It was found that individual animals circulate through the huddle, frequently exchanging locations in the group. By studying the huddle positions of an anesthetized pup and a marked control sibling, dynamics of the pup flow were clarified. Ordinarily, the direction of movement was actively downward, into the pile; immobile pups "floated" on the surface. When the nest temperature was raised to thermoneutral, the direction of pup flow reversed and an immobile animal sank to the depths of the huddle. Through individual competitive adjustments the huddle behaves as a self-regulating unit which provides warmth and insulation to all its active members.

From birth, the rat is a member of a social group, viz., the litter and mother. Interaction between rat pups initially consists of huddling behavior. Broadly defined,

huddling is the behavior that leads to the formation and maintenance of the litter aggregate (Alberts, 1978) and, similarly, produces the social clumps of adult rats observed under group-living conditions (Barnett, 1963; Calhoun, 1962; Steineger, 1950).

Most of this research was supported by Grant MH28355-01 from the National Institute of Mental Health to the author. Experiment 1 and portions of Experiment 3 were submitted as part of a doctoral dissertation to the faculty of Princeton University and were supported by a grant from the National Institute of Mental Health to Byron A. Campbell and a predoctoral fellowship from that agency to the author. The oxygen analyzer and laboratory space for Experiment 2 were generously made available by Henry D. Prange and David Robertshaw, Department of Physiology, Indiana University. The careful and creative assistance of Brad May made the metabolic experiments possible. I would particularly like to thank B. A. Campbell, J. C. Craig, M. I. Friedman, D. J. Howell, B. G. Galef, C. G. Mueller, P. K. Randall, and C. S. Sherrick for their support during various phases of the research.

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

Huddling is the major behavioral activity of the infant rat. Pups exhibit vigorous and persistent huddling behavior with a variety of animate and inanimate stimuli. Thermal, olfactory, photic, and several kinds of tactile cues all play a demonstrable role in the expression of huddling by rat pups (Alberts, 1978; Cosnier, 1965).

There are many functions clumping might serve during early life. In the wild, rat pups are born and reared in special burrows that are defended by the mother against rats and other intruders (Calhoun, 1962). Clumping, or any behavior that confines the pups to the maternal nest, would therefore aid in protection from predation and permit the dam

to leave the nest to forage. By keeping the pups together and easy to localize during the mother's periodic visitations, huddling may also increase the efficiency of parental investments, such as nursing. In adult animals at least, huddling is widely considered to be an important behavioral adjustment to cold temperatures (Whittow, 1971).

Despite the prominence of huddling in the life history of the rat and its broad phylogenetic representation (see Whittow, 1971, 1973), little is known about the development or the significance of the behavior. Cosnier (1965) concluded that gregariousness in infant rats is primarily a thermotactile response. Welker (1959) and Jeddi (1970) reached similar conclusions from their observations of neonatal dogs. Cosnier recognized the contribution of huddling to homeostatic functions in the fragile neonate and suggested that grouping is an important condition for survival and early development in the altricial rat.

Two of the experiments reported below were designed to examine the effect of huddling behavior on two measures of physiological function in rat pups: body temperature and oxygen consumption. In addition, three experiments are described which studied the behavioral dynamics of the group itself and of the individuals in the group during huddling.

Experiment 1

As noted before, huddling among adult animals is frequently considered to be an adaptive social defense to cold (Whittow, 1971). Many species of animals abandon solitary habits and live in close association with conspecifics during the colder months (Hart, 1971). Huddling attenuates convective heat loss by reducing the exposed surface area of an animal. Moreover, in furred or feathered forms, the behavior provides each participant with a localized area of thicker insulation. In addition, huddling with other warm bodies reduces heat loss by conduction to colder surfaces.

The parameters of body temperature regulation differ between immature rats and adults. For its size, the infant rat produces less and loses more body heat than does the

adult (Taylor, 1960). Thermogenesis by shivering is absent in the infant, and the limits of metabolic heat production are below that of the adult. Heat loss is rapid in pups because they lack insulative fur and subcutaneous fat and cannot exert control over vascular flow (Hull, 1973). Moreover, juveniles have a relatively greater heat-losing surface area than adults.

Together, these morphological and physiological attributes leave the rat pup with thermoregulatory capabilities so narrow that its body temperature tends to approximate that of the environment (Hahn & Koldovsky, 1966). As a result, many writers have termed the infant rat "poikilothermic," thereby likening the process of body temperature regulation in this small mammal to that of a reptile. The question can be raised, then, whether huddling can make a quantitatively significant contribution to body temperature defense to the rat pups when they lack the thermogenic and heat-conserving capabilities of homeothermic adults. Experiment 1 was performed to evaluate the effect of huddling on rectal temperature in rat pups of various ages.

Method

Subjects. A total of 96 rat pups were tested. Pups were tested only once each, at 5, 10, 15, or 20 days of age. All pups were born in the Princeton University colony, descendants of Sprague-Dawley rats from the breeding population, or were derived from adult rats purchased from Camm Research, Inc. Three days after birth (Day 0), litters were uniformly reduced to eight pups each and otherwise left undisturbed with the mother in standard plastic maternity cages. Purina Laboratory Chow and water were continuously available. Twenty-four pups were used in each age group.

Procedure. Colonic temperatures were measured to the nearest .10 °C with a Shulteis mercury thermometer. The bulb of the thermometer was sufficiently small to be accommodated by the youngest pups and was inserted to a depth of 10 mm. The thermometer's rapid rise time (hence short insertion period) was advantageous in minimizing stress-induced hyperthermic reactions.

Litters were removed from the home cage and initial rectal temperatures were recorded. Pups were marked for individual identification and then placed in Plexiglas compartments carpeted with home cage shavings and topped with hardware cloth. Four pups from each litter were housed singly (isolates) and four pups were kept together in a single compartment (huddlers). Temperature measurements were made hourly on each pup for the next 4 hr.

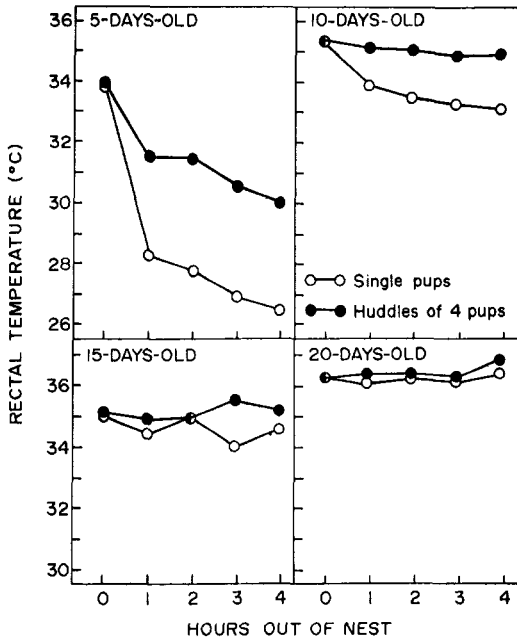


Figure 1. Rectal temperatures of rat pups at 24 °C in huddles of four siblings (closed circles) or alone (open circles). (The $n = 12$ for each condition at each age.)

