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A Comparative Analysis of Huddling in Infant Norway Rats and Syrian Golden Hamsters: Does Endothermy Modulate Behavior?

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In infant rats, huddling improves surface-to-volume ratios and provides metabolic savings during cold exposure. It is unclear, however, whether endothermy is also a necessary component of huddling. In the present experiment, huddles composed of infant Norway rats (2- or 8-day-olds), which produce heat endogenously, or Syrian golden hamsters (8-day-olds), which do not produce heat endogenously, were exposed to decreases in air temperature. Behavioral and physiological responses were monitored throughout the test. Rats, especially at 8 days of age, were better able to thermoregulate using huddling than hamsters, due in part to endogenous heat production. Furthermore, 8-day-old rats exhibited behavioral responses that promote heat retention, suggesting that both physiological and behavioral mechanisms contribute to effective thermoregulation during huddling in the cold.

Altricial infants are generally born into large litters that allow for aggregation of the group (Nedergaard, Connolly, & Cannon, 1986). When the mother is away from the nest and the litter is exposed to a cold environment, aggregation, or huddling, attenuates heat loss by reducing each infant's exposure to the cold (Alberts, 1978; Hull & Hull, 1982; Newkirk, Silverman, & Wynne-Edwards, 1995; Schank & Alberts, 1997; Schmidt, Barone, & Carlisle, 1986). Reducing heat loss with huddling is especially important for altricial infants that are born with relatively little fur and insulation (Nedergaard et al., 1986). Therefore, huddling can be viewed as an essential form of behavioral thermoregulation because endogenous heat production cannot sufficiently compensate for the infant's rapid heat loss (Leon, 1986; Satinoff, 1996).

Contrary to the conventional view that rapid heat loss negates any benefits of endogenous heat production for individual altricial infants, recent work has shown that, in the case of infant rats, heat production by brown adipose tissue (BAT) is sufficient to ameliorate many of the adverse effects of cold exposure (Blumberg & Sokoloff, 1998). Specifically, at moderate levels of cold exposure, heat produced by BAT helps to maintain cardiac rate, protects the expression of active sleep, and prevents emission of ultrasonic vocalizations (Blumberg & Sokoloff, 1998). In contrast, when exposed to extreme air temperatures, BAT thermogenesis can no longer increase to combat heat loss, resulting in bradycardia, behavioral arousal, and emission of ultrasonic vocalizations.

Given the importance of BAT for the production of heat in the individual and the importance of huddling for the retention of heat in the group, the following question is raised: Is BAT thermogenesis an emergency mechanism that is activated only when individual infants are isolated from the nest, or is it a necessary component of effective thermoregulation in the huddle? For many years, BAT thermogenesis has been viewed as an emergency mechanism because of a widespread assumption that huddling is sufficiently effective for heat retention to minimize the importance of endogenous heat production (Satinoff, 1996). This assumption was an unintended consequence of Alberts' (1978) compelling demonstration that huddling infants incur significant metabolic savings during cold exposure.

There are at least two potential approaches to assess the importance of BAT thermogenesis for huddling during cold exposure. One approach involves the pharmacological blockade of BAT thermogenesis in the infants of an endothermic species (Sokoloff & Blumberg, 1998). An alternative approach is to adopt a comparative strategy by analyzing huddling behavior in two species of altricial infants that differ in their ability to produce heat endogenously. We adopt this latter approach in the present article by comparing the effectiveness of huddling in infant rats (*Rattus norvegicus*) that produce heat endogenously at birth and infant Syrian golden hamsters (*Mesocricetus auratus*) that do not produce heat endogenously until the end of the second week postpartum (Blumberg, 1997; Hissa, 1968).

Although infant hamsters do not produce heat endogenously, they are capable of rapid orientation and locomotion toward a source of heat (Leonard, 1974). Indeed, infant hamsters exhibit more robust thermotactic responses than infant rats (Kleitman & Satinoff, 1982). Therefore, in infant hamsters, it appears that behavioral thermoregulatory responses compensate for inadequate physiological capabilities. It is not clear, however, whether the infant hamster, with its relatively developed behavioral abilities but lack of endothermy, is able to thermoregulate effectively in the context of the huddle (Leonard, 1982).

To examine the role of endothermy and its interaction with huddling, huddles of infant rats and hamsters were exposed to multiple subthermoneutral air temperatures. For this experiment, comparisons were made between different-sized huddles of rats

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and hamsters at 8 days of age. In addition, because the gestation period of Syrian golden hamsters is 16 days—6 days shorter than that of Norway rats—we controlled for gestational age by testing an additional group of 2-day-old rats. The results of the present experiment suggest that huddling during cold exposure is facilitated by physiological thermoregulation, as infant rats, especially 8-day-olds, maintained higher rates of oxygen consumption (one measure of endogenous heat production) and huddle temperature than did infant hamsters. Our results, therefore, suggest that both behavioral and physiological mechanisms are essential for effective thermoregulation during huddling.

Method

Subjects

Ninety-six 2-day-old rats (PD2 rats), 48 eight-day-old rats (PD8 rats), and 96 eight-day-old hamsters (PD8 hamsters) were used. Rat pups were from 35 litters born to Sprague–Dawley Norway rat females, and hamster pups were from 24 litters born to Syrian golden hamster females. Mothers and their litters were housed in standard laboratory cages (48 cm × 20 cm × 26 cm) in the animal colony at the University of Iowa where food and water were available ad libitum. Body weights ranged from 6.1–10.9 g for the PD2 rats, 14.3–22.4 g for the PD8 rats, and 7.6–14.7 g for the PD8 hamsters. All litters were culled to eight pups within 3 days after birth (day of birth = Day 0). Rats were maintained on a 12-hr light–dark schedule and hamsters were maintained on a 14:10-hr light–dark cycle (lights on at 0600).

