

Acute, Early Thermal Experience Alters Weaning Onset in Rats

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GERRISH, C. J., C. M. ONISCHAK AND J. R. ALBERTS. *Acute early thermal experience alters weaning onset in rats.* *PHYSIOL. BEHAV.* **64**(4) 463–474, 1998.—We hypothesized that first ingestion of solid food (weaning onset) would be accelerated in young rats with advanced thermoregulatory development. To manipulate the pups' thermoregulatory development, we exposed rat pups, but not their dams, to a Cold (10°C), Moderate (21°C), or Warm (31°C) ambience for 2 h/day from postnatal Day 2–14, expecting that early exposure to cooler temperatures would accelerate development of thermoregulatory capabilities and thus accelerate nest egression as well as onset of feeding. Contrary to expectation, cold exposure was associated with a profile of developmental delays in both growth and maturation. Pups in the Cold condition began feeding later than pups with Moderate or Warm thermal experiences. We then evaluated thermoregulatory status (mechanisms for heat production and temperature conservation) on Day 15–16 (just prior to weaning onset). Thermogenesis, measured by oxygen consumption, was unaltered by the thermal manipulation. In contrast, pelage development (insulation) was altered. Pups in the Warm condition had greater fur density and an increased frequency of longer hairs relative to pups in the Cold condition. Although the developmental response to early cold exposure was in the direction opposite to our predictions, the hypothesized relation of thermoregulatory development to weaning onset was supported: Thermoregulatory status correlated with weaning onset. © 1998 Elsevier Science Inc.

Weaning onset Rats Temperature Thermoregulatory development Fur growth Oxygen consumption
Thermal experience

THE dissolution of suckling and the concomitant emergence of independent feeding, together comprise weaning, one of the fundamental transitions in mammalian ontogenesis. Weaning is a complex, coordinate process involving regulated interaction between offspring and mother, as well as integrated physiological modifications within both juvenile and adult individuals. In Norway rats (*Rattus norvegicus*), a species important in laboratory studies of ingestive behavior, the young begin to reliably ingest solid food about Day 18 (52). Time spent suckling begins to decline around Day 20, while time spent ingesting solid food increases (5,52). By about Day 34 the young no longer suckle, and weaning is essentially complete.

In our recent studies of weaning in Norway rat, we found that environmental temperature helps determine when and for how long pups leave the nest (19). In those investigations, as in nature, pups encountered solid food only when they were away from the nest (2,54). With extra-nest temperatures between 10° and 30°C, there were orderly differences in first ingestion of solid food. Weaning occurred about 48 h sooner when food became available in a warm environment compared to when it was cold outside the nest. As the pups spent progressively more time outside the nest, they also spent progressively more time feeding. Moreover, when food was in a cool area, there was a strong positive correlation between duration spent outside the nest and duration feeding (19).

Effects of environmental temperature on behavior must involve

the thermal characteristics of an organism. In the case of Norway rats, pups are born with meager thermoregulatory capabilities in relation to the ambient temperatures that they typically encounter and which are relatively trivial for an adult rats' body temperature regulation. Infant rats display progressive, age-related increases in heat production capacity with a correlated decrease in the lower limit of thermoneutrality (3,48,51). Improved heat production and heat conservation mechanisms are evident during the weaning period. By Days 19–32, they are better able to maintain stable a core temperature during cold challenge, due to improved heat production and the development of insulation (3,50).

Data from other rodent species are consistent with the hypothesis that thermoregulatory development is related to weaning. McClure and Randolph (38) made a comparative study of the development of homeothermy in the altricial wood rat (*Neotoma floridana*) and the precocious cotton rat (*Sigmodon hispidus*). Cotton rats attain thermal independence (Days 10–12) earlier than do wood rats (Days 19–22). In both species, achievement of peak metabolic rate coincided with weaning.

The putative relation between body temperature regulation and weaning is inferred from ontogenetic correlations. If there is a strong causal relation, then one would predict that a change in the development of thermoregulatory capability would be accompanied by a similar change in weaning. The present study was designed to alter thermoregulatory development in Norway rat

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pups and observe possible changes in egression from the nest and in weaning.

When rat dams and their litters are maintained in a cold environment, development of the pups' thermogenic capabilities can be altered. For example, rats reared in severe cold (below 10–12°C) showed accelerated development of thermogenic responses compared to rats reared in a more moderate (23–23.5°C) environment (17). Pups reared under warm conditions (28–30°C) achieved comparable thermogenic capabilities later than those in the two cooler groups. Similarly, Křeček, Křečková, and Martínek (32) found that young rats reared in a 34°C environment were retarded in the development of thermoregulatory capability, relative to conspecifics reared at 18–20°C.

An alternative approach to exposing the dam and her young to a specific ambient temperature, is to expose only the offspring to the experimental temperatures during discrete daily bouts and maintain typical temperatures when the family unit is together. Such acute exposures avoid complications of temperature effects on the dams' behavior. Doi and Kuroshima (9) exposed pups from Days 0–14 to four such 1-h bouts each day in either a warm or cold temperature. For the remaining time and during 30-min intervals between each exposure, pups were returned to their mother (25°C ambience). Cold-exposed pups showed improved cold-tolerance relative to warm-exposed pups when tested at 3 weeks of age. Potentiated cold-resistance was maintained 19 weeks following termination of exposure. Similar effects have also been reported in rabbits (4), kittens (27), and human infants (22,23).

The main goal of the present study was to measure onset of weaning in pups in which thermoregulatory development was modified. It was hypothesized that acceleration or delay in thermoregulatory maturation would have corresponding effects on weaning. Individual litters were given discrete daily exposures to either a Cold, Moderate, or Warm temperature. These daily bouts of thermal experience began on Day 2 and lasted until Day 15, just before weaning onset. It was expected that thermoregulation would develop earlier in pups experiencing cooler temperatures, and that accelerated thermoregulatory capabilities would be associated with accelerated weaning.

We applied a relatively broad evaluative perspective on possible effects of thermal manipulation. It seemed important to determine whether such manipulations might affect aspects of pup development other than thermoregulation. We measured growth as well as a variety of developmental milestones. Thermoregulatory development was assessed both in terms of heat production (thermogenesis) as well as heat retention (insulation).

Experiment 1: Behavior, Growth, and Maturation Following Early Cold Exposure

Experiment 1 was designed to test the hypothesis that modifying the development of thermoregulation would modify the time of weaning onset. From Days 2 through 14, litters were exposed 2 h/day (on weekdays) or 1 h/day (on weekends) to either a Cold (10°C), Moderate (21°C), or Warm (31°C) environmental temperature. To avoid temperature-related effects transduced through the mother, rat dams were not exposed to the temperature manipulation.

On Day 14, litters and their mothers were moved into the test habitat, which consisted of a nest (21°C) connected via a tunnel to a field (16°C), containing powdered rat chow; mesh partitions insured that food intake by pups and the mother could be measured independently. Time-lapse video recordings were made of dams and their litters for 12 h/day from Day 15 to 23. During rapid playback, a computer-based program was used to score the behavior of the dam and two focal pups per litter. The amount of time

pups spent in the field was measured. Cold-exposed pups were expected to leave the nest sooner and ingest solid food sooner than would pups housed and tested identically, but which were acutely exposed to warmer temperatures.

Although only pups (not dams) were exposed to the thermal manipulations, we nonetheless evaluated whether maternal behavior might differ across groups in ways that could affect the outcome. To determine whether our manipulations of the pups affected maternal behavior, we measured the duration that dams spent in the field as well as the duration spent nursing. Time spent in the field by the dam is an important variable because the presence of adults in the vicinity of solid food influences pups' first feedings (12). There was a possibility that the young might wean earlier if their mother spent more time in the field. Duration spent suckling indicates whether pups might experience a nutritive deficit that could be associated with early weaning.

Finally, daily litter weight and developmental milestones were used to assess the effects of cold-exposure on growth and development. Two milestones were used: 1) eye-opening, which typically occurs before weaning onset; and 2) vaginal opening, which occurs after weaning.