The ambient temperature ranged from 23 to 24 °C. Relative humidity was not controlled but was monitored with a hygrometer and ranged from 40% to 60%.

Results and Discussion

The initial colonic temperatures of the pups did not differ, but after 4 hr out of the nest, the rectal temperatures of the isolated 5-, 10-, and 15-day-old pups were significantly below those of the littermates left in clumps of four (Mann-Whitney U test, two-tailed, p s = .000, .038, .006, respectively). The colonic temperatures of 20-day-old isolates, on the other hand, did not differ significantly from their huddling siblings after 4 hr ($p = .253$). Figure 1 depicts the median rectal temperatures of the pups for each hour of the experiment.

It can be seen in Figure 1 that huddling attenuated the rate of temperature loss in an age-related manner. Significant ($p < .05$) differences in rectal temperature between grouped and singly housed pups were found after the first hour out of the nest in the 5- and 10-day-olds. Fifteen-day-old isolates, however, did not have significantly lower

colonic temperatures until the third hour of the experiment. The 20-day-old pups (both the singles and huddlers) successfully defended their initial rectal temperatures for the entire 4-hr duration of the test period.

The 15- and 20-day-old pups did not show the same dramatic body temperature decreases as did the younger subjects. These data are entirely consistent with general descriptions of the ontogeny of thermoregulation in altricial mammals (Barnett & Mount, 1971; Hart, 1971; McCance, 1959) and with Cosnier's (1965) findings on surface temperature changes in isolated and grouped pups. During development, heat production ability increases, heat loss decreases with increasing size, and the insulation value of the pelage improves. The temperature challenge used here was probably not sufficiently severe to see a robust huddling effect in the older pups. In a pilot study (Alberts, 1973, unpublished), however, huddling in a very cold (10 °C) environment enabled 15- and 20-day-olds to defend their colonic temperatures more effectively than singleton littermates.

Huddling appears to provide the rat pup with an effective behavioral means of reducing loss of body heat and thus combating cold challenge. It is not possible to extend interpretation of these data to more natural conditions or even to estimate the extent to which huddling can augment temperature regulation in the litter situation. The conditions used in this experiment were less than optimal for the pups; very small clumps were used and the insulation of the nest was eliminated. Nevertheless, even the neonates derived marked thermoregulatory benefits from huddling with small numbers of other furless, rapidly cooling siblings.

Experiment 2

Rectal temperature is a useful but limited measure of temperature regulation. Although rectal temperature is often interpreted as an "average" body temperature, it is not representative of the organism as a whole and remains a regional temperature measurement. Moreover, while rectal temperature may yield information on the consequences of an organism's thermal re-

sponse, it does not reflect the strategy used. Body temperature is the difference between the amounts of heat produced and heat dissipated. Two animals may manifest equivalent body temperatures, but one may be losing heat more rapidly and therefore exert a greater metabolic effort to maintain the same colonic temperature.

A more direct measure of thermogenic effort is found in metabolic rate. Oxygen consumption (VO_2 : volume of oxygen consumed/body weight/time) is a standard technique for determination of metabolic rate and can be used to quantify thermogenesis. Homeotherms increase their metabolic rate in cold temperatures; thus, their rate of VO_2 is lower in warm temperatures, reaching its minimum in the range defined as the zone of thermoneutrality. In true poikilotherms, however, metabolism varies directly with ambient temperature and there is no zone of thermoneutrality.

Because the rectal temperature of a juvenile rodent is likely to decrease precipitously in a cold environment, the pup is often termed "poikilothermic" (Fairfield, 1948; Fowler & Kellogg, 1975). Taylor (1960) showed, however, that neonatal rats increase their metabolic heat production by 100% if the temperature challenge is not too severe to combat. Classifying the neonatal rat as "poikilothermic" is not accurate and can be seriously misleading (Hull, 1973).

Huddling behavior has been implicated as a behavioral means of reducing metabolic expenditure in adult homeotherms. Adult mice permitted to huddle in cold environments survive longer than singly housed mice (King & Connon, 1955; Sealander, 1952), presumably because huddling reduces metabolic expenditures (Howard, 1951; Pearson, 1960). Prychodko (1958) calculated that at -3°C , the nutritive energy requirement of a mouse in a huddle of five is reduced by almost 30%, a metabolic response equivalent to that of raising the ambient temperature 11°C .

The present experiment investigated whether huddling could conserve metabolic energy in rat pups. It was possible that the results of the previous rectal temperature experiment told only part of the story; even the "poikilothermic" neonates, whose body

temperatures dropped dramatically, may have been making metabolic responses not apparent from their colonic temperatures. Past research, using various measures of metabolic rate, has been inconclusive. Taylor (1960) found only meager differences in oxygen consumption by grouped and isolated pups, but his animals were tested at thermoneutral or very cold (10°C) temperatures. Cosnier (1965) reported that huddling reduced oxygen consumption by rat pups aged 1–20 days, at either 21 or 32°C . More recently, Bryant and Hails (1975) suggested that "before homeothermy is fully developed" in mice (*Mus musculus*), huddling may actually increase oxygen consumption. Their measures, however, appeared to be confounded by movement artifacts in the respirometer. The question of the metabolic role of huddling in juveniles therefore remains open. The study reported below utilized a procedure and an apparatus well suited to accurate measures of metabolic responses in neonatal rats.

Method

Subjects. A total of 96 Sprague-Dawley pups (12 litters) were used in this experiment. The litters were born in the Indiana University colony and were derived from a line of rats initially purchased from Laboratory Supply Inc., Indianapolis. The breeding and rearing procedures were as described in the previous experiment.

Apparatus. The respiratory chambers were constructed from clean commercial paint cans. These inexpensive containers are sturdy but malleable, are water tight, and with the use of stopcock grease, can be repeatedly opened and resealed. Hardware cloth baskets (1 cm mesh) approximately 2 cm smaller than the internal dimensions of the cans were constructed to prevent the animals from directly contacting the walls of the chamber. Legs, .5 cm in length, prevented contact with the floor. There were four such chambers made from 1-qt. (.95-l) cans and one chamber made from a 1-gal. (.004- m^3) can. Each lid was fit with two pipes (3.5 mm ID) which served for air inlet and exhaust. Air entered near the base of the chamber through the longer pipe and was exhausted from the shorter effluent near the ceiling. A mercury thermometer was also sealed into each chamber lid.

