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Competition and Cooperation among Huddling Infant Rats

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ABSTRACT: *Huddling is expressed by infant rats and continues to be an important behavior throughout adulthood. As a form of behavioral thermoregulation, huddling is thought to play an essential role in compensating for inadequate physiological thermoregulation early in development. Infant rats, however, are capable of heat production shortly after birth using brown adipose tissue (BAT) and exhibit thermogenesis in the huddle, suggesting that huddling does not obviate the need for endothermy during cold exposure. In the present experiment, 4-pup huddles of infant rats (2- or 8-day-olds) were exposed to two subthermoneutral temperatures, and BAT thermogenesis was inhibited in 0, 2, or 4 of the rats in each huddle. Inhibition of BAT thermogenesis compromised the pups' ability to maintain huddle temperature, but surprisingly did not result in enhanced huddling at either age. These results suggest that effective huddling during cold exposure requires the thermal resources provided by endothermy. Furthermore, the heat provided by BAT appears to shape behavioral interactions in the huddle during development.* © *2001 John Wiley & Sons, Inc.* Dev Psychobiol 39: 65–75, 2001

Keywords: *behavior; brown adipose tissue; development; huddling; infant; norway rat; thermoregulation*

It has been shown recently that infant rats rely on both physiology and behavior when huddling during cold exposure (Sokoloff, Blumberg, & Adams, 2000). Physiologically, huddling 8-day-old rats show increases in brown adipose tissue (BAT) thermogenesis, as evidenced by increased rates of oxygen consumption and the maintenance of high huddle temperatures. Behaviorally, these infants exhibit increased contact with multiple littermates and increased three-dimensional buddling (i.e., piling), both of which act to decrease rates of heat loss from the huddle by improving surface-area-to-volume ratios. In contrast, younger rats (i.e., 2-day-olds) show increased rates of oxygen consumption, indicative of BAT thermogenesis, but do not show behavioral changes that would aid in retaining the heat produced by each individual. Furthermore, 8-day-old hamsters do not yet show BAT thermogenesis (Blumberg, 1997; Hissa, 1968) and also fail to demonstrate effective huddling behaviors during cold exposure. It appears, then, that the heat produced by BAT plays an important role in effective huddling.

Adult mammals are noted for their ability to maintain body temperature by recruiting a suite of physiological and behavioral mechanisms (Adair, 1977; Corbit, 1970). Moreover, compromising physiological thermoregulation in adults results in the rapid recruitment of behaviors that compensate for a physiological deficit (Carlisle, 1968; Richter, 1943; Satinoff & Rutstein, 1970). The finding that younger infant rats and hamsters show poorer behavioral responses to cold exposure than the larger and more physiologically competent 8-day-old rats, however, poses a problem for the idea that huddling compensates for inadequate physiological thermoregulation (Leon, 1986; Satinoff, 1996). Therefore, one possible

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explanation for the difference in huddling behavior between infants of different ages and species is that huddling is not a typical thermoregulatory behavior that compensates for a physiological deficit, but rather reflects a synergism between physiology and behavior that results in effective thermoregulation.

If huddling during cold exposure is the product of an interaction between physiology and behavior, how might the heat produced by BAT fulfill a role in effective huddling? First, as evidenced by differences between endothermic infant rats and non-endothermic infant hamsters, the heat provided by BAT is clearly an important resource for effective huddling (Sokoloff et al., 2000). Second, the heat produced by BAT may play a role in guiding the contact behavior of individuals in the huddle; the importance of thermal stimuli for guiding the behavior of infants has been well documented (Alberts, 1978b; Alberts & Brunjes, 1979; Alberts & May, 1984). Contact behavior among heat-producing littermates is advantageous as it retards heat loss and thereby lowers the metabolic demands of cold exposure (Alberts, 1978a; Schmidt, Barone, & Carlisle, 1986; Stanier, 1975).