METHODS

Subjects

Subjects were 18 primiparous Sprague–Dawley dams and their young. The original stock was obtained from Harlan Laboratories (Indianapolis, IN). Dams were time-mated, and births were monitored; litters were typically born between 0900 hours and 1500 hours on gestation Day 22. Day of birth = Day 0. Litters were culled to eight pups, four males and four females, on Day 2. Nevertheless, in three litters, there were five males and three females (two Warm groups and one Moderate group), and one litter consisted of two males and six females (one Moderate group). Pups were housed with their mother and littermates in standard maternity cages [47 × 26 × 21 cm (length × width × height)] and were maintained on a 12:12 h light/dark cycle (2000 hours, lights off; 0800 hours, lights on).

Apparatus

Environmental Chamber

A Revco Refrigerated Incubator (BOD50, Asheville, NC, USA) was used as the environmental chamber for the temperature exposures. Air was continuously circulated inside the unit.

Housing

Pups were reared and tested in a tricompartiment habitat cage consisting of a nest area and two other, adjacent compartments, one of which was accessible only to pups and one of which only the mother could enter. The apparatus depicted in Fig. 1 includes the tricompartiment habitat, as a part of the larger, seminatural habitat. This design permitted separate feeding areas for the dam and her pups. A small slot [4 × 2.5 cm (length × height)] at floor level between the nest area and one of the adjacent compartments limited access to only the pups. Only the dam had access to the remaining compartment because she was able to jump over the barrier that separated her area from the nest area. Cramer et al. (5) provide a detailed description of this cage system.

A shelter (19 × 14 × 12 cm) was provided in a corner of the nest area; it was constructed of two sheet metal sides and a wire mesh top. It created a discrete nest site, used by the dam for nursing and brooding. The rear half of the top was covered with a metal plate to darken the area below; the front half of the top

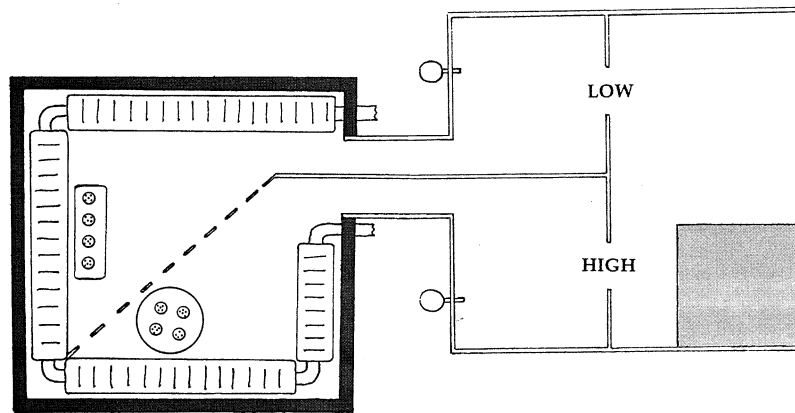


FIG. 1. Schematic diagram of the seminatural habitat (not to scale). The tricompartiment cage (right side) consisted of a nesting area containing a shelter. One wall of the nesting area had a "low" slot in it that permitted pups, but not the dam, to cross to an adjacent area. The dam could traverse a notch, open "high" on the wall and enter a separate area. From these separate, adjacent areas the dam and pups could enter the field through a tunnel. Powdered chow was available to the dam in a bowl (lower left) and for the pups in a trough (upper left). The field was temperature-controlled via the slant fin tubing, shown schematically around the field and, in turn, surrounded by insulation.

permitted direct observation of the animals. In addition, approximately 7 g of cotton batting were provided for nest material until behavioral observations began (Day 15).

Water was supplied ad libitum in each of the smaller compartments. In addition, powdered chow was provided ad libitum to dams in a glass dish [11 (diam.) \times 5 cm (height)] to prevent hoarding in the nest. From Days 2 to 14, the dams' food was placed in a separate area of the nest. When the field was connected to the nest on Day 14 and until the end of the test period, food for the dam and the pups was placed in the field.

Seminatural Habitat

Figure 1 shows the seminatural habitat in which the animals resided between Days 14 and 23. It consisted of the tricompartiment cage attached by a tunnel to a field (33.5 \times 33 \times 12.5 cm). The field was designed to maintain a specifiable ambient temperature and allow for separate food intake measurements for the dam and her litter. The field was surrounded by a slant fin tubing connected to a temperature-controlled water circulator, thus allowing regulation of field temperature. The field was insulated by Styrofoam (5.1 cm thick) on the sides and bottom. Two layers of Plexiglas covered the top of the field which allowed for complete viewing of the animals while also providing insulation. (For a detailed description of the field, see Ref. 19.) Field temperature was 16°C for animals in all conditions, and the nest was maintained at room temperature (21°C).

The field was divided by a wire mesh wall that ran diagonally from the opening of the tunnel to the far end of the field, separating the dam from her young in the feeding area, while providing full exchange of visual, olfactory, and auditory cues. The pups' feeding area contained a metal tray (15 \times 6 \times 3 cm) with five holes (2.5 cm diameter) in the top, filled daily with 120 g of powdered rat chow. A ramp attached to the food tray facilitated access to the feeding holes. To prevent loss from food spillage, the front of the tray had a 1-cm lip, while the remaining three sides were surrounded by a 3-cm high lip.

The field was connected to the habitat via a sheet metal tunnel (19 \times 10 \times 7 cm) divided longitudinally by a clear Plexiglas barrier (7 mm); the pup side was about 3.5 cm wide, while the dam's side was the remainder. Because the tunnel was raised 5.5

cm from the floor, pups were provided a sheet metal ramp [22.5 (length) \times 3.5 cm (width)] covered with a wire mesh screen to permit easy access to the field and food. To correct for fluctuations in food weight due to humidity, a glass dish filled with 60 g of powdered chow was placed in the corner of the dams' side of the field; a wiremesh shield protected it. Food intake measures were adjusted appropriately.

Field temperature was monitored by a thermistor located in the far corner of the pup side of the field. A solenoid was activated when the temperature was out of range, and the appropriate adjustments were made to the radiators that determined field temperature. Because the field and nest were maintained at different ambient temperatures, there was a 4°C difference between the tunnel entrance and the temperature at the most distal end of the field.

Procedure

Exposure

From Days 2 to 14 individual litters were placed in the environmental chamber for either 2 (on weekdays) or 1 h (on weekends) and exposed to one of three ambient temperatures: 31°C (Warm), 21°C (Moderate), 10°C (Cold). The entire litter was removed from the dam, weighed, checked for eye opening (day of eye opening = first day both eyes were fully unsealed) and placed in the corner of a maternity cage which was then put into the environmental chamber. Pups in the Warm condition only were covered with around 7 g of cotton batting because 31°C is below the approximate thermoneutral range for infants (approximate thermoneutral range for 5-day-old pups is 34–36°C) (3). This batting could be removed by the pups as they aged, and 31°C was toward the lower range of their thermoneutral zone (lower critical temperature for 13- to 15-day-old pups = 32.2°C) (48). Weekdays, exposures consisted of two, 1-h bouts separated by a 30-min period during which they were returned to their home cage and mother. Exposures occurred between 1200 and 1800 hours.

On Day 14 following temperature exposure, the animals tricompartiment cage was attached to the field, creating the "seminatural habitat" (Fig. 1). When assembled, there were several interconnected, functional areas. In the nest area, pups and dams

could engage in the full complement of mother–litter interactions. If pups left the communal area, then they first entered the pup side of the tricompartiment cage and then the tunnel which led to their half of the field and their solid food. Likewise, if the dam left the communal nest area, then she first entered her side of the tricompartiment cage and then the tunnel which led to her half of the field and her solid food. Once housed in the seminatural habitat, conditions were the same for animals regardless of prior exposure experience: field temperature was set to 16°C (a temperature that provided a slight cold challenge to weanlings, but was between the three exposure temperatures) and nest temperature was the room temperature (approximately 21°C).