Test Environment

A detailed description of the test environment is provided elsewhere (Blumberg & Stolba, 1996). Briefly, huddles of pups were tested in a double-walled glass chamber (height = 17 cm; i.d. = 12.5 cm). Air temperature inside the chamber was controlled by circulating temperature-controlled water through the chamber's walls. Access holes in the chamber wall and lid allowed for the connection of thermocouples and the passage of air into and out of the chamber. A round polyethylene mesh platform, fitted inside the chamber, supported the pups approximately 8 cm above the floor of the chamber.

Temperature Measurements

Air temperature (T_a) and the temperature of the chamber wall ($T_{chamber}$) were measured using calibrated chromel-constantan thermocouples (Omega, Stamford, CT) accurate to within 0.1 °C. Signals from the thermocouples were fed into a computerized data acquisition system (National Instruments, Austin, TX). T_a within the metabolic chamber was determined by using a thermocouple situated 4 cm beneath the platform. $T_{chamber}$ was measured by attaching a thermocouple to the glass wall on the inside of the metabolic chamber. For the measurement of huddle temperature, it was not possible to attach thermocouples to individual infants, as the thermocouple wires could interfere with behavior or the thermocouples themselves could be removed through the activity of other infants. Therefore, the temperature beneath the huddle was measured with three thermocouples attached to the underside of the mesh platform, each approximately 1.5 cm from the center. Huddle temperature (T_{huddle}) was defined as the highest value among the three thermocouples at each time point throughout the experiment.

Oxygen Consumption Measurements

Oxygen consumption was measured as described elsewhere (Blumberg & Stolba, 1996). Briefly, compressed air was passed through two lines. One line circulated air through the metabolic chamber at 300 ml min^{-1} pup^{-1} (Alberts, 1978). The air was then drawn from the chamber into the first channel of an electrochemical oxygen analyzer (Ametek, Pittsburgh, PA). A second line of air passed directly to the second channel of the oxygen analyzer. The percentage of oxygen present in each airstream was measured simultaneously and the percentage of O_2 difference between the two airstreams was computed to 0.001%. Data were then fed into the computerized data acquisition system and converted into a measure of oxygen consumption ($\dot{V}O_2$) in ml O_2 kg^{-1} min^{-1}.

Ultrasonic Vocalizations

Ultrasonic vocalizations were made audible and recorded with a bat detector (QMC, Ltd., London, United Kingdom, Model SM100) connected to a microphone sealed inside the lid of the metabolic chamber. The bat detector was tuned to a frequency of 42 kHz (±5 kHz).

Data Acquisition

Thermal and metabolic measures were acquired once every 15 s throughout the test by a customized data acquisition system for the Macintosh computer (LabView, National Instruments, Austin, TX). A minicamera situated above the chamber lid was connected to an S-VHS videorecorder and allowed for the recording of behavior throughout the test. The output from the bat detector was fed into one of the audio channels of the videorecorder, thus allowing for ultrasound to be recorded simultaneously with behavior.

Behavioral Data

Behavioral data were analyzed for 1 pup within each huddle. This pup was designated as the "focal pup" at the beginning of the experiment and was marked for identification before being placed in the chamber. The focal pup thus provided representative data for all of the infants in the huddle.

The test was divided into a 15-min baseline period at thermoneutrality (i.e., 35 °C) and a succession of 1-hr periods at subthermoneutral air temperatures (i.e., 30 °C, 25 °C, 20 °C, 15 °C, and 5 °C). For each 1-hr period, two 15-min periods were analyzed: The first 15-min period, designated the transition period, corresponded to the time of transition from one air temperature to the next, and the fourth 15-min period, designated the stability period, corresponded to the time when air temperature had stabilized. The middle 30 min were not scored.

For quantitative assessment of huddling, two measures were used: contact and three-dimensional huddling. *Contact* was defined as the duration of each 15-min period in which the focal pup maintained at least one headlength of contact with one or more of the other infants in the huddle, and *three-dimensional huddling* was defined as the portion of each 15-min period during which the focal pup was on top of, underneath, or in between any of the other infants in the huddle.

Sleep and awake behaviors were also scored for the focal pup in each huddle. Our previous work with individual infants indicates that these behaviors provide information as to the intensity of the cold challenge being experienced (Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998). *Active sleep* was defined on the basis of the occurrence of myoclonic twitching (Blumberg & Lucas, 1994). *Twitching* is defined as phasic, rapid, and independent movements of any area of the pup's body, especially the limbs and tail (Blumberg & Lucas, 1994; Blumberg & Stolba, 1996; Gramsbergen, Schwartze, & Prechtl, 1970).

Awake behavior was divided into two categories: stationary and translational. *Stationary awake behavior* was defined as the duration of each 15-min period in which the focal pup exhibited coordinated movements that did not involve locomotion or a change in body position in relation to the rest of the huddle; these behaviors included stretching, kicking, yawn-

ing, postural elevation of the head and/or torso, facial wiping, and mouthing. In contrast to stationary awake behavior, *translational awake behavior* was defined as the occurrence of forward locomotion, punting, or righting.

Interrater and Intrarater Reliability

Simple regression analyses were performed on randomly chosen segments of behavioral data for stability and transition periods at multiple values of $T_{chamber}$. These comparisons were made for all three groups of infants and all three huddle sizes. Interrater reliabilities for sleep and awake behaviors, contact, and dimension were consistently high, $rs \geq 0.84$. Intrarater reliabilities for the same measures were typically higher, $rs > 0.98$.

Procedure

On the day of testing, 2, 4, or 6 pups, all with visible milk bands, were removed from their home cage and weighed. (Due to their large size in relation to the chamber, huddles of 6 PD8 rats were not studied.) The focal pup was marked for identification. Huddles were composed equally of male and female infants, and focal pups were represented equally by both sexes.