If the endothermic capabilities of each individual infant rat play a role in establishing contact between littermates within the huddle, then huddling behavior should be disrupted when some infants in the huddle are capable of heat production and some are not, especially as cold exposure progresses. Specifically, heat-producing pups should actively seek other heat-producing pups and actively avoid non-heat-producing pups. Therefore, to assess the effect of unequal endothermic capabilities on huddling, huddles of 2- or 8-day-old rats comprised of 4 littermates were sequentially exposed to two subthermoneutral air temperatures. Huddles were assigned to one of three groups. In the first group (balanced saline), all 4 pups were treated with saline. In the second group (balanced chlorisondamine), all 4 pups were treated with chlorisondamine, a ganglionic blocker that inhibits BAT thermogenesis (Blumberg, Sokoloff, & Kirby, 1997). In the third group (unbalanced), 2 pups were treated with saline and 2 pups were treated with chlorisondamine. The behavior of representative focal pups in each huddle was scored, and infrared thermography was used to measure huddle temperature.

METHODS

Subjects

Ninety-six 2-day old (PD2) rats and ninety-six 8-day-old (PD8) rats were used. Rat pups were from 43 litters born to Sprague-Dawley Norway rats housed in standard laboratory cages ($48 \times 20 \times 26$ cm) in the animal colony at the University of Iowa. Food and water were available ad libitum. Body weights ranged from 5.6 to 9.9 g for the PD2 rats and 13.0 to 22.4 g for the PD8 rats. All litters were culled to 8 pups within 3 days after birth (day of birth = Day 0). The animal colony was maintained on a 12:12 hr light; dark cycle (lights on at 0600 hr).

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. Air temperature inside the chamber was controlled by circulating temperature-controlled water through the chamber's walls. Access holes in the side of the chamber allowed for the connection of thermocouples and the passage of air through the chamber at a rate of 1,200 ml min^{-1} (300 ml min^{-1} pup^{-1}; Alberts, 1978a). A round polyethylene mesh platform allowed the infants to move freely within the chamber.

Temperature Measurements

The temperature of the chamber ($T_{chamber}$) was measured using a calibrated chromel-constantan thermocouple (Omega, Stamford, CT) accurate to within 0.1°C. Outside the chamber the thermocouple was connected to a handheld digital thermometer (Omega). $T_{chamber}$ was measured by attaching the thermocouple to the glass wall on the inside of the metabolic chamber.

Infrared Thermography

The infrared (IR) thermography system (FLIR Systems, Portland, OR) consists of an IR camera, computer, and monitor for viewing images. The thermal images are acquired by a computer system that controls the camera as well as image acquisition (ThermaCAM Researcher 2000, FLIR Systems). The system is capable of storing images to hard disk at rates of up to 60 images/s. IR thermography allows for accurate and noninvasive measurements of surface skin temperature (Blumberg, Efimova, & Alberts, 1992).

To accurately measure absolute skin temperature using IR thermography, determination of the skin's emissivity was necessary. (Emissivity is the ratio of the radiant energy emitted by a surface to the energy emitted at the same temperature by a black body

radiator.) To accomplish this, the skin of PD2 and PD8 rats ($n = 4$ at each age) was heated to at least 40°C, and an emissivity value was obtained. Across a range of skin temperatures, values acquired using IR thermography were compared to those acquired using a calibrated thermocouple attached to the skin. Finally, for each age, average emissivity values were obtained, and a regression equation was derived with the thermocouple temperature as the independent variable and the IR temperature as the dependent variable. The equation then was used to adjust the values obtained using IR thermography.

Data Acquisition

Thermal data ($T_{chamber}$ and IR images) were acquired by the experimenter once every 15 min throughout the test. $T_{chamber}$ was recorded by hand, and 3 to 5 successive IR images were saved on hard disk. Continuous videorecording of behavior throughout the test was accomplished using a minicamera situated above the chamber lid.

Procedure

On the day of testing, 4 pups, all with visible milk bands, were removed from their home cage and weighed. Huddles were comprised equally of both male and female infants. Each pup was marked for identification. Huddles were placed in the metabolic chamber maintained at 35°C. The pups were allowed to acclimate in the chamber for at least 45 min. After approximately 30 min of acclimation, the pups received a subcutaneous injection of either the ganglionic blocker, chlorisondamine hydrochloride (5 mg/kg; Ciba Geigy Corp., Summit, NJ) or saline at an injection volume of 1 μl/g body weight. Two huddle conditions (i.e., balanced and unbalanced) and two treatment conditions (i.e., saline and chlorisondamine) were tested. Specifically, the balanced saline group consisted of 4-pup huddles in which all 4 infants were treated with saline. The balanced chlorisondamine group consisted of 4-pup huddles in which all 4 infants were treated with chlorisondamine. Finally, the unbalanced group consisted of 4-pup huddles in which 2 pups were treated with chlorisondamine and 2 pups were treated with saline.