The small, 1-qt. chambers were used to test single pups and huddles of two. Huddles of four and eight were run in the larger, 1-gal. can. Another respiratory chamber, adapted from a 5-gal. (.02- m^3) commercial aquarium was fit with a Plexiglas lid and arranged internally in a manner similar to the cans described above. This chamber permitted subjects to be directly observed. The results of this experiment using the two

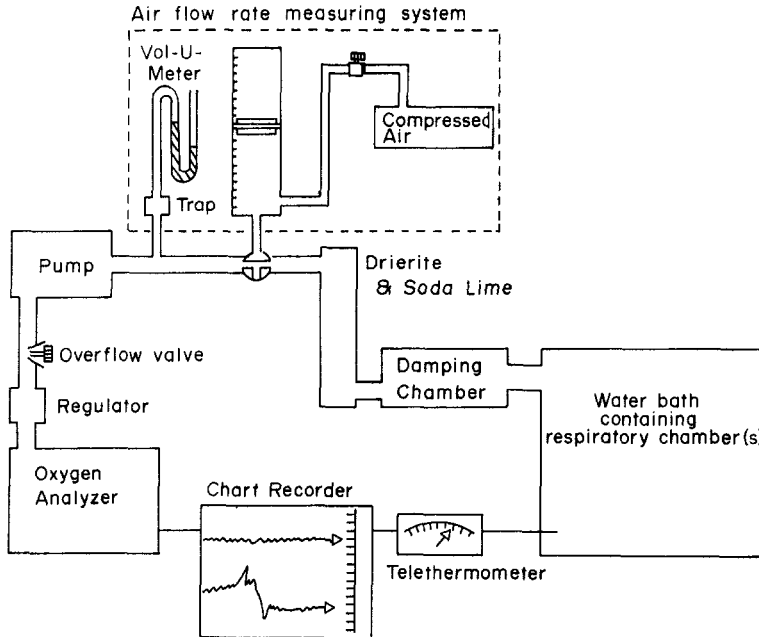


Figure 2. System for analysis of oxygen consumption.

types of chambers were the same, so no distinction is made between them in the discussion below. The testing chambers were submerged to within 5 cm of their top in a temperature-controlled water bath. Air temperature in the chambers was either 28 or 30 °C, as described below.

The system used to measure oxygen consumption is depicted in Figure 2. A Neptune Dyna-pump drew atmospheric air through the respiratory chambers and into a damping chamber which also served as a manifold and water trap. Total flow rate, divided among the five respiratory chambers or through the larger glass chamber, was maintained at 1,200 ml/min. Still under negative pressure, the air passed through a column of desiccant (Drierite) and CO₂ absorbant (soda lime).

The air flow rate-measuring system consisted of a 250-ml displacement flowmeter (Vol-U-Meter, Brooks Instrument Div., Emerson Electronics), a water manometer, needle valves, and a source of compressed air. The calibration apparatus was connected to the gas-analyzing system by a T valve, as shown in Figure 2.

Air left the pump under positive pressure. An adjustable overflow valve was open on-line so that a constant flow of air, regulated at 5 in. (12.7 cm) of water (Matheson regulator) entered an Applied Electronics oxygen analyzer (Model S-3A, Sunnyvale, California), accurate to .001%. In addition to a digital display of percentage of O₂ entering the analyzer, the output was transcribed continuously onto a Linear Instruments (Irvine, California) stereo chart recorder. The full pen excursion of 26 cm, corresponding to 1.0% oxygen, yielded a high degree of sensitivity. A second channel on the chart recorder monitored the bath temperature by means of a Yellow Springs telethermometer.

Procedure. The entire system was calibrated at the

beginning of each experimental session. Air pressure and flow rate were adjusted with the displacement flowmeter and water manometer. After flushing the entire system with atmospheric air for 15 min, the oxygen analyzer and chart recorder were calibrated.

Rat pups were weighed individually and then placed in a single, large chamber as an intact litter ($n = 8$). When 5-day-old huddles were tested, the pups were placed in a glass finger bowl (11 × 5 cm). Pilot data and direct observation indicated that these neonates had difficulty maintaining a unified clump in the apparatus without a concave substrate. After a 15-min habitation period, VO₂ was recorded continuously for 75 min. The litter was then subdivided into two huddles of four pups each and tested similarly. Half of the litter remained with the dam while the other four pups were being tested. By subdividing again, clumps of two pups each and singletons were tested. Removable perforated partitions in the 5-gal. chamber allowed simultaneous testing of the entire litter, with the pups separated or in clumps of two, four, and eight. Litters were tested both in descending and ascending order of group size.

The 10-, 15-, and 20-day-olds were tested at 28 °C, a temperature below thermoneutral (Taylor, 1960) but not stressful to the pups. Five-day-olds performed erratically at this temperature in pilot studies, probably because the neonate's thermoneutral zone is somewhat higher (Taylor, 1960). Therefore, the ambient temperature of the test chamber was increased to 30 for the 5-day-olds.

Upon completion of the last test, the empty chambers were resealed, and for 15 min the system was again flushed with atmospheric air and the calibrations were checked. As is typical in such long-term metabolic measures, rate of oxygen consumption was calculated

Table 1
Mean Rate of Oxygen Consumption (in ml/g/hr) of Developing Rats Tested Alone and in Huddles of Littermates of Various Numbers

Age (in days)	No. of pups in group			
	Single	2	4	8
5	3.38	2.76	2.31	2.24
5	2.66	2.56	2.35	2.25
5	3.05	2.86	2.10	1.71
5	3.50	3.41	2.77	2.21
9	3.46	—	2.40	2.10
9	3.70	—	3.10	2.49
10	3.46	2.59	2.45	2.10
11	2.71	—	1.90	1.74
15	2.51	—	1.84	1.70
15	2.15	—	1.90	1.62
15	2.37	1.74	1.67	1.48
16	2.23	—	1.69	1.47

Note. Five-day-olds were tested at an ambient temperature of 30 °C, and the 9- to 15-day-olds were tested in a chamber at 28 °C. Not all litters were tested with only two pups/huddle, but in each of the three age groups shown, some litters were tested in ascending and descending order of huddle size. Pups in each litter were tested alone and in groups of various sizes. Thus, the mean given is for eight pups in each case.

from an extended period of stability. A 20-min plateau period from the last half of each session was used, and 10 VO₂ readings, 2 min apart, were taken from the chart records. Values were corrected to standard temperature and pressure and are expressed below as milliliters of oxygen consumed/gram/hour.

Results

In this experiment, huddling significantly reduced oxygen consumption by rat pups. The magnitude of the metabolic savings was related to the size of the huddle. The mean volume of oxygen consumed (VO₂) by *individual* pups was 2.93 ml/g/hr across all ages tested. In huddles of eight siblings, VO₂ by the *same pups* averaged 1.93 ml/g/hr, yielding an overall mean reduction of 33.8%. The largest reduction in VO₂ observed, 43.9%, was in a litter of 5-day-olds. Table 1 presents a summary of the results obtained in this experiment. It can be seen in Table 1 that the 12 litters studied in the present experiment displayed a consistent pattern of results. Maximum rates of oxygen consumption were obtained from singletons, and lower VO₂ was associated with huddling, as a function of the number of pups per clump. Although the absolute rates of oxygen consumption differed considerably among some

of the litters tested, the within-litters variability in VO₂ was negligible, ranging from .01% to .06% O₂.