Huddles were then placed in a metabolic chamber maintained at 35 °C. The infants in the huddle were allowed to acclimate for 45 min, after which videotaping began and the first 15-min period of behavioral data was acquired. After 15 min, the temperature was decreased to 30 °C and the infants were allowed to stabilize for 1 hr. This procedure was repeated for changes in temperature to 25 °C, 20 °C, and 15 °C. In addition, because PD8 rats are larger and produce substantial amounts of heat with BAT thermogenesis, these huddles were also tested at 5 °C. After exposure to the final temperature, the infants were removed from the chamber and returned to their home cage.

The protocol used here, in which air temperature is decreased sequentially, produces a confound between air temperature and time. To avoid this confound, we could have exposed huddles to multiple air temperatures using a balanced design, but such a design would introduce new problems. First, a balanced design would require huddles to experience variable temperature transitions of 5 °C, 10 °C, 15 °C, or 20 °C, thus introducing the magnitude of the temperature transition as a new variable. Second, because these transitions would involve heating as well as cooling, the direction of the temperature transition would also be introduced as a new variable. Furthermore, because pups of different ages and species would respond very differently to these variable temperature transitions in a balanced design, the confounding of air temperature and time would remain. Finally, the sequential protocol used here and in previous research (Blumberg, 1997; Blumberg, Sokoloff, & Kirby, 1997; Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998) has proven to be effective for revealing the orderly patterns of behavioral and physiological responding during thermal challenge.

Data Analysis

Video records were scored by an experienced observer. For each value of $T_{chamber}$, two scoring passes were made through each 15-min segment of data. One pass consisted of scoring active sleep (i.e., myoclonic twitching), awake behaviors, and ultrasonic vocalizations. The other pass consisted of scoring contact and three-dimensional huddling. Each behavior was scored by making key presses on a computer keyboard. These key presses were recorded by a custom program written in HyperCard for the Macintosh (Claris, Santa Clara, CA), which created raw data files that consisted of each key press for the individual behaviors and the time that the key press occurred.

The raw data for active sleep, awake behaviors, contact, and three-dimensional huddling were then analyzed with custom programs written in HyperCard that calculated the duration of each behavior for the 15-min period. Converted data were then imported into StatView 4.5 for the Macintosh (SAS Institute, Cary, NC) for statistical analysis. For ultrasonic vocalization data, the total number of individual pulses was calculated for each 15-min period. Although all other behaviors scored are representative of only the focal pup, the measure of ultrasound production represents the combined vocalizations of all of the infants in the chamber.

At each temperature, a mean value for thermal and metabolic data was calculated from 60 data points acquired over the appropriate 15-min period corresponding to the acquisition of behavioral data (in a few cases for $\dot{V}O_2$, fewer than 60 data points were available for analysis). $T_{chamber}$ was used instead of T_a due to the small, but significant, effect of the presence of multiple littermates on the measurement of T_a. Finally, thermal and metabolic data (i.e., $T_{chamber}$, T_{huddle}, and $\dot{V}O_2$) were imported into StatView 4.5 for analysis.

The physiological and behavioral data were analyzed with repeated-measures analysis of variance (ANOVA). Single factor ANOVAs and Fisher's protected least significant difference were used as post hoc tests when significant main effects and/or interactions were obtained. Alpha was set at $p < .05$. For missing data, values were interpolated from appropriate group means. These instances of interpolation were rare (≤ 1.5% of all data cells). All means are presented with their standard errors.

Results

Physiological Measures

Figure 1 shows T_{huddle} and $\dot{V}O_2$ for all huddles of infants for the stability period at each value of $T_{chamber}$. As expected, T_{huddle} decreased during cold exposure, PD2 rats: $F(4, 84) = 3448.3$; PD8 rats: $F(5, 70) = 788.7$; PD8 hamsters: $F(4, 84) = 25{,}190.8$; $ps < .0001$, although values of T_{huddle} were higher for huddles composed of more than two pups, PD2 rats: $F(2, 21) = 8.2$; PD8 rats: $F(1, 14) = 18.3$; PD8 hamsters: $F(2, 21) = 28.6$; $ps < .005$. Moreover, in contrast to the PD8 rats, both PD2 rats and PD8 hamsters exhibited greater decreases in T_{huddle} with each successive decrease in $T_{chamber}$, regardless of huddle size.

The bottom row in Figure 1 shows $\dot{V}O_2$ for all huddles of infants. As was expected, due to the ability to produce heat through BAT thermogenesis, both groups of infant rats showed increases in $\dot{V}O_2$ as $T_{chamber}$ decreased, PD2 rats: $F(4, 84) = 324.2$; PD8 rats: $F(5, 70) = 138.8$; $ps < .0001$. For the PD8 rats, it is also clear that the increase in $\dot{V}O_2$ differed between the two huddle sizes as $T_{chamber}$ decreased; specifically, the initial increase in $\dot{V}O_2$ was higher in the 2-pup huddles and remained so until the $T_{chamber}$ of 20 °C was reached ($ps < .05$). Lower $\dot{V}O_2$ for the 4-pup huddles, at values of $T_{chamber}$ above 20 °C, is consistent with the idea that there is a metabolic savings as more infants are added to the huddle (Alberts, 1978). In contrast, hamster pups showed progressive decreases in $\dot{V}O_2$ as $T_{chamber}$ decreased, as would be expected from the absence of endothermy and the direct suppression of cellular metabolism by the cold, $F(4, 84) = 973.8$, $p < .0001$.