At the end of the 45-min acclimation period, videotaping began, and a 15-min period of behavioral data was acquired at thermoneutrality (35°C). After 15 min, the lid of the chamber was removed, and the first thermographic images were acquired. (The experimenter was able to obtain images and replace the chamber lid within 10 s.) The temperature of the chamber then was decreased to 30°C for PD2 rats and 25°C for PD8 rats. Thermographic images were acquired at 15, 30, 45, and 60 min after the temperature decrease began. This procedure was repeated for the final change in temperature to 20°C for PD2 rats and 15°C for PD8 rats. After the test, the infants were removed from the chamber and returned to their home cage.

Behavioral Data

For the analysis of behavioral data, the test was divided into a 15-min baseline period at thermoneutrality (35°C) and successive 60-min periods at two subthermoneutral air temperatures (i.e., 30°C and 20°C for PD2 rats or 25°C and 15°C for PD8 rats). For each 60-min period, only the fourth 15-min period was scored. Because 45 min are sufficient for individual pups to stabilize at new air temperatures (Sokoloff & Blumberg, 1997), this period was designated the ''stability'' period.

Behavioral data were analyzed as described by Sokoloff et al. (2000). Briefly, for each huddle, data were analyzed for 1 pup from each treatment condition (i.e., 1 chlorisondamine- and/or 1 saline-treated pup). This pup was designated as a focal pup and was chosen randomly by the experimenter when behavioral scoring was conducted; focal pups were represented equally by male and female infants. For overall comparisons of huddle condition, a focal pup was randomly selected from each huddle, except for unbalanced huddles where 2 focal pups were selected—1 for each treatment condition (i.e., 1 chlorisondamine- and 1 saline-treated pup).

Two measures of huddling were scored: three-dimensional huddling and contact. Three-dimensional huddling was scored by determining when the focal pup was situated on top, underneath, or between the other littermates in the huddle. Contact behavior was scored by determining the number of littermates with which the focal pup was in contact at any given time during the 15-min stability period. In addition, active sleep (e.g., myoclonic twitching), stationary awake (e.g., yawning and stretching), and translational awake (e.g., forward locomotion and righting) behaviors were scored. These behaviors provide a useful index of the thermal success of the huddle during cold exposure (Sokoloff & Blumberg, 1998; Sokoloff et al., 2000).

Data Analysis

Videorecords were scored by experienced observers blind to huddle condition and treatment condition. For

each $T_{chamber}$ two scoring passes were made through each 15-min segment of data, one for the scoring of active sleep and awake behaviors and the other for scoring three-dimensional huddling and contact.

The behaviors of the focal pups were coded by key presses made by the observer during video playback. The key presses were recorded using an event recorder program written in HyperCard for the Macintosh. The raw data for active sleep, awake behaviors, three-dimensional huddling, and contact behavior were analyzed using programs written in HyperCard that calculated the duration of each behavior for each 15-min period. Converted data then were imported into StatView 4.5 for statistical analysis.

To assess inter- and intrarater reliability, simple regression analyses were performed on randomly chosen segments of behavioral data for Stability periods at each of the values of $T_{chamber}$. These comparisons were made for both ages of infants and for all huddle and treatment conditions. On average, interrater reliabilities yielded $rs \geq .90$. The average intrarater reliabilities for the same measures yielded $rs \geq .96$.

A mean value for $T_{chamber}$ was calculated from two data points acquired over the appropriate 15-min period corresponding to the acquisition of behavioral data. Huddle temperature (T_{huddle}) was analyzed by exporting thermal data from the IR images. Specifically, the temperature values (°C) of each pixel comprising the exposed surface of the huddle could be selected and imported into StatView 4.5 to obtain the average T_{huddle}.