Some dams were initially disturbed by the new habitat. To facilitate acclimation to the field, a piece of dark cloth was placed over the top of the communal portion of the nest. Furthermore, wire mesh was placed on the floor of the dam's compartment in the nest, and a white light bulb (15 W) illuminated the dam's compartment to discourage her from bringing pups to her side. On Day 15, the cloth was folded to allow observation of the animals in the nest area. The animals were left undisturbed in this habitat except for daily maintenance (see below).

Behavioral Observations and Data Collection

Behavioral observations and inspections of eye opening were conducted on pups in the Warm and Cold groups. Only daily food intake and weight gain were monitored for pups in the Moderate condition.

On Day 15, two pups from each litter (one male and one female) with body weights in the gender's mid-range for the litter served as focal pups and were distinctly marked (Pilot Super Color Marker, SC-6600). Beginning on Day 15, the animals' activities were recorded via time-lapse video for 12 h a day (Gyrr no. TLC1400, 12:1 record:playback ratio). One camera was directed on the field, while the other was directed on the nest (Panasonic WV-BL90). A screen splitter provided simultaneous viewing of both areas of the habitat. Pups were primarily observed during lights off because adult rats are nocturnal, and the dam is known to influence pups' first feedings primarily during dark hours (12). Recording began at 2100 hours and continued to 0900 hours. Twenty-five-Watt red bulbs illuminated the habitat during the dark phase. Pilot studies indicated that data from these 12 h reflected relative durations of behaviors observed over 24 h.

Recordings were made daily from Days 15 to 22. Beginning on Day 15, pups and the dam were removed from the habitat at about 1530 hours for daily maintenance and data collection. Individual pups were weighed and checked for eye opening. Focal pups were remarked, and the pups' food tray and the blank were weighed. Before the animals were returned to the habitat, food and water were replenished, the field was vacuumed and blanketed with fresh bedding, and finally, a sliding door was inserted into a slit in the top of the tunnel on the nest side. The door prevented pup field excursions due to experimenter handling. The door was removed once pups settled (approximately 10–15 min). This procedure required about 1 h.

During playback, an observer used a computerized program to quantify the following:

- 1) *Suckling*: duration that the focal pups spent attached to the nipples of the dam. Brief detachment from the nipple after milk-letdowns, i.e., nipple shifts, were included in the total duration of time spent sucking.
- 2) *Dam in field*: duration that the dam spent in the field. Criterion was that more than half of a body length was in the field.
- 3) *Focal pups in the field*: duration that each focal pup spent in

the field. Criterion was that more than half of a body length was in the field.

Instances in which the dam carried the pups into the field were not included in the analysis. This was only observed once, but because a focal pup was involved, only the data for the remaining focal pup that was not carried into the field was included. On three occasions equipment malfunction resulted in either the loss of an entire or partial tape session. If a session was entirely lost, then values of the proportion of time that the particular behaviors were active from the day before and after the tape loss were averaged. If there was partial loss, then we simply evaluated the proportion of time that a particular behavior was active for the duration of the shortened test period.

Reliability

Three coders scored tapes. For reliability, two tapes were scored repeatedly. Scores were within 96.8% of those by the primary coder.

Data Analysis

Food intake was calculated as a percentage of litter mass at the end of each 23-h feeding session. To assess relative differences between groups in the timing of weaning onset, we assigned the following quantitative criterion to define this term: for each litter, weaning onset was defined as the day when the litter consumed at least 0.5% of the total litter weight; amounts less than this were not included in data analyses.

For all the behavioral observations, values were averaged for each pair of focal pups within a litter to obtain a single mean value per day per litter. Temporal scores were converted to proportion of time active during the 12-h test period. Criterion for the onset of nest egression was the first of two consecutive days in the field.

Day of eye and vaginal opening were calculated for not only the focal pups, but the entire litter. The percentage of pups in the litter with both eyes fully opened or a complete vaginal opening was averaged across litters to obtain a mean percentage for each day.

Unless otherwise stated, the data were analyzed using repeated measures ANOVAs. Fisher's least-squared difference (LSD) were used for post hoc analyses at the 0.05 level.

RESULTS AND DISCUSSION

Food Intake

Weaning onset in the three groups did not support the hypothesis that Cold-exposed pups would wean earlier. In fact, the data were the opposite of our expectations. Mean onset for independent feeding was on Day 18.2 ± 0.17 for pups in the Warm condition, Day 18.5 ± 0.22 for pups in the Moderate condition, and Day 19.2 ± 0.17 for pups in the Cold condition. First ingestion was earlier by pups in the Moderate and Warm conditions compared to pups in the Cold condition [one-way ANOVA: $F(2, 15) = 7.4, p = 0.006$; LSD = 0.57].

Figure 2 (top) illustrates group differences in the litters' daily food intake expressed as a proportion of body weight. These results mirror the pattern seen for weaning onset. A repeated-measures ANOVA conducted on data from Days 19 (when the majority of litters reached the criterion for weaning onset) to 23 indicated significant differences in daily food intake as a percentage of litter weight between the Cold, Moderate, and Warm pups [$F(2, 13) = 10.9, p = 0.002$]. Technical problems resulted in data loss of food weights on Days 20 and 21 for two litters in the Moderate condition; the overall analysis was conducted with only four litters for the Moderate condition. Collapsed across Days

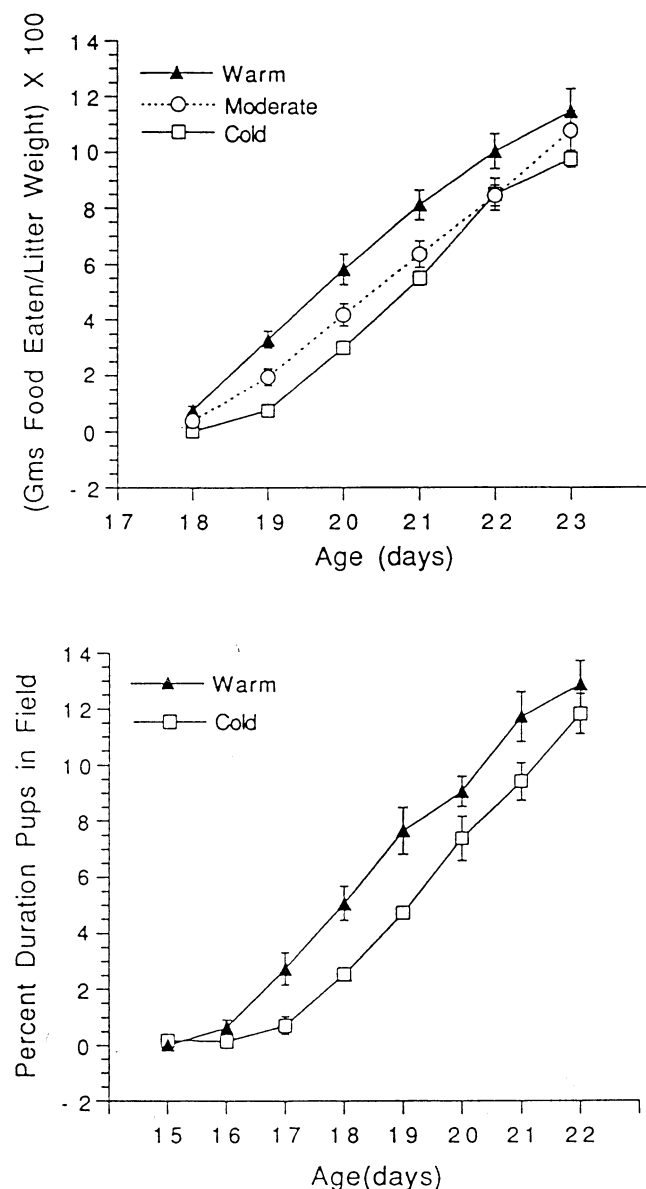


FIG. 2. (Top) Amount of food eaten as a proportion of litter weight for Warm-, Moderate-, and Cold-exposed pups. (Bottom) Proportion of time that Warm- and Cold-exposed pups spent in the field.