The results shown in Figure 1 suggest that PD8 rats gain more thermoregulatory advantage during huddling in the cold than do PD2 rats and PD8 hamsters. This interpretation of the data, however, would be more reasonable if a comparison could be made between all three groups of infants. Because individual PD2 rats weighed less than PD8 hamsters, which weighed less than PD8 rats ($9.7 < t_{46} < 28.6$, $p < .0001$), the following groups were compared: 2-pup huddles of PD8 rats (37.1 ± 1.6 g; $n = 8$); 4-pup

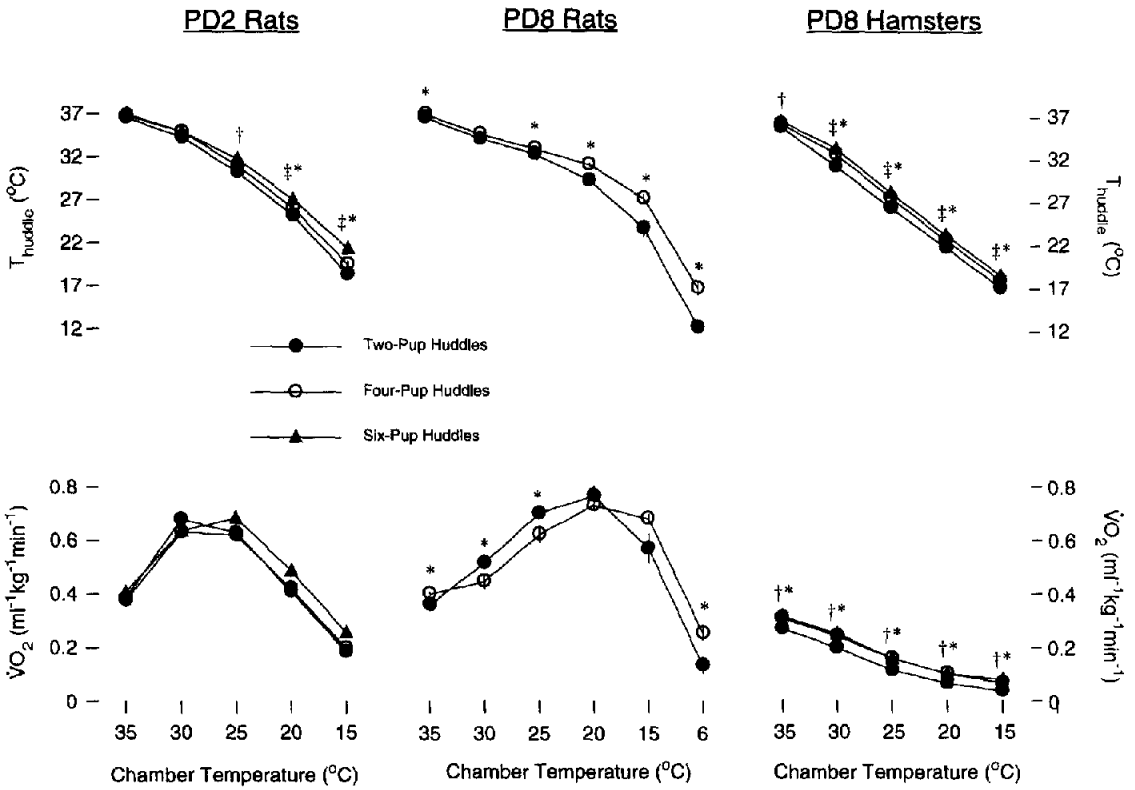

Figure 1. Huddle temperature (T_{huddle}) and oxygen consumption ($\dot{V}O_2$) for 2-, 4-, and 6-pup huddles of postnatal day (PD)2 and PD8 rats and PD8 hamsters. In general, PD8 rats showed a lower rate of heat loss at each chamber temperature. Both PD2 and PD8 rats increased $\dot{V}O_2$ as the chamber temperature decreased, indicative of increased heat production by brown adipose tissue thermogenesis. In contrast, PD8 hamsters showed linear decreases in $\dot{V}O_2$ as temperatures decreased. Values are means (± *SEM*). ‡ 6-pup huddles significantly different from 2- and 4-pup huddles. † 6-pup huddles significantly different from 2-pup huddles. * 4-pup huddles significantly different from 2-pup huddles.

huddles of PD8 hamsters (44.3 ± 2.0 g; $n = 8$); and 6-pup huddles of PD2 rats (45.8 ± 0.7 g; $n = 8$). Although the weights for these huddle compositions were not equivalent (i.e., two-pup huddles of PD8 rats weighed less than the other two groups), it will become clear that these differences in huddle mass cannot account for the results described below.

The top left-hand plot of Figure 2 shows T_{huddle} for 2-pup huddles of PD8 rats, 4-pup huddles of PD8 hamsters, and 6-pup huddles of PD2 rats. T_{huddle} was highest for the PD8 rats even though the huddle was only composed of 2 pups ($ps \leq .001$). T_{huddle} for 6-pup huddles of PD2 rats was higher than T_{huddle} for 4-pup huddles of PD8 hamsters ($ps < .001$). The lower left-hand plot of Figure 2 shows $\dot{V}O_2$ for the mass-equated huddles. As discussed above, both groups of infant rats showed increases in $\dot{V}O_2$. In PD8 rats, however, this increase was sustained at lower temperatures, whereas PD2 rats, like PD8 hamsters, showed progressive decreases in $\dot{V}O_2$ at each $T_{chamber}$ value below 25 °C.

The results described above indicate that huddling PD8 rats are more effective thermoregulators even when huddle mass is controlled. For the behavioral comparisons described below, however, equating huddles based on size (i.e., the number of infants in the huddle) is more appropriate because our behavioral measures (e.g., number of infants in contact with the focal pup) are sensitive to the number of infants within the huddle. Therefore, the physiological data were reanalyzed for huddles composed of 4 infants. The right-hand column of Figure 2 shows the plots for T_{huddle} and $\dot{V}O_2$ for 4-pup huddles of PD8 rats, PD2 rats, and PD8 hamsters. Again, differences in T_{huddle} and $\dot{V}O_2$ between the groups were significant, $F(2, 21) > 263.1, p < .0001$. More surprising, perhaps, is the result that PD2 rats, which are smaller and have less fur than PD8 hamsters, had higher values of T_{huddle} than the infant hamsters at all values of $T_{chamber}$ ($ps < .005$).