IR images also provided a means for assessing the surface area of the platform covered by the huddle. The exposed surface area of the huddle was obtained by analyzing IR images with NIH Image (v. 1.62; U.S. National Institutes of Health, Washington, DC). An outline of the huddle was created by tracing around the exposed surface of the pups using a drawing tablet (Wacom Technology Corporation, Vancouver, WA). Percent changes in huddle surface area (ΔHuddle Spread) were obtained by subtracting the baseline (35°C) huddle surface area from its value at each time point during the experiment and multiplying this value by 100.

The physiological and behavioral data were analyzed using repeated measures analysis of variance (ANOVA). Single factor ANOVAs and Fisher's PLSD were used as post hoc tests when significant main effects and/or interactions were obtained. The α was set at .05. For missing data, values either were interpolated from appropriate group means or from the averages of individual values immediately preceding and following the missing value. These instances of interpolation were rare (behavioral data: 1.4% of cells for PD8 huddles; T_{huddle} and surface area: $<1\%$ of all cells), and the degrees of freedom were adjusted when necessary to compensate for missing data.

RESULTS

Inhibition of BAT thermogenesis in unbalanced and balanced chlorisondamine huddles resulted in a significant reduction in T_{huddle} during cold exposure. The plots in the top row of Figure 1 present the data for T_{huddle} for all huddles of infants. A single-factor ANOVA indicated that there was no significant difference in T_{huddle} at 35°C between any groups at either age. As $T_{chamber}$ decreased below 35°C, however, T_{huddle} began to differ between the groups, PD2 rats: $F(2, 21) = 22.3$; PD8 rats, $F(2, 21) = 50.9$, $ps < .0001$. Specifically, for PD2 rats (top left-hand plot), T_{huddle} for the balanced saline huddles was significantly higher than T_{huddle} for unbalanced and balanced chlorisondamine huddles during the 60-min period of exposure to 30°C ($p < .001$). At 20°C, however, T_{huddle} did not differ between balanced saline and mixed huddles, although the difference approached significance ($p < .07$). Similar differences were seen in T_{huddle}, for PD8 rats, with balanced saline huddles exhibiting significantly higher values for T_{huddle} than both mixed and balanced chlorisondamine huddles at both 25°C and 15°C (top right-hand plot; $ps < .005$).

The plots in the bottom row of Figure 1 present the data for the percent change in the exposed huddle surface area (ΔHuddle Spread) for all huddles of infants. A single-factor ANOVA indicated that there was no significant difference in the exposed huddle surface area at 35°C between any of the groups at either age. At both ages, all three huddle conditions showed decreases in ΔHuddle Spread as $T_{chamber}$ decreased, PD2 rats, $F(1, 21) = 24.3$; PD8 rats, $F(1, 21) = 70.3$, $ps < .0001$.

For PD2 rats (bottom left-hand plot), there were no differences between groups in ΔHuddle Spread despite the significant differences in T_{huddle}. This also was true for huddles of PD8 rats (bottom right-hand plot) at 25°C. During exposure to 15°C, however, balanced saline huddles exhibited a significantly larger ΔHuddle Spread than the other two groups ($ps < .05$). These results for both groups of rats suggest that huddles comprised of pups that lack BAT thermogenesis do not compensate for the lack of heat production by further decreasing ΔHuddle Spread.

Figure 2 presents IR images of the huddles of PD2 and PD8 rats at the end of 60 min of exposure to