19–23, litters in the Warm group ate an average of $7.7 \pm 0.44\%$ of their body weight, whereas litters in the Moderate group ate an average of $6.6 \pm 0.34\%$ of their body weight, and, finally, litters in the Cold condition ate an average of $5.4 \pm 0.23\%$ of their body weight. Post hoc analyses indicated that pups in the Warm condition ate more food relative to body weight, followed by pups in the Moderate condition, and, lastly, followed by pups in the Cold condition (LSD = 1.05). As expected, there was a main effect for age; food intake relative to body weight increased with age [$F(4, 52) = 243.2, p = 0.0001$]. The Group \times Day interaction was not significant [$F(8, 52) = 1.3, p = 0.26$].

These results are contrary to the predicted outcome and indicate that the warmer the exposure temperature, the earlier weaning

onset occurred, and the greater the amount of food consumed relative to body weight.

Behavioral Observations

Earlier weaning was expected to be associated with earlier departures from the nest. Mean day of first egression was 16.4 ± 0.20 for pups in the Warm condition and 17.3 ± 0.33 for pups in the Cold condition [two-tailed $t(10) = 2.4, p = 0.04$], confirming this expectation.

Figure 2 (bottom) illustrates that the proportion of time that pups spent in the field showed a daily pattern similar to that for day of first egression. Mean proportion of time in the field, averaged across Days 17 (when the majority of litters reached the criterion for onset of nest egression) to 22 was $8.2 \pm 0.42\%$ for pups in the Warm condition and $6.1 \pm 0.35\%$ for pups in the Cold condition. A repeated measures ANOVA indicated a main effect for exposure condition [$F(1, 10) = 14.7, p = 0.003$]. There was also a significant main effect for day [$F(5, 50) = 101.1, p = 0.0001$], indicating that as pups matured they spent a greater proportion of time in the field. The Group \times Day interaction was not significant [$F(5, 50) = 0.68, p = 0.64$]. Again, these results are contrary to original expectations, but display a consistent pattern: pups in the Warm condition appeared relatively advanced in departure from the nest and time in the field.

A repeated measures ANOVA (Days 15–22) indicated that there were no differences between groups in the proportion of time that the dams spent in the field [main effect for exposure condition: $F(1, 10) = 2.6, p = 0.14$]. There was, however, a main effect on the daily duration of bouts spent in the field. Dams spent less time in the field as the pups matured [$F(7, 70) = 2.3, p = 0.035$]. The Group \times Day interaction was not significant [$F(7, 70) = .471, p = 0.85$]. Because the dams' in both conditions spent similar time in the field, the finding of earlier nest egression and first ingestion of solid food for the Warm pups was thus independent of the proportion of time that the dam spent in the field.

A repeated measures ANOVA (Days 15–22) indicated that suckling durations were similar for pups in each condition [main effect for group: $F(1, 10) = 0.17, p = 0.69$]. There was a main effect showing that suckling declined with age [$F(7, 70) = 6.0, p = 0.0001$], but the Group \times Day interaction was not significant [$F(7, 70) = 1.1, p = 0.36$]. These results suggest that group differences in weaning onset were not due to differences in suckling behavior, i.e., a nutritive deficit. Body weight data described below further refute the possibility that nutritive deficits contributed to earlier weaning by pups in the Warm group.

Litter Weight

To determine if pups' body weights were affected by the exposure procedure and rearing in the tricompartment nest, we included two additional groups. One group consisted of six litters that were housed in standard maternity cages and not given the exposure procedure (NX-Maternity), while the other group consisted of six additional litters that were housed in standard maternity cages and given Moderate exposure (X-Maternity). During the beginning of the Exposure period (Days 2–8; Fig. 3a), mean litter weights of all pups gradually increased [$F(6, 150) = 4293.0, p = 0.0001$]. There was no main effect for group [$F(4, 25) = 2.4, p = 0.07$]. There was, however, a significant Group \times Day interaction [$F(24, 150) = 5.5, p = 0.0001$]. Figure 3 (top) shows that litter weights were initially similar. Differences emerged with the exposure experiences. During the latter half of the exposure phase (Days 9–14), mean litter weight was 219.1 ± 4.0 g for the Cold pups, 224.3 ± 3.1 g for the Moderate pups, 235.3 ± 5.3 g for the Warm pups, 238.2 ± 7.8 g for the X-Maternity pups, and $248.6 \pm$

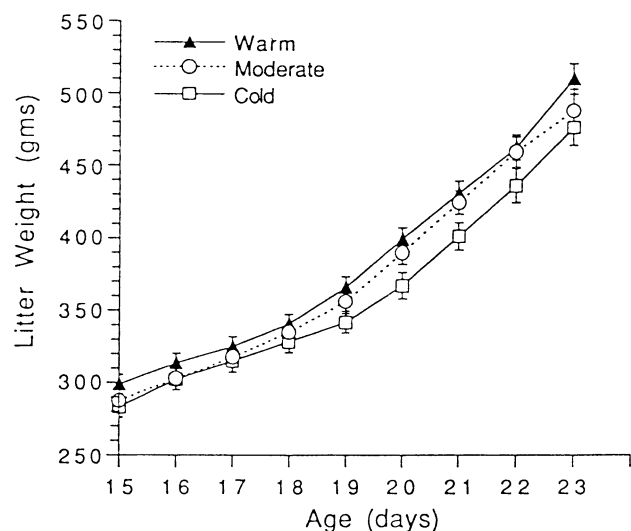
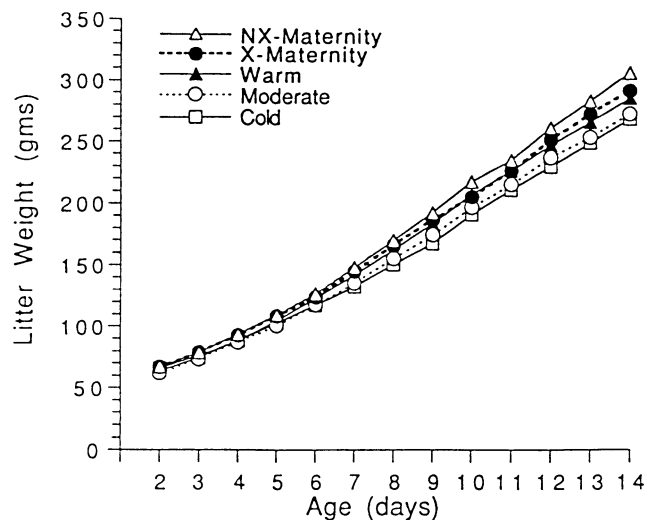


FIG. 3. (Top) Mean litter weights in the various conditions. Refer to text for description of conditions. (Bottom) Mean litter weight for Warm-, Moderate-, and Cold-exposed groups.

1.6 g for the NX-Maternity pups. A repeated measures ANOVA indicated a significant main effect for group [$F(4, 25) = 5.9; p = 0.002$]. Post hoc analyses indicated that pups in the Cold group had a significantly lower body weight than pups in the Warm, X-Maternity, and NX-Maternity groups, and that pups in the Moderate group had a significantly lower litter weight than pups in the NX-Maternity group ($LSD = 14.05$). The main effect for day was also significant [$F(5, 125) = 2698.1, p = 0.0001$]. Finally, there was a Group \times Day interaction [$F(20, 125) = 2.7, p = 0.0004$]. Hence, these results suggest that as exposure continued, the most dramatic difference in litter weight was due to cold-exposure and represented by a relative decrease in litter weight of Cold-exposed pups.