Behavioral Measures

Figure 3 presents the data for three-dimensional huddling, contact, and stationary and translational awake behaviors during the stability periods. The top left-hand plot of Figure 3 shows the amount of time spent by the focal pups in three-dimensional huddling. As stated before, this behavior was defined as the focal pup being either on top of, underneath, or in between other members of the huddle. All three groups of infants showed an increase in the duration of three-dimensional huddling as $T_{chamber}$ decreased, $F(4, 84) = 33.2, p < .0001$. PD8 rats, however, showed longer durations of three-dimensional huddling than the other two

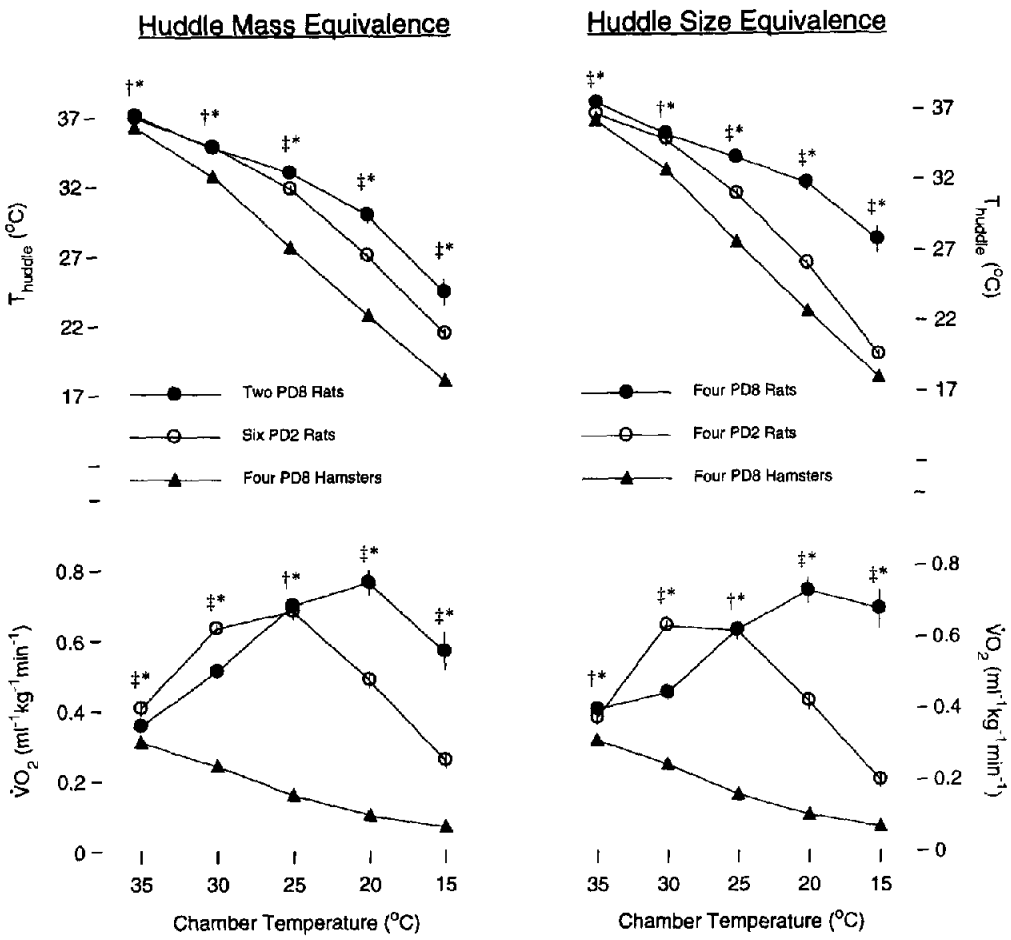

Figure 2. The left-hand plots show huddle temperature (T_{huddle}) and oxygen consumption ($\dot{V}O_2$) for mass-equated huddles, that is, 2-pup huddles of postnatal day (PD)8 rats, 4-pup huddles of PD8 hamsters, and 6-pup huddles of PD2 rats. The right-hand plots show huddle temperature and $\dot{V}O_2$ for 4-pup huddles. In general, PD8 rats showed a lower rate of heat loss than PD2 rats and PD8 hamsters, but PD2 rats showed a lower rate than PD8 hamsters. Again, both PD8 and PD2 rats showed increases in $\dot{V}O_2$ as the chamber temperature decreased, whereas PD8 hamsters showed linear decreases. Values are means (± *SEM*). ‡ PD8 rats significantly different from PD2 rats and PD8 hamsters. † PD8 rats significantly different from PD8 hamsters. * PD2 rats significantly different from PD8 hamsters.

groups of infants ($ps < .05$). The lower left-hand plot of Figure 3 shows the amount of time that the focal pups were in contact with the other 3 infants in the huddle. At baseline (35 °C) and 25 °C, PD8 rats spent more time in contact with their littermates than did the PD8 hamsters and PD2 rats ($ps < .005$).

For all groups, stationary awake behavior increased as $T_{chamber}$ decreased, $F(4, 84) = 155.0$, $p < .0001$. PD8 rats, however, showed less stationary awake behavior than the other two groups at each subthermoneutral $T_{chamber}$ ($ps < .0001$). For translational awake behavior, PD8 hamsters differed from the other two groups, with the focal hamster pups exhibiting more locomotor behavior at lower values of $T_{chamber}$ ($ps < .05$).

Figure 4 presents the behavioral data for the transition periods. The top left-hand plot of Figure 4 shows the amount of time spent by the focal pups in three-dimensional huddling. Again, PD8 rats spent more time in three-dimensional huddling than the other two groups ($ps < .01$), but all groups showed increases in the behavior as $T_{chamber}$ decreased, $F(3, 63) = 63.4$, $p < .0001$. Similar to the results found for the stability periods, as shown in the lower left-hand plot of Figure 4, PD8 rats spent more time in contact with all 3 littermates than did the PD8 hamsters and PD2 rats ($ps < .005$). For stationary and translational awake behavior, the results for the transition period were again similar to those for the stability period. PD8 rats showed the least amount of stationary awake behavior ($ps < .05$), and PD8 hamsters showed the greatest amount of translational awake behavior ($ps < .05$).