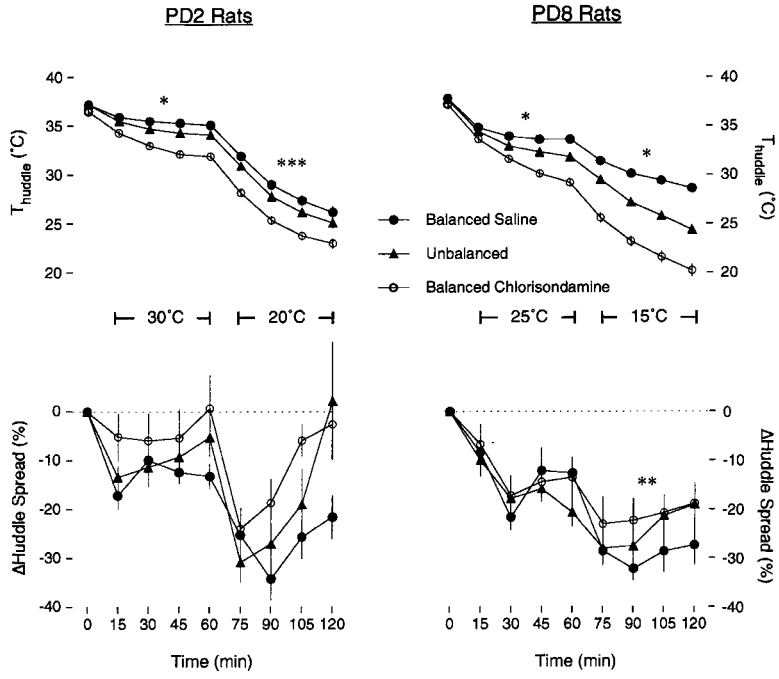

FIGURE 1 Huddle temperature (T_{huddle}) and percent change in huddle surface area (ΔHuddle Spread) for 4-pup huddles of PD2 and PD8 rats. When the temperature of the chamber decreased below 35°C, balanced chlorisondamine and unbalanced huddles were colder than balanced saline huddles at both ages. Similarly, the largest and most sustained ΔHuddle Spread was shown by balanced saline huddles. Mean ± *SEM*. *All three groups significantly different from one another for the 60-min period of cold exposure. **Balanced saline huddles significantly different from the other two groups for the 60-min period of cold exposure. ***Balanced saline and mixed huddles significantly different from balanced chlorisondamine huddles for the 60-min period of cold exposure.

20°C and 15°C, respectively. Pixels coded in white represent temperatures ≥31°C, and pixels coded in black represent temperatures ≤15°C (for more temperature values of pixel colors, refer to the figure legend). Consistent with Figure 1, the IR images in Figure 2 confirm the thermal advantage of balanced saline huddles. Interestingly, for PD8 rats, saline-treated pups from unbalanced huddles appear cooler than saline-treated pups in balanced saline huddles, and chlorisondamine-treated pups from unbalanced huddles appear warmer than chlorisondamine-treated pups from balanced chlorisondamine huddles. This suggests that the heat produced by the saline-treated pups is a thermal resource for the chlorisondamine-treated pups.

For all focal pups at both ages, active sleep (i.e., myoclonic twitching) decreased as $T_{chamber}$ decreased, $F(2, 55) > 7.3$, $ps < .01$. For PD8 rats, regardless of huddle composition, saline-treated focal pups slept more than chlorisondamine-treated pups, $F(1, 28) = 19.9$, $p = .0001$. Conversely, stationary awake behavior increased during cold exposure, $F(2, 55) > 32.3$, $ps < .01$. For PD8 rats, saline-treated focal pups showed less stationary awake behavior than chlorisondamine-treated pups, $F(1, 28) = 20.1$, $p = .0001$. Finally, for both ages, although overall levels of translational awake behavior were very low, saline-treated focal pups showed more translational awake behavior than chlorisondamine-treated focal pups during exposure to the final $T_{chamber}$ (20°C for PD2 rats and 15°C for PD8 rats; $ps < .05$).

The results presented in Figures 1 and 2, along with the results from the analysis of sleep and awake behaviors, suggest that BAT thermogenesis is a necessary resource for effective huddling during cold exposure. Balanced saline huddles not only showed higher values of T_{huddle} but also showed larger reductions in the exposed surface area of the huddle. Furthermore, differences in the amount of sleep and awake behaviors are consistent with previous results in which the inhibition of BAT thermogenesis resulted in less sleep and more awake behavior in individual rats during cold exposure (Sokoloff & Blumberg, 1998).