Percentage of reduction in oxygen consumption, by comparison of VO₂ by singletons with that of the same pups clumped together in groups of two, four, or eight, enables one to evaluate the proportional reduction in metabolic rate produced by huddling. Again, a highly consistent pattern of results was obtained. For the three age groups tested: 4–5-, 9–11-, and 15–16-day-olds, rate of oxygen consumption averaged 32.5%, 36.8%, and 32.2% less, respectively, in pups huddled in groups of eight than when they were separately measured. Huddled in groups of four, pups in these age groups consumed 23.9%, 26.5%, and 23.0% less oxygen, respectively, than their rates taken singly. Relatively few huddles of two pups were tested, as can be seen in Table 1, but it was apparent that even two pups, the smallest huddle possible, decreased VO₂.

It should be recalled that the ambient temperature of the testing environment was varied for the different age groups (see Method) in order to conform to the shift in the level of the juveniles' thermoneutral zone during early development. Thus, additional age-wise comparisons of metabolic savings would not be profitable here.

Discussion

The results of the present experiment show that huddling provides the rat pup with an efficient means of reducing the expenditure of metabolic energy (oxygen consumption). This phenomenon was found in rat pups ranging from 5 to 16 days of age. The magnitude of the metabolic savings measured in the present experiment was considerable, averaging about 34% for a litter of eight pups. Figure 3 shows the pups' oxygen consumption when alone and in littermate groups of different numbers.

The effect of huddling on metabolic rate found here is the opposite of what would be predicted if the rat pup was truly poikilothermic. Metabolic rate in a poikilothermic animal is a direct function of temperature; VO₂ increases with increasing body temperature. It was shown in Experiment 1 that

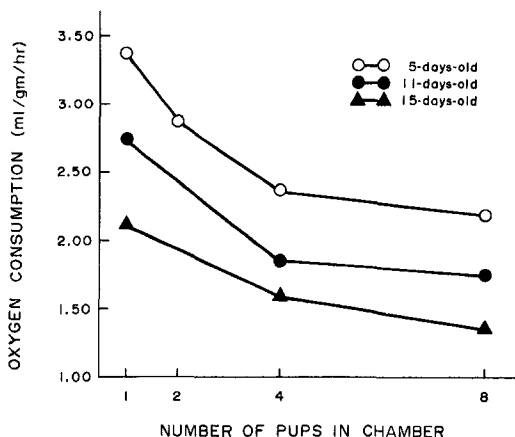


Figure 3. Rate of oxygen consumption by rat pups of various ages tested alone and in huddles of different sizes.

huddling reduces heat loss and thus keeps pups warmer. In the present experiment (Figure 3) pups had lower metabolic rates when they were warmer in huddles than when heat loss was increased by placing the animals in smaller groups or in isolation.

Overall, the findings of this study are in basic agreement with the existing data on the effects of grouping on oxygen consumption in rats (Cosnier, 1965; Taylor, 1960). However, unlike earlier research, the present experiment tested huddles of various sizes and, in doing so, strengthened the assertion that the resultant metabolic effects can be attributed to huddling per se. Studies that simply compare grouped and single animals may obtain results reflecting relatively nonspecific differences, such as those caused by isolation distress (see Randall & Campbell, 1976), rather than processes characteristic of grouping. The group-size-related reduction in oxygen consumption found here strongly suggests that the phenomenon is related to some specific aspect of huddling.

The present findings show more consistent metabolic reductions across ages than did the previous studies. In contrast to the earlier studies, the ambient temperatures used here were only moderately challenging to the pups, i.e., 2–4 °C below thermoneutral. In many past experiments, rats have been tested at severely cold temperatures, which may rapidly induce torpor, or in thermo-

neutral environments, which, by definition, minimize metabolic responses.

The findings of Experiments 1 and 2 can be explained by the consequences of clumping on surface area. Rat pups, like other physical entities, lose heat at a rate proportional to their surface/volume ratio. Huddling reduces the exposed body surface area of each pup in the group, thereby decreasing the surface/volume ratio and heat loss. The huddle, in effect, increases the body size of the animals in it. With reduced heat loss accompanying increased size, metabolic rate can decrease, as it does for homeotherms in general (Schmidt-Nielson, 1975).

Experiment 3

Experiments 1 and 2 were concerned with the physiological consequences of huddling but did not address the behavioral mechanisms by which these results are obtained. An important question therefore arises: Are the thermal and metabolic consequences of huddling merely the passive effects of a stereotyped tendency to aggregate, or can huddling be viewed as an active *regulatory* behavior on the part of the group? The significance of this question involves a fundamental bias in our view of the altricial infant and the nature of postnatal development.

A commonly held view of the infant as a helpless, reliant, and passive organism emphasizes the asymmetry of the parent-offspring relation and focuses on the juveniles' needs and incapacities. This conceptual framework has spawned a body of data that, not surprisingly, demonstrates the relative inability of juveniles to solve problems common to adults. Much of the early work on the ontogeny of temperature regulation, for instance, consists of demonstrations that infant rodents, tested in the same manner as their adult counterparts, suffer relatively large decreases in body temperature and manifest reduced oxygen consumption (Conklin & Heggeness, 1971). Consequently, we have a persisting tradition of inaccurate reference to the neonate as "poikilothermic."

Experiment 3 sought to examine the extent to which huddling might be an active regulatory behavior rather than a fixed re-

sponse resulting in a heap of otherwise passive pups. Such an empirical demonstration would warrant a shift in emphasis and awareness, to focus more on the regulatory abilities of the infant and the functional organization of neonatal behavior. It was reasoned that if huddling is modulated by environmental temperature, then one would expect a clump to form as a body of minimal surface area at low ambient temperatures and, with increasing heat, the surface area of the clump would increase. Previous observers have noted that immature rats and mice group and disband as a function of temperature (Cairns, 1972; Cosnier, 1965). The present experiment describes a method used to investigate group regulatory dynamics of huddling on the form of the clump.

Method

Subjects. A total of 48 pups were tested. Three groups of four littermates each were tested at 5, 10, 15, and 20 days of age. Animals were bred and maintained as described in the experiments above.

Procedure. To examine the surface area of the clump as a function of ambient temperature required a method of measurement of clump size (surface area) and a means of changing the animals' environmental temperature.

Surface area measurements of clumps of pups were obtained as follows: A tripod-mounted 35-mm camera was positioned directly above the animals' chamber (described below). Photographic transparencies were taken of subjects when they were clumped and when they were not in contact. Tracings of the pups were later made from projections of the slides from a fixed distance. The circumference of the clumps was measured with a standard commercial odometer (map measurer). For purposes of correction for age-related differences in pup size, distance of camera to cage, and enlargement during projection, 6–10 measures of individual pups were made from each roll of film (all parameters were constant within each roll). An average circumference of an individual pup was derived from the tracings of 6–10 individuals. This average pup circumference was arbitrarily assigned the value 1.0. The total circumference of the four individual pups in an observation cage which were not in contact with one another was therefore 4.0.