Figure 5 shows the amount of time each focal pup spent in active sleep, as measured by myoclonic twitching, during the stability and transition periods. For both periods, the amount of active sleep decreased in the cold, stability: $F(4, 84) = 157.9$; transition: $F(3, 63) = 144.9$, $ps < .0001$. In general, PD8 rats spent more time in active sleep than PD2 rats, which spent more time in active sleep than PD8 hamsters (stability: $ps < .05$; transition: $ps < .05$).

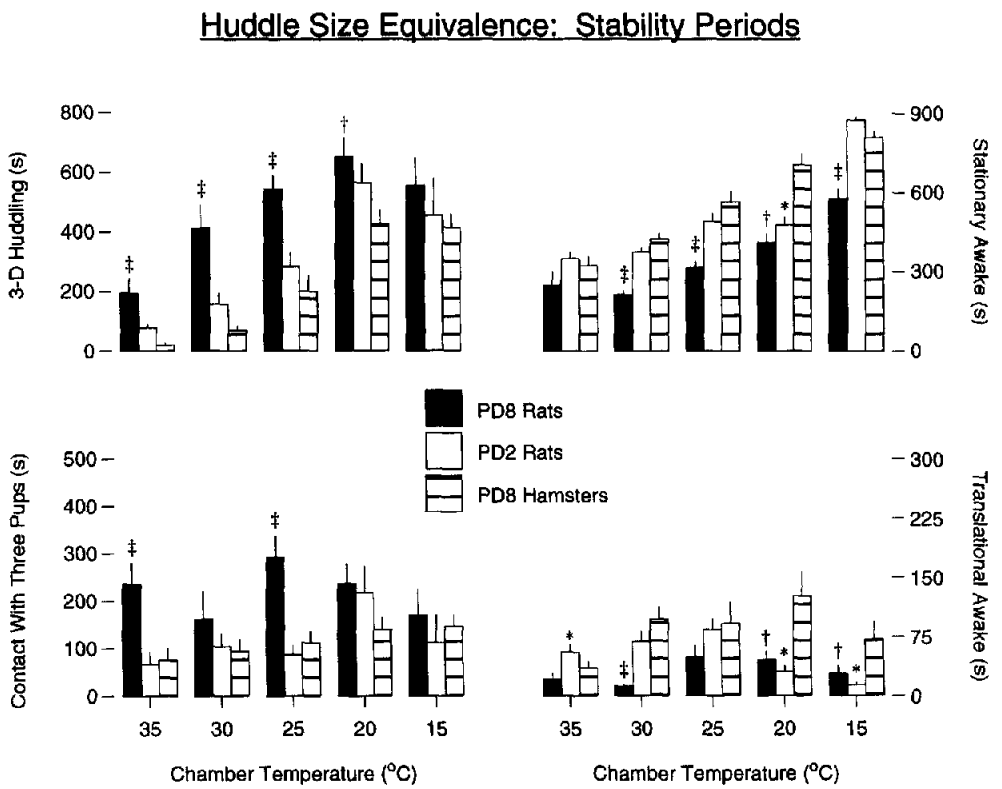

Figure 3. Three-dimensional huddling, contact, and stationary and translational awake behavior for the focal pups of the 4-pup huddles of postnatal day (PD)2 and PD8 rats and PD8 hamsters for the last 15-min period of each hour of cold exposure (stability). PD8 rats exhibited more three-dimensional huddling and less stationary awake behavior than both PD2 rats and PD8 hamsters. PD8 hamsters showed more translational awake behavior than both groups of infant rats. Values are means (± *SEM*). ‡ Significantly different from the other two groups. † Significantly different from PD8 hamsters. * Significantly different from PD8 rats.

Discussion

Infant Norway rats and Syrian golden hamsters differ in their ability to produce heat endogenously using BAT (Sokoloff & Blumberg, 1998). In the present experiment, this difference between rats and hamsters was reflected in higher values of T_{huddle} and higher $\dot{V}O_2$ during cold exposure for all huddle sizes of infant rats tested. The significance of endogenous heat production is made especially salient by the finding that PD2 rats maintained higher values of T_{huddle} than did the heavier, larger, and furred PD8 hamsters. However, although infant rats of both ages were better than hamsters at maintaining T_{huddle} and $\dot{V}O_2$ in the cold, PD8 rats were superior to PD2 rats. Indeed, 2-pup huddles of PD8 rats were superior to 6-pup huddles of PD2 rats even though their biomass was significantly less.

Consistent with the physiological differences between huddling infants noted above, behavioral differences were observed as well. First, there was a clear difference between infant rats and hamsters in the amount of motor activity observed. Specifically, PD8 hamsters exhibited more translational awake behavior during both stability and transition periods than either PD2 or PD8 rats. This increased motor activity may have been caused by their greater rates of cooling and, in addition, may have exacerbated those greater rates of cooling by preventing effective aggregation among individuals. Second, PD8 rats were more effective at forming and maintaining three-dimensional huddles than either PD2 rats or PD8 hamsters, thus helping to explain their superior resistance to cooling. This superiority is further demonstrated by the ability of PD8 rats to remain asleep at lower temperatures.

The finding that PD8 rats huddle more effectively than PD8 hamsters may seem counter intuitive given that infant hamsters are considered more effective behavioral thermoregulators than infant rats (Kleitman & Satinoff, 1982; Leonard, 1974). This behavioral superiority is consistent with the fact that infant hamsters do not produce heat endogenously before the third week postpartum (Blumberg, 1997; Hissa, 1968), thus placing a premium on behavioral thermoregulation (Leonard, 1982).