Analysis of litter weight immediately after termination of exposure (Days 15–19; Cold, Moderate, and Warm pups only; Fig. 3

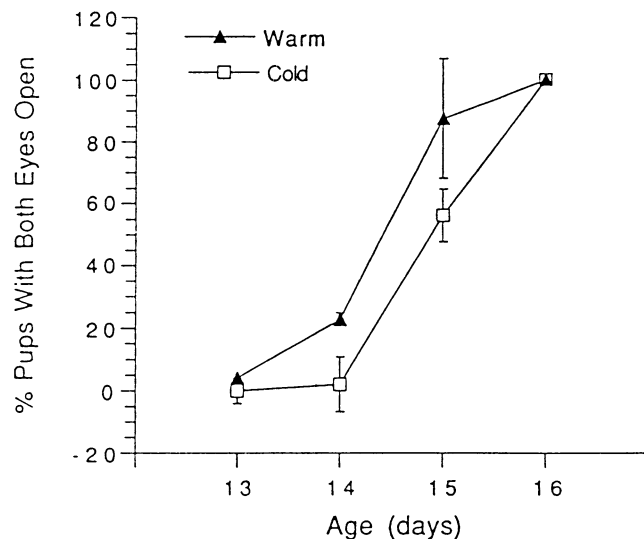


FIG. 4. Proportion of pups with both eyes open in Warm- and Cold-exposed groups.

bottom) indicated no differences between groups [$F(2, 15) = 1.4, p = 0.28$], but there was a significant repeated measures effect [$F(4, 60) = 515.5, p = 0.0001$] and a significant Group \times Day interaction [$F(8, 60) = 3.0, p = 0.007$]. Thus, the groups showed daily differences in litter weight. On Days 20–23, differences were no longer apparent [main effect for group: $F(2, 15) = 2.6, p = 0.10$; Group \times Day interaction: $F(6, 45) = 1.1, p = 0.39$]. Pups, nevertheless, continued to gain weight as they matured [main effect for day: $F(3, 45) = 354.8, p = 0.0001$].

These results suggest that litter weight loss could be attributed primarily to cold temperatures rather than aspects of the exposure procedure or housing. Reduced body weights due to cold exposure were strongest during the latter half of exposure (Days 9–14) and then were gradually ameliorated and finally disappeared. These results indicate that earlier first ingestion of solid food for Warm-exposed pups clearly was not due to lowered body weight or a nutritive deficit.

Eye Opening and Vaginal Opening

Figure 4 shows differences in the timing of eye opening as a function of exposure condition. The mean proportion of pups per litter with both eyes open, averaged across Days 14 (when at least half of the litters had both eyes open) to 15 (the day before all litters showed complete eye opening) was only $29.1 \pm 9.6\%$ for Cold pups compared to $55.2 \pm 7.8\%$ for Warm pups. A repeated measures ANOVA indicated a trend toward a main effect for group [$F(1, 10) = 4.4, p = 0.06$]. The main effect for day was significant indicating the expected pattern of an increase in eye opening with age [$F(1, 10) = 32.2, p = 0.002$]. The Group \times Day interaction was not significant [$F(1, 10) = 0.25, p = 0.63$]. These results are consistent with an overall pattern of delayed growth and development for the Cold pups relative to the Warm pups.

Analysis of proportion of females in litters with full vaginal opening was conducted on Days 34 (when at least half of the litters displayed full vaginal opening) to 37 (the day before all litters showed full vaginal opening). Results indicated that there were no differences between the groups and that the Group \times Day interaction was not significant [main effect for group: $F(1, 8) = 0.02, p = 0.90$; Group \times Day interaction: $F(3, 24) = 1.2, p = 0.34$].

There was a main effect for day indicating an expected pattern of an increase in the number of females with complete vaginal opening as they matured [$F(3, 24) = 39.4, p = 0.0001$]. Temperature exposure did not affect onset of vaginal opening.

Experiment 2: Metabolic Heat Production Following Early Cold Exposures

The present study was designed to examine the relation between ambient temperatures experienced acutely during infancy and the juvenile pups' thermogenic capabilities. In numerous studies, mammalian young exposed to cold ambient temperatures subsequently demonstrated improved cold-tolerance (e.g., 4,9,17,22,23,32), i.e., maintenance of core temperature in cool temperatures and/or increased heat production capabilities at either moderate or cool temperatures. For example, Doi and Kuroshima (9), exposed rat pups but not the rat mothers to cold temperatures. From birth to Day 14 they housed one group of litters with the dam at room temperature (25°C) except during four, 1-h periods each day during which the pups were exposed to 5°C. These periods of cold exposure were separated by 30-min intervals during which pups were returned to the dam. Other litters were housed with the dam and received similar handling but were not exposed to cold temperatures. On Days 21, 35, 42, 63, and 147, the pups' thermogenic capabilities were tested by anesthetizing the animals and placing them in a 5°C room for 90 min. EMG recordings from neck muscles indicated that cold-exposed pups showed less shivering and a more modest decrease in colonic temperature during an acute cold exposure test compared to non-cold-exposed pups. Furthermore, at 2, 4, and 7 weeks following cold-exposure, unanesthetized pups showed a greater calorogenic response to norepinephrine injection, measured by maximal increase in oxygen consumption from baseline, relative to non-cold-exposed pups. The authors concluded that early cold exposure produces long-lasting effects on thermogenic capability characterized by increased nonshivering thermogenesis (9).

In the present study, we applied a temperature exposure protocol similar to that used by Doi and Kuroshima (9), but modified with shorter and less severe cold temperature exposures (10°C instead of 5°C). To assess pups' thermogenic capabilities, we measured oxygen consumption first around thermoneutrality (31.5°C) and then at a mild cold challenge (18°C); this was posited to simulate temperatures that pups might encounter upon leaving the warmth of the huddle. Because this study was focused on the relation of thermoregulatory capabilities to the timing of weaning onset, pups were tested on Days 15–16, which is around the time they begin to emerge from the nest and just prior to first ingestion of solid food.

Methods

Subjects

Subjects were 16 pups (10 males and six females), 15–16 days old, randomly selected from 16 different litters bred and reared as described in Experiment 1. Litters were born to dams that had previously given birth to 0–3 litters. Different pups from these litters were used in Experiment 3. The day after birth was counted as Day 1.

Housing

Dams and their litters were housed in standard maternity cages with wood chips on the floor. Approximately 7 g of cotton batting was provided for nest material. Food and water were supplied ad libitum.

Procedure

Exposure

The exposure procedure was the same as that used in Experiment 1, except that litters were exposed either to a Cold (10°C) or Warm temperature (31°C); there was no Moderate condition.

Oxygen Consumption Apparatus

The apparatus and methods used to measure oxygen consumption is described in detail elsewhere (20). Briefly, pups were tested in a double-walled glass cylinder [(17.5 × 9.0 cm (height × width)] which permitted viewing of the animals' behavior. The open end of the chamber was sealed with a rubber stopper. Two ports passing through the stopper directed air flow into and out of the chamber. A circulating water bath (Lauda RM6) pumped heated or cooled water between the glass walls of the chamber, thus regulating air temperature within it. A thermistor within the chamber monitored ambient temperature.

A dual channel oxygen analyzer (Ametek, S-3A) was used to measure oxygen consumption. Two lines directed air from a tank of certified compressed dry air (20.94–21.00%) at a rate of 350 mL/min (Omega Flowmeter; FMA-5606). One line passed through the animal chamber after which it exited through a tube of desiccant and was drawn at a rate of 90–120 mL/min through one cell of the oxygen sensor. Pressure changes due to movement of the animal were minimized via a 2-cm diameter opening in the line prior to the sensor which allowed excess air to escape. The second airstream was drawn at the same rate through the second cell of the oxygen sensor. Oxygen drawn through the sensors was heated to ionization temperature in two separate electrochemical cells. The system computed the difference in oxygen between the two cells and provided a continuous digital display of amount of oxygen consumed by the test subject. The output of the oxygen analyzer, the thermocouple inside the chamber, and the subject's rectal temperature (T_r , see below) were interfaced with a Macintosh SE computer. A computer program (Omegalog, Stamford, CT, USA) provided a record of oxygen consumed and temperature inside the chamber each minute.