For measurement of the exposed surface area of huddles, tracings were made of clumps which eliminated all areas of overlap between animals. An example of this is shown in Figure 4. The left portion of the figure is a tracing of a clump of 10-day-old pups, drawn to represent the actual appearance of the pups in contact and depicting their postures in relation to one another. The right portion of the figure illustrates the exposed surface area of this clump. Note that in the latter tracing, portions of a pup's body that are in contact with another

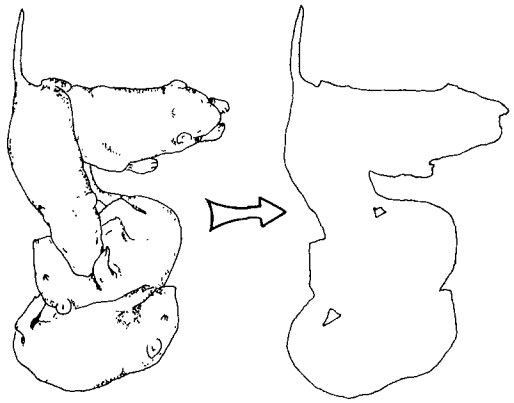


Figure 4. Conversion of a tracing of a huddle into an outline reflecting the exposed huddle "surface." (Portions of pups contacting or overlapping with other pups were eliminated in the conversion, leaving an outline of exposed pup surface.)

pup have been eliminated so that the drawing represents the exposed surface area of the group viewed from above. The linear circumference in this tracing, corrected for average pup size, represents an estimate of total exposed surface area of the clump. With this measure, clump surface could theoretically range in value from a minimum of 1.0 (if pups were stacked perfectly on top of each other when photographed from above) to a maximum of 4.0 (if a clump of four pups was completely dispersed with no pup touching another).

Control of ambient temperature in the test cage was accomplished by changing the temperature of a water bath in which the observation chamber was partly submerged. Changes in chamber temperature effected in this manner are gradual and uniform.

Molded opaque plastic observation cages were weighted and partially immersed in a commercial 20-gal. aquarium. The water level reached to within approximately 2 mm of the top of the cage. The cage was covered with a perforated sheet of clear Plexiglas. The cage floor was insulated from the water below it by a layer of corrugated paper covered by woodshavings taken from the subject's home cage.

Cold ambient temperatures were produced by adding ice to the aquarium water; warm chamber temperatures were achieved by removing ice, siphoning water, and replacing it with sufficient quantities of warm or hot water to reach desired temperatures in the observation cage. Cage temperature was monitored with a Yellow Springs telethermometer (Model 73TD) and an air temperature probe.

The experiment involved placing groups of four littermates into the partially submerged observation cage. After a 15-min habituation period the ambient temperature of the cage was recorded, and a photograph was taken from above the cage. Thereafter, every 15 min and for the duration of the experiment, a temperature reading and a photograph were taken. Following each 10-min period, the water temperature was altered, as described above, so that the chamber temperature in-

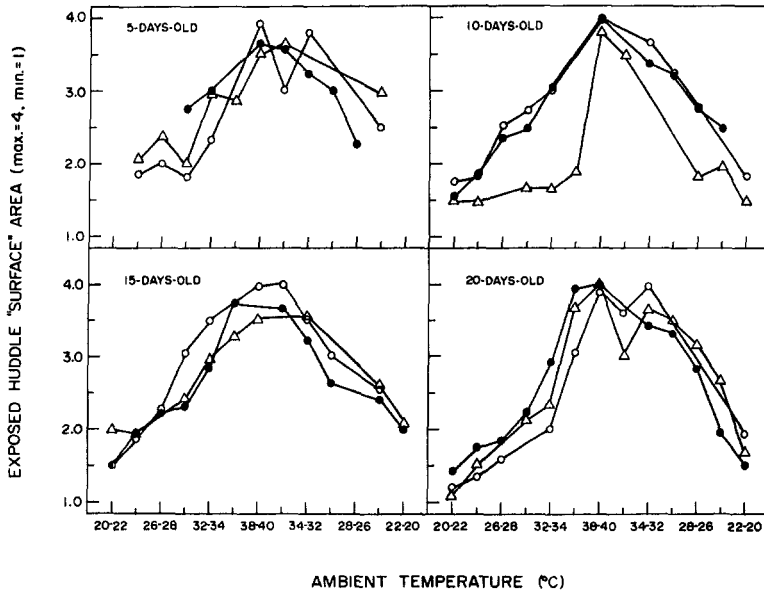


Figure 5. Regulation of the exposed huddle "surface" area by rat pups of various ages. (Each continuous line depicts the average exposed surface area of a clump of four siblings at various points during a cycle of temperature change, shown along the abscissa. The exposed surface of a clump ranged from 1.0 to 4.0, by the method of conversion described in the text.)

creased until it reached 40 °C, at which time the water temperature was decreased gradually until the ambient temperature in the cage was 20 °C. The entire 20–40–20 °C cycle lasted 1.5–2 hr. Some of the youngest subjects were tested in a somewhat narrower, and potentially less debilitating, temperature cycle, beginning around 26 °C and reaching only 37 °C before descending.

Results and Discussion

The most conspicuous and significant finding in this experiment was that groups of rat pups interacted so as to adjust the total exposed surface area of their clumps in correspondence to the ambient temperature. As the temperature of the animal chamber rose and fell, the total exposed surface area of the huddles increased and decreased. The volume or mass of each huddle remained constant, of course, so that the surface/volume ratio of the clumps was changed in accordance with the ambient temperature.

Figure 5 illustrates the results, showing the alterations in "clump surface area" as a function of ambient temperature in huddles of 5-, 10-, 15-, and 20-day-old pups. Each single continuous line represents the behavior of a single clump during the temperature cycle. The graphs depict the clump

sizes at 3.0 °C intervals. Therefore, most of the points on the graphs actually represent the average of several photographs taken when the chamber temperature was within the range specified on the abscissa.

The method of measurement utilized in the present study appears to be a useful means of studying a novel dimension of the behavior of groups. The technique reveals that young rats interact so as to regulate the size of the clump. The rate of temperature change varied considerably in this experiment due to the relatively crude method of heating and cooling the water bath; some of the differences between groups were undoubtedly produced by inconsistencies in the temperature cycle.

The positive correlation of huddle surface to ambient temperature can be seen at each age. It should be noted that all of the clumps studied were observed to disband completely at the peak temperatures and eventually reform as the temperature of the chamber declined. Despite some of the variations seen in Figure 5, it is clear that rat pups 5 days of age and older are capable of adjusting themselves and the clump to conform to changes in environmental temperature.

It is likely that this form of "group regulatory behavior" can regulate heat loss and, correspondingly, modulate the pups' metabolic rate. This would suggest that the physiological consequences of huddling are not passively derived from the clump. Instead, it suggests that the behavior is a well-tuned, integrated activity which regulates the group as a whole, resulting in thermoregulatory and metabolic benefits shared by the group members.

Experiment 4

A huddle of infant rats appears to alter its form and structure in an adaptive fashion. By means of behavioral interaction, rat pups regulate the exposed surface area of the huddle in response to the ambient temperature. Although the litter aggregate can be profitably viewed as a regulative unit, it is necessary to examine the behavior of the individual pups that constitute the huddle to understand how group regulation might be accomplished.