How can we reconcile the superiority of infant hamsters on a thermocline with their inferiority in the huddle? Perhaps the reconciliation lies in the availability, or lack thereof, of a warm target stimulus in the environment. For the ectothermic infant hamster in the nest, this target stimulus is most likely the mother; indeed, in hamsters, the mother rarely leaves her young during the first week postpartum (Leonard, 1974). When the mother is absent, however, there is no source of heat. In this instance, each individual hamster competes for access to the warmest region of the group, that is, the bottom of the huddle (Leonard, 1982); in turn, this com-

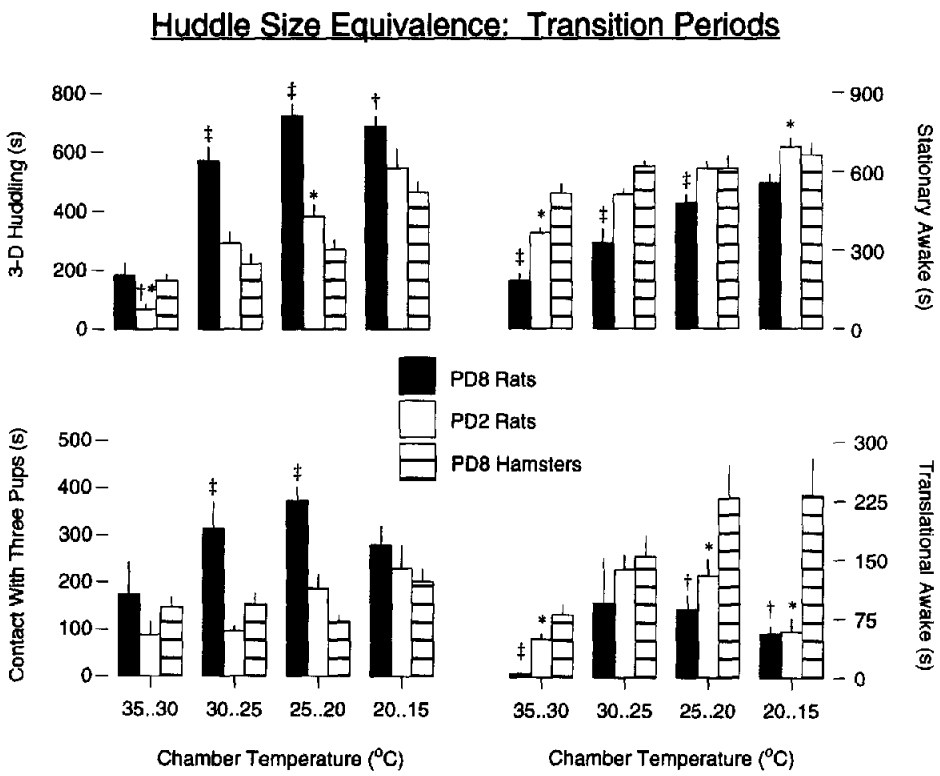

Figure 4. Three-dimensional huddling, contact, and stationary and translational awake behavior for the focal pups of the 4-pup huddles of postnatal day (PD)2 and PD8 rats and PD8 hamsters for the 15-min period corresponding to a change in the temperature of the metabolic chamber (transition). Again, PD8 rats exhibited more three-dimensional huddling and less stationary awake behavior than PD2 rats and PD8 hamsters. They also spent more time in contact with the other 3 infants in the huddle. Again, PD8 hamsters showed more translational awake behavior than the rats. Values are means (± *SEM*). ‡ Significantly different from the other two groups. † Significantly different from PD8 hamsters. * Significantly different from PD8 rats.

petition produces a constantly active, and hence unstable, huddle.

As shown here, with the increases seen in $\dot{V}O_2$, infant rats differ from infant hamsters in that they produce heat with BAT, even during huddling. Because heat is produced and distributed more widely among huddling infant rats, there is no single warm spot that can become a site of competition. As shown by Alberts (1978), individual infants will dive to the center of a huddle or climb out of the huddle depending on the needs of the moment. Cooperation can then emerge because the needs of each individual differ over time, including the occasional preference for remaining on the outside of the huddle. In contrast, the needs of infant hamsters are always the same—without a source of heat, infant hamsters must continuously seek the warmest region available, and no single individual will ever prefer the outside of the huddle. Thus, the presence or absence of endothermy appears to be the basis for the cooperative huddling exhibited by infant rats and the competitive huddling exhibited by infant hamsters.

As discussed earlier, for individual rats, moderate air temperatures are defined as those air temperatures that are associated with progressive increases in BAT thermogenesis and oxygen consumption (Blumberg & Sokoloff, 1998). In addition, as described earlier, infants exposed to such moderate temperatures maintain cardiac rate, remain asleep, and do not emit ultrasonic vocalizations. In contrast, at extreme air temperatures, BAT thermogenesis can no longer compensate for heat loss, thus resulting in rapid cooling and suppression of oxygen consumption. At this time, the cardiac rate plummets, the pups wake up, and they begin to vocalize.

We can also apply the definitions of moderate and extreme cold exposure to huddling infants. Figure 6 presents data from 2- and 4-pup huddles of PD8 rats during the final 15 min at each air temperature (i.e., the stability periods). It can be seen that increased rates of oxygen consumption were sustained at 20 °C for 2-pup huddles and 15 °C for 4-pup huddles. This figure also presents the data for individual week-old rats from a previous experiment (Sokoloff & Blumberg, 1998). In contrast with the data for huddling infants, the transition between moderate and extreme air temperatures is 24–26 °C. Thus, it is clear that the addition of one or three littermates extends the region of moderate cold exposure for an individual infant rat by approximately 5 °C and 10 °C, respectively. From Figure 6, the metabolic savings shown by Alberts (1978) is evident at the moderate air temperatures of 30 °C and 25 °C. Interestingly, as shown in the lower plot of Figure 6, ultrasound production increases dramatically at the transition to extreme air temperatures when oxygen consumption decreases, just as it does for individual infants. This finding of increased ultrasound production in the cold, even within the social context of