Oxygen Consumption and Core Temperature Measurements

After litters were separated from the dam and the nest, T_r was read from a randomly selected subject. T_r was measured with a thermocouple wire, the tip of which was coated with a thin layer of silicone caulk, inserted 1 cm into the rectum. Litters were separated from the dam for 2 h, after which T_r was again measured, and the pup was placed in a small polyethylene mesh cage (7.0 × 5.5 × 3.5 cm) and put into the metabolic chamber. The cage was raised approximately 4 cm from the floor of the glass chamber. The construction of the cage minimized movement by the subject and prevented the subject from contacting the walls of the chamber, thus allowing full circulation of air around the animal.

Air temperature in the metabolic chamber was 31.5°C (in the lower range of the thermoneutral zone [lower critical temperature = 32°C] for 17- to 19-day-old pups) (48). After a 25-min stabilization period, baseline resting metabolic rate was collected for 10 min. During the next 15–20 min the temperature was then decreased to approximately 18°C. Pups remained in the test chamber for 1 h following the start of the cooling phase.

Data Analysis

For each subject, mean rate of oxygen consumption was measured for the 10-min period prior to the temperature drop and for the 40-min period following the drop in temperature. In a few

cases, technical problems forced us to calculate oxygen consumption over a shorter period; test duration did not vary between subjects. Data were analyzed separately for the different temperatures of 31.5°C and 18°C using one-tailed, two-sample *t*-tests.

RESULTS AND DISCUSSION

One-tailed *t*-tests indicated no differences in resting metabolic rate between Warm- and Cold-exposed pups at temperatures close to thermoneutrality or during a cold challenge [31.5°C: $t(14) = 1.05$, $p = 0.16$; 18°C: $t(14) = 1.05$, $p = 0.16$]. Mean resting metabolic rate at 31.5°C was 31.39 ± 1.05 mL/kg/min for Cold-exposed pups and 30.03 ± 0.76 mL/kg/min for Warm-exposed pups. At 18°C, mean resting metabolic rate was 67.26 ± 1.36 mL/min/kg for Cold-exposed pups and 65.00 ± 1.72 mL/kg/min for Warm-exposed pups. These indicate equivalent thermogenic capability of Day 15–16 Cold- and Warm-exposed pups tested at these temperatures.

The lack of difference in rate of oxygen consumption between the two groups contradicted our expectations, which were based on Doi and Kuroshima's report (9). A number of factors could account for the unexpected outcome. Milder temperatures and shorter exposures were employed in our study. Furthermore, our test procedures differed from theirs. We did not test anesthetized animals nor did we assess calorogenic response to pharmacological manipulations (norepinephrine). Finally, Doi and Kuroshima (9) did not analyze thermogenic capability on Days 15–16, just before pups' first ingestion of solid food. Recall that their results, indicating greater thermogenic capability after cold exposure were obtained when testing older pups (Days 21, 35, 42, 63, and 147) that were already reliably ingesting solid food and had either begun to show a decline in time spent suckling or were no longer suckling (5,52). Any or all of the procedural differences could account for the different outcomes. The procedures and parameters that we chose were driven primarily by the factors seen as behaviorally and developmentally relevant to the weanling rat pup. It is possible that the rat pups initial response to early cold-exposure is to slow growth, development, and aspects of thermoregulatory development. Perhaps it is not until after first ingestion of solid food that measurable differences in thermoregulatory capability emerge.

T_r was lower for Cold-exposed than Warm-exposed pups: $36.0 \pm 0.22^\circ\text{C}$ versus $36.8 \pm 0.21^\circ\text{C}$, respectively [two-tailed $t(14) = -2.5$, $p = 0.03$]. Before placement in the metabolic chamber, T_r s were similar for both groups: $36.4 \pm 0.26^\circ\text{C}$ for Cold-exposed pups and $36.2 \pm 0.26^\circ\text{C}$ for Warm-exposed pups [$t(14) = 0.38$, $p = 0.71$]. Likewise, T_r upon removal from the metabolic chamber was also similar between the two groups: $35.0 \pm 0.23^\circ\text{C}$ for Cold-exposed pups and $34.9 \pm 0.18^\circ\text{C}$ for Warm-exposed pups [$t(14) = 0.34$, $p = 0.74$]. Cold-exposed pups' rectal temperature decreased by $0.7 \pm 0.26^\circ\text{C}$ from the nest to the pre-metabolic measurement, which was less than the $1.6 \pm 0.34^\circ\text{C}$ loss for Warm-exposed pups. This difference approached significance [$t(14) = -2.1$, $p = 0.056$]. Similarly, temperature loss from the nest to postmetabolic chamber measurements showed a trend toward a greater loss for Warm- as compared to Cold-exposed pups. Cold-exposed pups lost $1.1 \pm 0.31^\circ\text{C}$ during this interval, while Warm-exposed pups lost $1.9 \pm 0.35^\circ\text{C}$ [$t(14) = -1.8$, $p = 0.089$]. The smaller drop in core temperature for Cold-exposed pups presumably reflects their lower temperature upon removal from the nest. The nonsignificant difference between pre- versus posttest T_r was $2.4 \pm 0.19^\circ\text{C}$ for Cold pups and $2.3 \pm 0.28^\circ\text{C}$ for Warm pups [$t(14) = 0.11$, $p = 0.91$]. The lower core temperature for Cold-exposed pups was consistent with the trend of slowed energy expenditure relative to Warm-exposed pups.

Experiment 3: Development of Insulation Following Early Thermal Exposure

Thermoregulation involves both heat production and heat loss. The results from Experiment 2 indicated no differences in heat production in pups exposed daily to cold. It remains possible, however, that the temperature exposure regimes induced differences in heat retention (insulation).

The fur coat of most mammals serves as a major source of insulation. Fur is usually composed of an upper layer of coarse guard hairs or over hairs, which cover an undercoat of fine hairs or under hairs. Under hair density determines insulation; under hairs trap air that is warmed by the body. For many species of animals, insulation varies seasonally. In voles (*Microtus agrestis*), for example, the density of hairs per unit area increases during the winter months, mostly due to a greater number of under hairs (30). Similarly, the weight of under hair clipped from same-size patches from voles' mid-dorsum area was significantly heavier during the winter than the summer (30). Seasonal changes in insulation have been found in other small mammals such as deer mice, field mice, and shrews (1,24,47).

Environmental factors such as day length and temperature are known to influence hair growth (29). Exposing voles to short days and low temperatures in the summer resulted in a dense, fine winter coat, whereas exposing voles to long days in autumn resulted in a sparser summer coat (31). Lee and Zucker (34) manipulated vole dams' pre-conception photoperiod and found that vole dams 3 weeks postpartum responded with a greater number of follicles per follicle bundle in short-day lighting compared to long-day lighting. Finally, deer mice (*Peromyscus*) showed a decrease in fur density when exposed to warmer temperatures in the winter and an increase in fur density when exposed to cooler temperatures in the summer (47).

To our knowledge, seasonal changes in Norway rat pelage have not been studied, although there is evidence for other seasonal effects in rats. For example, wild female rats in natural settings show seasonal variation in pregnancy and/or lactation; the typical pattern is a reduction in pregnancy and/or lactation in winter, with peaks in spring and early autumn (8,21,36). Similarly, female laboratory rats demonstrate seasonal fecundity. Birth and survival rates were higher in the late spring to early autumn and lower in the winter (33,44). Male Norway rats living under controlled laboratory conditions nevertheless demonstrate seasonal variations in gonadal, pituitary, pineal, and thyroid activity (40–42,45,55). The stimuli and mechanisms responsible for these seasonal variations in laboratory rats remain to be determined.

The present experiment examined the hypothesis that rat pups with prior cold experience develop greater thermal insulation than do Warm-exposed pups. To relate potential effects of cold exposure to weaning onset, we evaluated fur growth on Day 16, just before weaning onset. At this age, the first wave of hair growth is still in progress (Gerrish, C. J., personal observation, 1994). It is well known that many mammals undergo cycles of hair growth during which the follicles actively produce hair, or hair is either retained as dead or "club" hair, or molt occurs (28,29). In black rats (*R. rattus*), the first cycle of hair proliferation continues until about the third week postpartum and then rests until Week 4 (43). Thus, in seasonal animals, the hair cycle varies with timing of the activity onset, number and amount of hair(s) produced by each active follicle, and number of club hairs that are shed or retained (29). Based on measures commonly used to evaluate pelage growth (30,31,34,35,37), we determined fur weight, length, and follicle density in Cold- and Warm-exposed pups.