Not all positions in the huddle provide equivalent insulation or allow for rapid heat dissipation. At cool ambient temperatures, a place in the interior of the clump, minimizing body exposure, for instance, would be advantageous for the individual; a peripheral position on the huddle surface would be the least effective site from which to derive the insulative benefits of clumping. Conversely, dissipation of body heat by convection or evaporation would be more efficient on the surface of the huddle than in the core. If the pups maintained constant positions in the litter, then the observed temperature-dependent changes in huddle size would have little or no effect on these pups located on the periphery of the group. Informal observation, however, indicated that pups do change their position in the huddle. The study described below was designed to examine some of the individual behavioral dynamics within the huddle that could contribute to both group and individual regulation.

Method

Subjects. A total of 36 pups were used. The rats were reared as described above and were tested in six groups

of six littermates each when they were 10–12 days of age.

Apparatus. Huddles of rat pups were studied in a bowl-shaped nest. The nest was made from a polypropylene funnel, cut horizontally to form a truncated cone, 11.5 cm high (top diameter, 15.7 cm; bottom diameter, 6.5 cm) with a 60° sloping wall. The modified funnel fit snugly into a 24-cm-high Plexiglas cylinder. The bottom of the nest was covered with woodshavings from the subject's home cage.

A closed-circuit television camera was positioned above the nest and a close-up lens provided a clear and detailed picture of the pups in the nest; normally two or three pups were fully visible from above. Test sessions were recorded on a time-lapse videotape recorder (Hitachi-Shibaden Model 512-U) and scored during rapid playback. The time-lapse video system and scoring apparatus are described in detail elsewhere (Alberts, 1978).

Procedure. Six pups were removed from their home cage immediately before testing. Two of the pups, selected randomly, were marked for individual identification. One pup was marked with a solid stripe (about 4 mm wide), extending along the dorsal midline from the level of the shoulders to the hips. The second pup was marked similarly, with a dashed line covering the same body region. The entire group of pups was placed into the nest of the testing apparatus, and after a 20-min habituation period, the activity of the clump was recorded by time-lapse videotape for 2 hr. Recordings were made at 5 fields/sec and were viewed for scoring at 60 fields/sec (12:1 record/playback ratio).

The videotapes were scored by viewing the experiment during high-speed playback and measuring the time-spent-exposed on the clump by each of the marked pups. The identification marks were used to define the pups' exposure; when the entire dorsal marking was visible, the experimenter activated an electromechanical timer and counter. Thus, time-spent-exposed refers to only the dorsal mark on the pup. Body regions could be exposed or buried in the clump without being scored as such. The electromechanical scoring apparatus was programmed to sum the total duration of exposure and the number of appearances on the huddle surface for successive 7.5-min intervals. Some of the videotapes were scored by two independent observers to assess the reliability of the measures. Values obtained in these tests differed less than 5% between observers. In addition, all the videotapes were viewed again and scored with an Esterline-Angus event recorder which provided a record of the number and the duration of each bout of exposure.

Results and Discussion

The time-lapse record revealed that the huddle is a restless, almost continuously active mass of bodies. Pups appeared and disappeared from view on the surface of the huddle. Figure 6 is a composite of drawings made of a clump of six 10-day-olds in a glass funnel, depicting the movements studied in



Figure 6. Four views of the flow of pups through the huddle. (The drawings were taken from photographic transparencies of a clump of six 10-day-old siblings in a glass funnel. The arrows depict the movements that were analyzed in Experiments 4 and 5.)

this experiment. The drawings are from photographs taken at various angles, modified with arrows to emphasize the dynamic nature of the pile. Experimental data were collected from a video camera located directly above the group. From the individually marked pups in the pile, quantitative data were derived to describe these movements.

Figure 7 shows four representative individual pups studied in this experiment. Each graph in Figure 7 depicts the record of a single, individually marked animal. Each point shows the percentages of time during consecutive portions of the test that the dyed region on the subject's back was visible. It can be seen in the figure that the pups periodically emerged from and disappeared into the huddle. The flow of pups in and out of the depths of the clump appeared as convection currents of bodies circulating through the group.

Note that the individual pups described in Figure 7 could remain visible or obscured while other pups in the same clump change position. The individual pups labeled a and c in Figure 7, in fact, were littermates in the same clump. By superimposing panels 7a and 7c one can visualize part of the complexity and rapidity of behavioral dynamics within the huddle. Positions in the clump

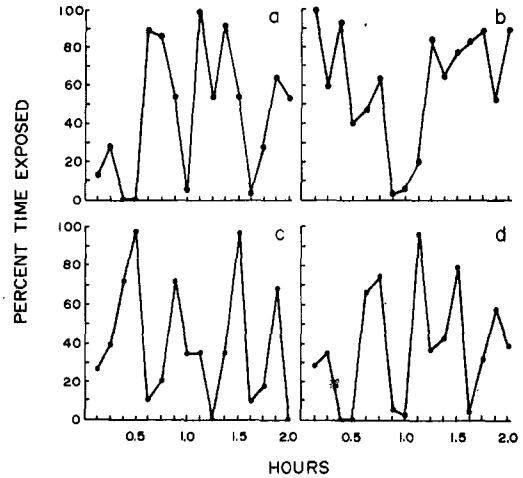


Figure 7. Time spent exposed by individual pups on the surface of the huddle during successive 7.5-min intervals of 2-hr observations. (The graphs depict the cyclic pattern of appearance and disappearance of pups on the huddle surface. Pups in panels a and c were littermates; their patterns of movement can be compared by superimposing those panels in the figure.)

changed even more frequently than is apparent in Figure 7 because those graphs show cumulative time exposed per 7.5-min block. The average number of bouts of exposure on the huddle surface was 25/2-hr session (range = 18-47).

The rapid cycles of relocation in the huddle suggest that the pups' behavior, in addition to regulating clump size (exposed surface area), enables them to share both the costs of providing insulation and the benefits of the thermal protection.

Experiment 5

Individual rat pups are observed to appear and disappear periodically among their siblings in the huddle. The movements of the litter establish "convection currents" of bodies in which individual pups flow through the clump. The purpose of Experiment 5 was to examine more precisely the organization of the behavioral interchange in the huddle.

There are several kinds of responses that could mediate the exchange of position in the huddle. Movement of pups could be predominantly downward, into the clump. The apparent flow of bodies would be actuated

by peripherally located animals diving and burrowing under the other pups in the group. Alternatively, the phenomenon of periodic appearance of pups could be created by animals emerging upward from the group and climbing upon siblings, thereby providing insulation until they were covered by a similar response made by a pup from below. Finally, a third possibility is that there is a complex form of cooperation among the group members of the clump, based on a sharing of the benefits and demands of huddling. Although it is difficult to specify the behavioral mechanisms that could orchestrate a cooperative group process, this hypothesis should not be immediately rejected.

Experiment 5 consisted of two manipulative studies using the basic observational technique described in the previous experiment. The fate of an anesthetized rat pup in a clump of active siblings was studied under different temperature conditions. The general hypothesis for this experiment was that the flow of pups in the huddle is based primarily on regulatory adjustments made by individual pups for themselves. If true, then at cool temperatures individual pups should move more deeply into the group. An immobilized pup would therefore remain on the surface of the clump. Conversely, this hypothesis would predict that under warm ambient conditions, pups would tend to maximize their own exposed surface area, creating an upward movement. Consequently, an immobilized pup would remain beneath the active group members.