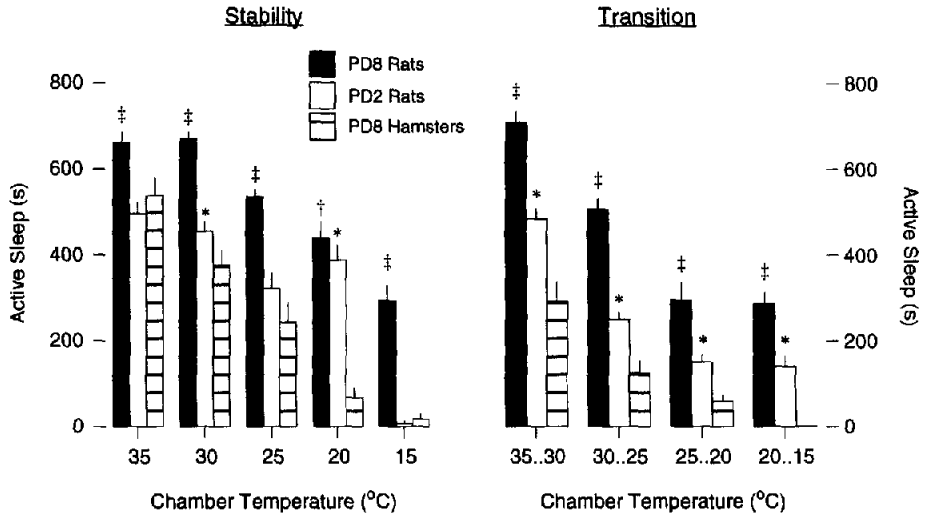

Figure 5. The amount of time spent in active sleep by the focal pups of the 4-pup huddles of postnatal day (PD)2 and PD8 rats and PD8 hamsters for both the stability and transition periods. PD8 rats spent more time in active sleep than PD2 rats and PD8 hamsters throughout the experiment. In addition, PD2 rats spent more time in active sleep than PD8 hamsters, especially during the transition periods. Values are means (± *SEM*). ‡ Significantly different from the other two groups. † Significantly different from PD8 hamsters. * Significantly different from PD8 rats.

huddling, is consistent with previous findings in infant rats (Blumberg, Efimova, & Alberts, 1992b).

The comparative method used here is effective for directing our attention to the role that BAT thermogenesis may play in the expression and effectiveness of huddling. Other experimental approaches, however, will be useful for assessing the role of BAT thermogenesis in huddling. One approach, for example, entails pharmacological blockade of BAT thermogenesis in infant rats, an approach that has been used effectively to assess the contributions of BAT thermogenesis to the behavioral and physiological responses of individual infants (Blumberg et al., 1997; Sokoloff & Blumberg, 1998). This pharmacological approach avoids confounds that may arise due to species-typical behavioral differences. A second approach involves testing huddles comprised of both infant rats and infant hamsters: If the competitive, highly active huddling behavior of infant hamsters truly results from a lack of multiple heat sources within the huddle, then infant hamsters should exhibit more "rat-like" huddling behavior when allowed to huddle with infant rats. In addition, for future studies, infrared thermography will be used to provide a more accurate and reliable measure of huddle surface temperature (Blumberg, Efimova, & Alberts, 1992a).

Finally, the power of Alberts' (1978) description of the efficacy of huddling in infant rats had the unintended consequence of leading some to conclude that group aggregation, by reducing heat loss, is sufficient for successful thermoregulation in the cold. Viewed in this light, endothermy, such as with BAT thermogenesis, becomes an emergency mechanism to be used when a pup is isolated from its littermates. In contrast, the present findings highlight the essential contribution that endothermy makes to successful thermoregulation in the huddle. These findings also suggest that species differences in endothermy play a central role in modulating behavioral interactions in the huddle, perhaps deter-

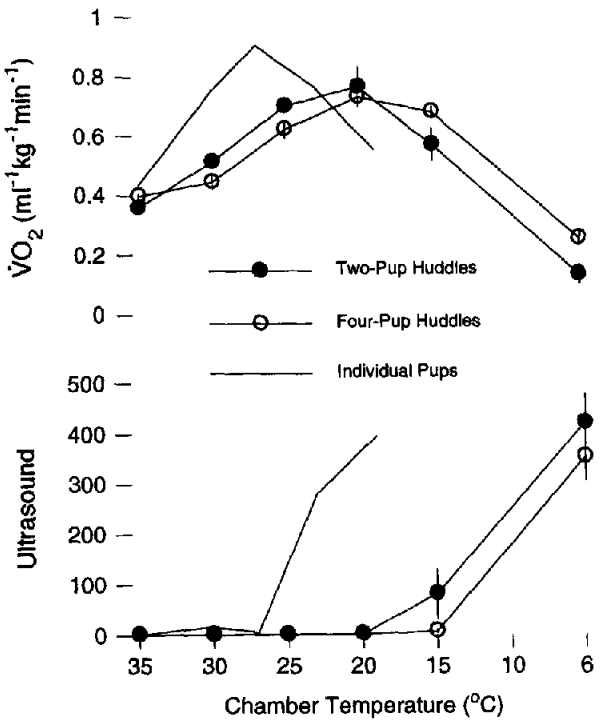

Figure 6. Oxygen consumption ($\dot{V}O_2$) and ultrasound production in Postnatal Day 8 rats (2-pup huddles: filled circles; 4-pup huddles: open circles) during cold exposure. The solid line shows mean $\dot{V}O_2$ and ultrasound production data for a group of individual week-old rats from a previous experiment (Sokoloff & Blumberg, 1998). The addition of 1 or more littermates extended the region of moderate cold exposure, as evidenced by a right-shift of the curves for $\dot{V}O_2$ and ultrasound production.

mining whether littermates behave cooperatively or competitively. In turn, species differences in endothermy may have cascading effects not only on behavioral interactions in the huddle, but on many aspects of behavioral development as well.

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