Subjects

Twenty-four rat pups were used to study pelage weight at 16 days old (with the exception of one Cold-exposed subject that was 17 days old). Pups were randomly selected from different litters. Half were Cold-exposed and half were Warm-exposed (see below). Follicle density and fur length were analyzed in a subset of six males and six females. The majority of litters were born to dams that were not time mated. Dams had given birth to between zero and three litters previously. Day after birth was termed Day 1. Litters were culled to eight pups, four males and four females in Day 2. Stock and housing conditions were the same as reported in Experiment 1.

Housing

Dams and their litters were housed in standard maternal cages. Dams were provided with approximately 7 g of cotton batting to use for nest material. Food and water were supplied ad libitum.

Procedure

Exposure

The exposure procedure was the same as in Experiment 2 except that for two pups analyzed for fur density, cold-exposure began on Day 1 instead of Day 2.

Fur analysis

Pups were sacrificed and weighed. To determine relative hair weight, a 2 cm² patch of hair from the posterior dorsal surface of the pup was shaved and weighed on a Mettler balance accurate to 0.0001 g (30,31). A small patch of skin was then cut from the shaved patch and stored in aqueous Bouin's fixative. Additionally, several fur samples were plucked from the interscapular region to measure fur length.

To determine follicle density, the skin sample was embedded in paraffin, sectioned at 10 μ m, and counterstained with hematoxylin and eosin (35). Sections were placed under a grid, 360 \times 360 μ m, at 20 \times magnification. All guard hair and under hair follicles lying within the borders of the grid were tallied from 25 different fields for each animal.

To determine hair length, permanent slides were made by placing hairs on a slide, and securing a coverslip on top of the slide with a drop of Super glue. The slides were drawn at 19 \times magnification using a projection microscope. Drawings of individual hairs were measured with a digitizing tablet, and a computer-based morphometry system (Sigmascan). This method provides a precise measure of hair length, even if the hairs are not straight. Twenty-five guard and under hairs were drawn for each subject.

Data Analysis

In black rats (*R. rattus*) first hair cycle differentiation and hair growth both develop topographically beginning on the dorsum and spreading to the head, rump, and ventrum (43). Because the subjects in the present study had not completed the first cycle it was likely that their follicles and hairs would appear at varying stages of the cycle. Therefore, χ^2 tests were used to determine whether there were regional differences in follicle density and if there were differences in hair lengths between groups. This analysis relied on interval categories of fur growth (lengths, follicle densities), quantitatively defined. Each interval category encompassed a range of values and is labeled in Fig. 5 (top, middle, bottom) with the mid-point of that range. In addition, Student's *t*-tests (two-tailed) were used to analyze mean fur weight, follicle density, and fur length in Warm- versus Cold-exposed pups.

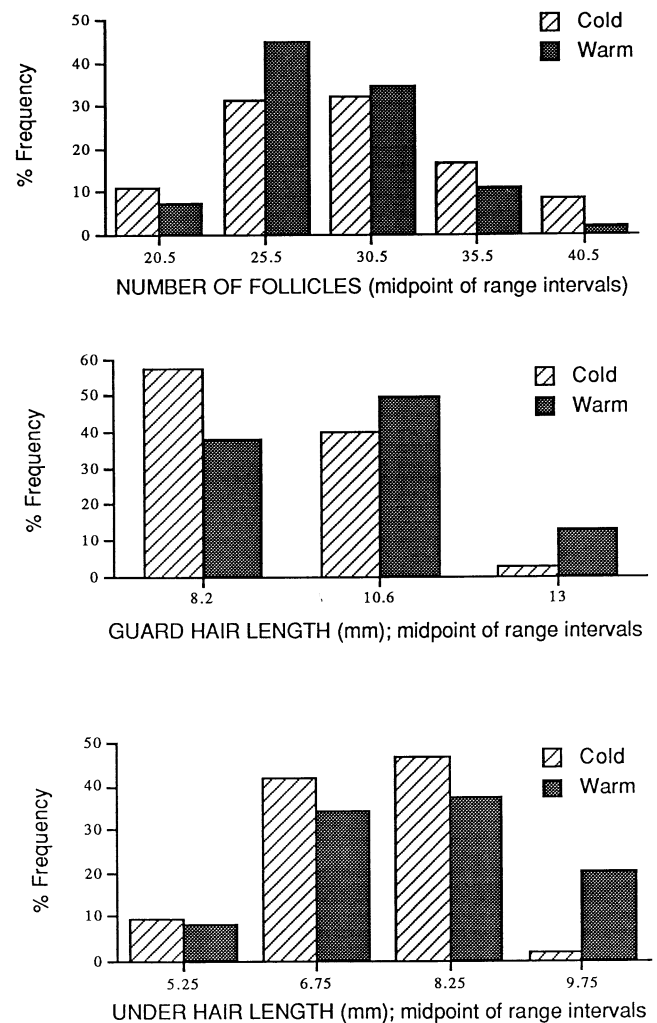


FIG. 5. (Top) Under hair follicle frequency distribution for Cold- and Warm-exposed pups. (Middle) Guard hair length frequency distribution for Cold- and Warm-exposed pups. (Bottom) Under hair length frequency distribution for Cold- and Warm-exposed pups.

RESULTS AND DISCUSSION

χ^2 analyses of total follicle densities (i.e., guard hair and under hair combined) indicated different distributions of follicle density between groups [χ^2 (7) = 14.12, $p < 0.05$]. These differences in total follicle density were not due to between-group differences in guard hair follicles [χ^2 (3) = 0.31, $p > 0.05$]. Rather, the distribution of under hair follicles was markedly different between groups [χ^2 (4) = 12.73, $p < 0.05$]. Figure 5 (top) shows that the distribution of under hair densities of Cold-exposed pups was skewed toward the right, indicating there are more patches of densely packed follicles in comparison to Warm-exposed pups. In Fig. 5 (top), for example, densities of 35.5 and 40.5 follicles per grid were 47.14% and 333.5% more frequent for Cold-exposed pups. Thus the difference in total follicle density is due to more patches of densely packed under hairs. These results are consistent with the notion that Cold-exposed pups would show greater insulation.

Distributions of guard hair and under hair lengths revealed significant differences between Cold and Warm-exposed pups

[guard hair: $\chi^2(2) = 17.13, p < 0.05$; under hair: $\chi^2(3) = 26.03, p < 0.05$]. Contrary to initial expectations for both hair types, Warm-exposed pups showed a greater frequency of longer hairs relative to Cold-exposed pups. In Fig. 5 (middle), for example, longer guard hairs (those in the 10.6- and 13.0-mm midpoint ranges) were 23% and 374.5% more frequent in Warm-exposed pups. For under hairs (see Fig. 5 (bottom)), at the 9.75-mm midpoint range, Warm-exposed pups had 930% more longer hairs compared to Cold-exposed pups. Thus, the different patterns of fur length distribution between groups is similar to the overall pattern of delayed growth and development for Cold-exposed relative to Warm-exposed pups found in Experiment 1.

Researchers interested in evaluating the development of fur growth should note that simple comparisons of mean follicle density and mean fur length revealed no differences between the groups. Mean total follicle density was 31.05 ± 1.34 for Cold-exposed pups and 30.12 ± 0.90 for Warm-exposed pups [$t(10) = 0.57, p = 0.58$]. Mean under hair follicle density was 28.83 ± 1.31 for Cold-exposed pups and 27.92 ± 0.90 for Warm-exposed pups [$t(10) = 0.57, p = 0.57$], while mean guard hair follicle density was 2.24 ± 0.09 for Cold-exposed pups and 2.21 ± 0.09 for Warm-exposed pups [$t(10) = 0.26, p = 0.80$]. Data for fur length indicated that mean guard hair length was 9.34 ± 0.23 mm for Cold-exposed and 9.95 ± 0.45 mm for Warm-exposed pups [$t(10) = -1.19, p = 0.26$], while mean under hair length was 7.36 ± 0.15 mm for Cold-exposed pups and 7.75 ± 0.42 mm for Warm-exposed pups [$t(10) = -0.87, p = 0.41$]. Thus, differences between groups in follicle density and fur length are revealed in frequency distribution patterns rather than by means.