Method

Subjects. A total of 12 litters were used. Rats were 10–12 days of age when tested. Litters were reared in the Indiana University colony as described above, but only six pups from each litter were tested.

Procedure. Six robust littermates were removed simultaneously from their home cage. Two pups, selected randomly, were marked for individual identification. The markings were similar to those used in Experiment 4 except that additional lines were placed laterally, equal in length and parallel to the dorsal mark. The lateral marks enabled the experimenter to use the same criteria as in the previous experiment for defining exposure on the clump, even when a pup rolled onto its side and obscured the dorsal mark.

All six pups, including the marked subjects, were

placed in the bowl-shaped nest described in Experiment 4. The nest was contained in a tall, waterproof, Plexiglas cylinder which was submerged in a water bath to a level 10 cm above the nest. The temperature of the water provided an ambient temperature of either $24 \pm .5$ °C (cool) or 36 ± 2 °C (warm) in the nest. Nest temperature was monitored by a Yellow Springs telethermometer (Model 41 TD) and an air probe.

After a 15-min habituation period the two marked pups were removed from the clump for injections. One pup was anesthetized with Equi-Thesin (.023 ml/kg, ip) and the other pup was given a control injection of isotonic saline, equal in volume to the anesthetic injection. After the anesthetic had taken effect, the two marked pups were placed together on top of the clump of littermates.

Time-lapse videotape recordings were made continuously for the next 2 hr. The record/playback ratio used was 12:1 as described in the previous experiment. Videotapes were viewed later, during rapid playback, and the duration of exposure of each of the marked animals was measured for eight successive intervals (7.5 min each) of the entire 2-hr test.

Results and Discussion

In every case, the immobilized rat pup returned to a “cool,” 24 °C nest did not show the typical pattern of periodic disappearance and reappearance in the huddle. Instead, the anesthetized pup “floated” on the surface of the clump, remaining visible for most of the 2-hr test. In contrast, the saline-injected littermate control resumed circulating through the group. The left panel of Figure 8 presents these results, showing that the immobilized pups were visible for a mean of 86% of the 2-hr test whereas the control pups, tested simultaneously, were visible for only 32% of the test period.

Immobile pups returned to their litter in a warm (36 °C) surround rapidly sank to the bottom of the pile where they remained, out of view, for most of the test session. Again, the littermate control pups intermittently disappeared and reappeared. The right panel of Figure 8 shows the clear difference between the amount of time spent on the huddle surface by active and immobile pups. In a warm nest, anesthetized pups sank into the pile and were seen for a mean of only 28% of the test. Active control pups in the same litter were exposed an average of 62% of the session. The differences between the anesthetized and control pups were statistically significant in both studies ($ps < .05$, sign test, two-tailed).

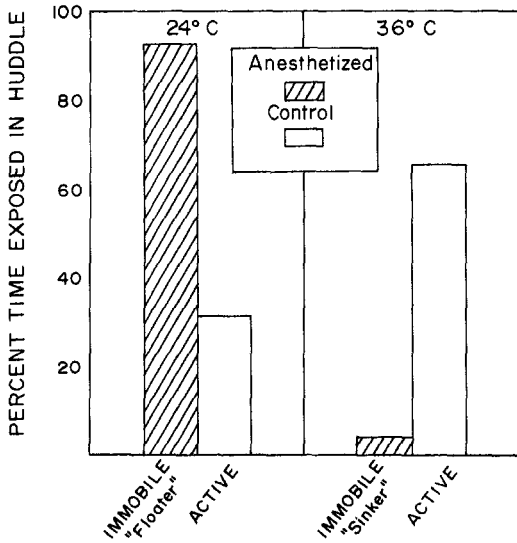


Figure 8. Median percentage of time spent exposed in a huddle by an anesthetized pup and a littermate control in nests maintained at cool and warm temperatures ($n = 6$ for each bar).

Figure 9 is a more detailed view of the outcome of this experiment, showing the patterns of movement observed in representative litters. The dashed lines in each panel of the figure depict the behavior of the active control pups in the two conditions. As in the previous experiment (see Figure 7) these pups periodically moved in and out of view in the huddle. Their immobilized littermates, however, were considerably dif-

ferent. In a cool nest (left panel) the immobile subject, like the sibling control, began the test session on the surface of the litter. As can be seen in Figure 9, the anesthetized pup remained on the surface of the huddle, providing insulation to the circulating litter but receiving virtually none. The immobile pup depicted in the left panel of Figure 9, like most of the anesthetized animals tested, eventually rolled onto its side and drifted to the wall of the nest. This pup disappeared from view briefly, for a total of about 4.2 min of the 2-hr test, before it was again lifted to the surface by the movements of its littermates.

The fate of an immobilized pup in a huddle at a cool ambient temperature suggests that the direction of the flow of pups is actively downward and that the appearance of a pup on the surface of the group is the result of displacement from the core. The anesthetized "floater" is maintained on the periphery of the clump by the lifting and pushing of the litter.

In contrast, the immobile pup in a warm litter environment rapidly sank to the bottom of the pile. The right panel of Figure 9 compares the performance of the experimental and control pups after their return to the warm nest. The experimental pup sank into the clump and remained obscured from view by the cycling litter above. The inactive pup represented by the solid line in the right panel of Figure 9 briefly became visible when

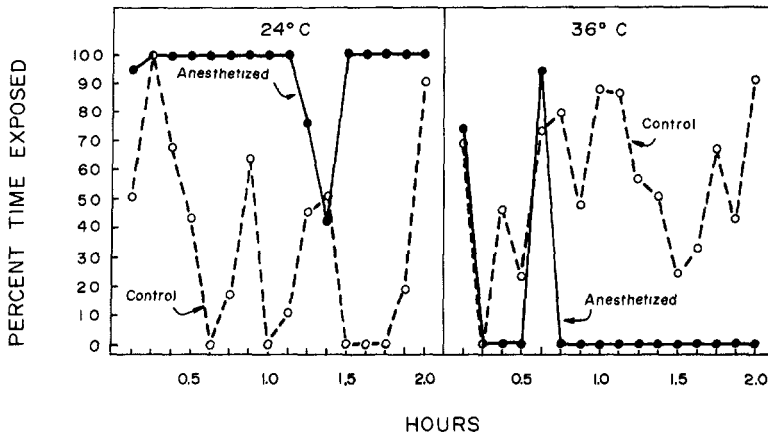


Figure 9. Examples of the movements in the litter of individual immobile and control pups at cool and warm nest temperatures. (Each point shows the percentage of time a pup's identification mark was visible during successive 7.5-min portions of the 2-hr observation.)

the siblings had almost all oriented along the side of the nest and effectively left the anesthetized pup exposed. Thus, in a warm nest the overall direction of active flow of bodies is the reverse of that seen in the cool nest. Pup flow is upward; the rats emerge and pile onto the littermates. An inactive member of the huddle thus becomes a "sinker" and disappears from view.

It is interesting to note that even within the confines of the funnel-type nest used here, pups increased the total exposed surface area of the group. This increase is apparent if the time-spent-exposed by rats in a funnel nest at 24 (Figures 6 and 9, left panel) is compared with that by rats in the 36 °C nest (Figure 9, right panel).

Welker (1959) and Cosnier (1965) both observed altered behavioral responsiveness in neonates as a function of temperature. Likewise, the overall pattern of results found here, namely, temperature-dependent changes in the organization and direction of the flow of bodies through a huddle, appears to be based on rather simple regulatory responses made by individual pups in the litter. Cooler pups burrow downward, into the insulation of the group. Their movements displace other pups to the periphery. In contrast, warm pups maximize their own exposed body surface area and thus actively ascend from beneath other pups to the surface, changing the overall direction of movement.

General Discussion

Huddling by rat pups functions to reduce heat loss and to conserve the expenditure of metabolic energy. In these respects, litters of infant rats resemble many species in which adults aggregate seasonally as a social defense against cold. Despite their small size, poor insulation, and meager thermogenic ability, rat pups nevertheless derive quantitatively significant thermoregulatory benefits from huddling.

The metabolic savings engendered by huddling is undoubtedly of great significance in the energy budget of development. By huddling, pups utilize far less metabolic energy for thermogenesis and can therefore channel this energy into processes of growth

and development. The sole source of the litter's nutritive energy is, of course, the mother's milk. Energy conservation from huddling allows the dam's finite supply of nutritive energy to contribute more efficiently to the weaning of her young, yielding a reproductive advantage for this behavior. This general interpretation is supported by the finding that low ambient temperatures depress growth rate in young rodents (Barnett & Mount, 1971).

The important physiological consequences of huddling are not simply the result of piling bodies upon one another. The pup's mode of activity in the litter leads to an elegant form of "group regulatory behavior" whereby the huddle acts as an adjustable unitary body, responding adaptively to changes in ambient temperature. Arranged loosely in warm temperatures and tightly cohesive in the cold, the huddle expands and contracts, maximizing and minimizing the heat-dissipating surface area of the clump (Experiment 3). By doing so, heat loss is significantly reduced (Experiment 1), which enables the pups to save considerable amounts of metabolic energy in the processes of thermogenesis and body temperature regulation (Experiment 2). Moreover, the pups exchange positions in the huddle and, in effect, share the costs and benefits of the group activity (Experiment 4).

Thus, the huddle behaves as an active, dynamic, regulatory unit. In particular, the results of Experiments 3-5 showed the adaptive modifiability of the huddle, produced by individual adjustments of the group members.

Together, these considerations support a perspective that contrasts sharply with the characterization of the altricial infant as a helpless or entirely dependent creature. In groups, infant rats were found to demonstrate exquisite behavioral regulatory abilities and to manifest a metabolic strategy characteristic of homeotherms rather than the so-called "primitive" poikilothermic response pattern. The findings reported here underscore the critical importance of including the typical ontogenetic milieu in our studies of development. For the developing rat the milieu is the huddle; the results can reveal both quantitative and qualitative

capacities that are not apparent in the individual pup.

References

- Alberts, J. R. Huddling by rat pups: Multisensory control of contact behavior. *Journal of Comparative and Physiological Psychology*, 1978, 92, 220-230.
- Barnett, S. A. *A study in behaviour*. London: Methuen, 1963.
- Barnett, S. A., & Mount, L. W. Resistance to cold in mammals. In A. H. Rose (Ed.), *Thermobiology*. New York: Academic Press, 1971.
- Bryant, D. M., & Hails, C. J. Mechanisms of heat conservation in the litters of mice (*Mus musculus* L.). *Comparative Biochemistry and Physiology*, 1975, 50A, 99-104.
- Cairns, R. B. Fighting and punishment from a developmental perspective. In J. K. Cole & D. D. Jensen (Eds.), *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.: U.S. Department of Health, Education, and Welfare, 1962.
- Conklin, P., & Heggeness, 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.
- Fairfield, J. Effects of cold on infant rats: Body temperatures, oxygen consumption, electrocardiograms. *American Journal of Physiology*, 1948, 155, 355-365.
- Fowler, S. J., & Kellogg, C. Ontogeny of thermoregulatory mechanisms in the rat. *Journal of Comparative and Physiological Psychology*, 1975, 89, 738-746.
- Hahn, P., & Koldovsky, O. *Utilization of nutrients during postnatal development*. Oxford: Pergamon Press, 1966.
- Hart, J. S. Rodents. In G. C. Whittow (Ed.), *Comparative physiology of thermoregulation* (Vol. 2). New York: Academic Press, 1971.
- Howard, W. E. Relation between low temperature and available food to survival of small rodents. *Journal of Mammalogy*, 1951, 32, 300-312.
- Hull, D. Thermoregulation in young mammals. In C. G. Whittow (Ed.), *Comparative physiology of thermoregulation* (Vol. 2). New York: Academic Press, 1973.
- Jeddi, E. Confort du contact thermoregulation comportementale. *Physiology and Behavior*, 1970, 5, 1487-1493.
- King, J. A., & Connon, H. Effects of social relationships upon mortality in C57BL/10 mice. *Physiological Zoology*, 1955, 28, 233-239.
- McCance, R. A. The maintenance of stability in the newly born. *Archives of Disease in Childhood*, 1959, 34, 459-470.
- Pearson, O. P. The oxygen consumption and bioenergetics of harvest mice. *Physiological Zoology*, 1960, 33, 152-160.
- Prychodko, W. Effect of aggregation of laboratory mice (*Mus musculus*) on food intake at different temperatures. *Ecology*, 1958, 39, 500.
- Randall, P. K., & Campbell, B. A. Ontogeny of behavioral arousal in rats: Effect of maternal and sibling presence. *Journal of Comparative and Physiological Psychology*, 1976, 90, 453-459.
- Schmidt-Nielson, K. *Animal physiology*. London: Cambridge University Press, 1975.
- Sealander, J. A., Jr. The relationship of nest protection and huddling to survival of *Peromyscus* at low temperature. *Ecology*, 1952, 33, 63-71.
- Steiniger, F. von. Beitrage zur soziologie und sonstigen biologie der wanderratte. *Zeitschrift für Tierpsychologie*, 1950, 7, 356-379.
- Taylor, P. M. Oxygen consumption in new-born rats. *Journal of Physiology*, 1960, 154, 153-168.
- Welker, W. I. Factors influencing aggregation of neonatal puppies. *Journal of Comparative and Physiological Psychology*, 1959, 52, 376-380.
- Whittow, G. C. (Ed.). *Comparative physiology of thermoregulation* (Vol. 1). New York: Academic Press, 1971.
- Whittow, G. C. Evolution of thermoregulation. In G. C. Whittow (Ed.), *Comparative physiology of thermoregulation* (Vol. 3). New York: Academic Press, 1973.

Received March 7, 1977 ■