To correct for differences in body weight between subjects, fur density was divided by body weight for each animal to determine the percentage of body weight that the fur sample comprised. Significant differences were found between the groups. Fur density was $0.060 \pm 0.003\%$ of body weight for Cold-exposed pups and $0.075 \pm 0.003\%$ of body weight for Warm-exposed pups [$t(22) = -3.63, p = 0.0015$]. These results further support the general pattern found in Experiment 1 of delayed growth and development in Cold-exposed relative to Warm-exposed pups.

Overall, measurements of fur length and fur weight indicated greater insulation for Warm-exposed pups. We also found that Cold-exposed pups demonstrated a higher frequency of more densely packed follicles which suggest a propensity toward greater insulation. Pups of this age were still in a hair growth cycle. Perhaps when the hair growth cycle was complete [around Week 3 in black rats, *R. rattus* (43)], the Cold-exposed pups relatively greater follicle density would result in superior insulation. Likewise, it is possible that thermogenic maturation of Cold-exposed pups might increase relative to Warm-exposed pups but at a later age. Early cold-exposure may enhance maturation of thermoregulatory capabilities, but not until later, even after weaning when the young engage in more prolonged forays outside the nest.

General Discussion

The present investigation was conducted to understand better the relations between a young mammal's basic behavioral development and its thermoregulatory status (19). Such developmental relations between behavior and physiology can be seen in the weaning process; we have used onset of independent feeding by young rats to measure weaning.

The hypothesized relation between weaning and thermoregulatory status arose from an earlier finding that rat pups begin to ingest solid food on about Day 17 if the ambient temperature of the feeding site is 30°C and on Day 19 if the ambient temperature of the feeding site is 10°C (19). In that study and in Experiment 1

herein, rats lived in a "seminatural habitat," that provided them with a nesting area at a physiologically moderate temperature, as might be found in a natal nest within a burrow (49) and a separate but accessible outside area with a separately regulated temperature, enabling us to simulate the thermal challenges a pup might encounter upon leaving the nest to go to a food cache elsewhere in a burrow system or forage outside (2).

The temperature at a feeding site affects weaning by influencing the likelihood and duration of pups' egression from the nest (19). While pups are outside the nest, they are exposed to numerous, different proximal influences, including social cues (see 10,11 for review) that influence their movements, behavior, and encounters with food (13,18).

The influence of temperature on behavior inevitably involves a combination of ambient temperature with the thermoregulatory status of the organism. In the case of a young, small, developing mammal, thermoregulatory status is exceptionally dynamic—it is the summation of body size, body composition, capacity to mobilize energy for heat production, and a variety of mechanisms for heat retention. Each of these factors changes rapidly and profoundly during postnatal ontogenesis (26). The weanling-aged rat pup is, in fact, at the nexus of morphological and physiological changes that enhance its ability to generate and conserve heat. Maturation of these factors, we think, *thermally emancipates* the juvenile and permits it to venture from and remain outside the nest for sufficient time to be exposed to new stimuli and engage in novel behaviors—including independent ingestion.

The purpose of Experiment 1 was to use daily, acute temperature exposures to alter development of thermoregulation and then evaluate a predicted alteration in weaning onset. The exposure regime had a profound effect on the pups' development: An entire suite of behavioral and maturational factors were shifted by early thermal experience. Egression from the nest and initial ingestion of solid food were delayed. These results were likely not due to either the behavior of the dams or alteration in the pups' suckling behavior. Recall that there were no differences between groups in either of these measures. Furthermore, eye opening was delayed in Cold-exposed pups relative to Warm-exposed subjects. Daily, acute experiences with cold were also associated with lower body weight, lower Tr (Experiment 2) and lighter fur weight (Experiment 3). Cold-exposed pups displayed a syndrome of delayed growth and maturation; weaning decelerated. Thus, our general hypothesis of a link between maturation of body temperature control and weaning received support. The fact that the developmental delay was directionally opposite from our initial prediction, if anything, underscores the coherence within the pattern of results.

Interestingly, other variables can delay early growth and development in laboratory and feral animals. Meadow voles (*Microtus pennsylvanicus*) given ad libitum food respond to decreased day length with lowered food intake, reduced body weight, and increased pelage density (6,7), which is presumed to serve as a means of conserving energy by reducing winter foraging time and thermoregulatory effort (6,7). Hill (25) reported unpublished data indicating that field mice (*Peromyscus leucopus*) given daily exposure to 10°C from Days 2 to 15–16 showed delayed eye opening, ear opening, lowered body temperature (although these mice could have been in daily torpor), and were smaller than mice exposed to 28°C. Finally, rats born during winter months achieved puberty later (42,46) and showed reduced body weight (42) as opposed to those born during the summer, even under controlled laboratory conditions. It is possible that pups in the present study might have used a seasonally sensitive mechanism to slow growth, development, and, hence, energy expenditure.

Although comparison of thermogenic capabilities on Days 15–16 failed to reveal an effect of thermal experience (Experiment

2), measurements of fur weight and fur length indicated that Warm-exposed weanlings were better insulated and thus had enhanced overall thermoregulatory status, at least around Day 16 (Experiment 3). We noted several indices suggesting that more thorough developmental testing might reveal a different, dynamic picture of temperature-induced changes.

We have suggested that the weaning transition is driven by events and processes unrelated to ingestion. It is known that diminished maternal milk availability does not instigate first ingestion of solid food (53). Instead, weaning is determined by factors that permit egression from the nest, which, in turn, allow the young to explore alternative nutritive resources that will soon be vital to their existence. Environmental temperature and thermoregulatory development emerge as critical determinants of the age that the young leave the confines of the nest. Thermal factors are also important to the timing of the repeated bouts of nest egressions and independent feedings that occur on any given day. Gerrish and Alberts (20) found that at the time of weaning onset, pups were more likely to leave the nest and ingest solid food just following a nursing bout than at other times. We also found that recently suckled pups displayed greater rate of oxygen consumption rates than did nonsuckled pups. These results suggest a thermogenic effect of milk that might contribute to nest egression by providing an immediate source of heat as the young encounter and explore the extra-nest environment. Finally, suckled pups showed increased play behavior relative to nonsuckled pups. Play represents another nonnutritive behavior that moves the pup about its environment and is thus likely part of egression from the nest.

After the young exit the nest a number of factors direct their activities toward solid food. For example, the young are attracted to the presence of adults in the vicinity of food (12,13) and to adult residual odor cues left around a food dish (14). In the absence of

adult presence, weaning is delayed (12). Furthermore, once in the vicinity of solid food, pups are more likely to ingest foods that bear chemosensory similarity to mother's milk (15,16). Finally, pups rapidly learn about the nutritive content of food through postingestive consequences of consuming it (39).

In conclusion, we have found that early thermal experience and its effect on thermoregulatory development represent general, non-ingestive factors that are directly linked to the timing of first nest egression and first ingestion of solid food. Rat pups displayed a striking response to early cold-exposure: growth, the achievement of developmental milestones and aspects of thermoregulatory development were modified. We propose that similar to seasonal animals responding to changes in day length, Cold-exposed rat pups responded to their previous thermal experience in a fashion that conserves metabolic expenditures. Cold-exposed rat pups delay first nest egression and weaning onset. Warm-exposed rat pups, on the other hand, showed more mature thermoregulatory capabilities and, in turn, demonstrated earlier first egression from the nest and first ingestion of solid food. Clearly, early thermal experience and developing thermoregulatory capabilities are critical factors in the timing of weaning onset and should be included in analysis of the weaning process.

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