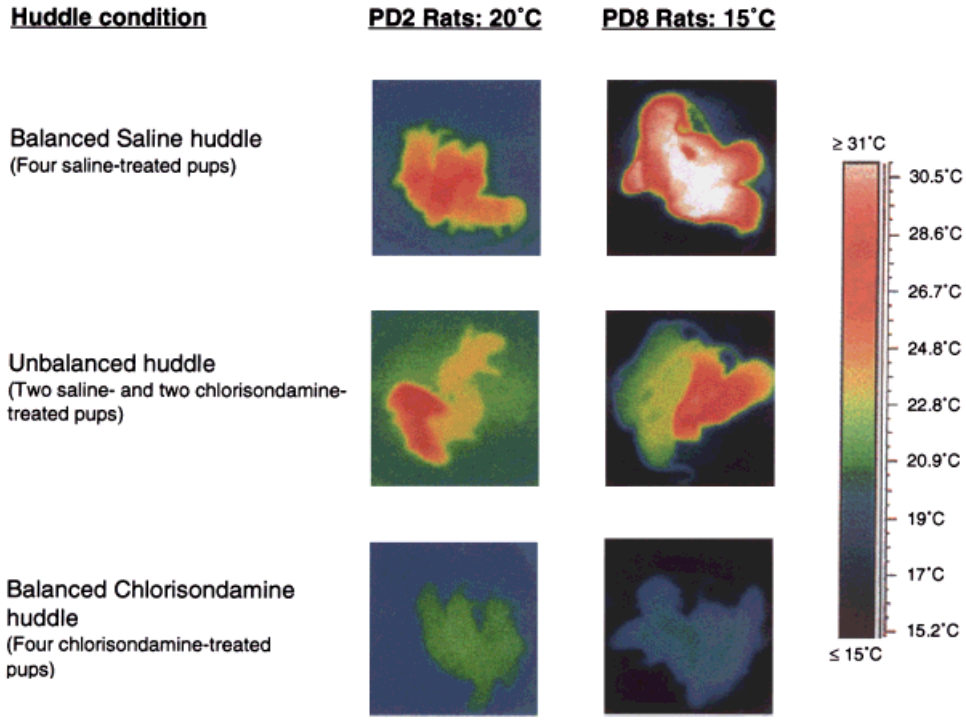

FIGURE 2 Representative infrared (IR) images of PD2 and PD8 huddles at 20°C and 15°C, respectively. Balanced saline huddles (top row) show higher huddle temperatures than the other two huddle conditions. In unbalanced huddles (middle row), saline-treated pups are easily distinguishable from chlorisondamine-treated pups. Finally, balanced chlorisondamine huddles (bottom row) are barely warmer than the ambient environment.

PD2 and PD8 rats differed in their expression of three-dimensional huddling. For PD2 focal pups, the amount of three-dimensional huddling did not increase significantly until exposure to 20°C ($p < .0001$; this also was true for 3-pup contact, $p < .005$; data not shown). In contrast, as shown in Figure 3, all PD8 focal pups showed significant increases in three-dimensional huddling when $T_{chamber}$ decreased below 35°C ($p < .0001$).

The behavioral data presented thus far revealed negligible differences between the balanced saline, balanced chlorisondamine, and unbalanced groups. For PD8 rats, however, an interesting difference in contact behavior emerged. Figure 4 presents 3-pup contact (recall that 3-pup contact is a measure of the amount of time in which a focal pup is in contact with the 3 other pups in the huddle) for balanced and unbalanced huddles (the results are collapsed because there was no significant difference between treatment conditions for this measure). Similar to the data for three-dimensional huddling (see Figure 3), PD8 pups progressively increased 3-pup contact as $T_{chamber}$ decreased, $F(2, 41) = 10.2$, $p < .01$. Repeated measures ANOVA revealed a significant interaction between huddle condition and $T_{chamber}$, $F(2, 41) = 6.7$, $p < .01$. At 25°C, focal pups from unbalanced huddles exhibited more 3-pup contact than focal pups from balanced huddles ($p < .05$). More importantly, this pattern was reversed at 15°C; focal pups from balanced huddles exhibited more 3-pup contact than focal pups from unbalanced huddles ($p < .05$), indicating that unbalanced huddles were beginning to disintegrate as $T_{chamber}$ decreased from 25°C to 15°C.

Given that unbalanced PD8 huddles showed decreased 3-pup contact at 15°C, we performed further analyses to explore the basis of this decrease. To do this, we examined contact behavior between a focal pup and 2, rather than 3, pups in the huddle. For all focal pups in the present experiment, 2-pup contact was the predominant contact behavior during each stability period (6.98 ± 0.93 min), regardless of $T_{chamber}$.

As shown in Figure 5, for both a chlorisondamine- (C) and saline-treated (S) focal pup, there were two possible combinations by which it could establish contact with 2 of the remaining 3 pups in the huddle. Each focal pup could be in contact with a mixed pair

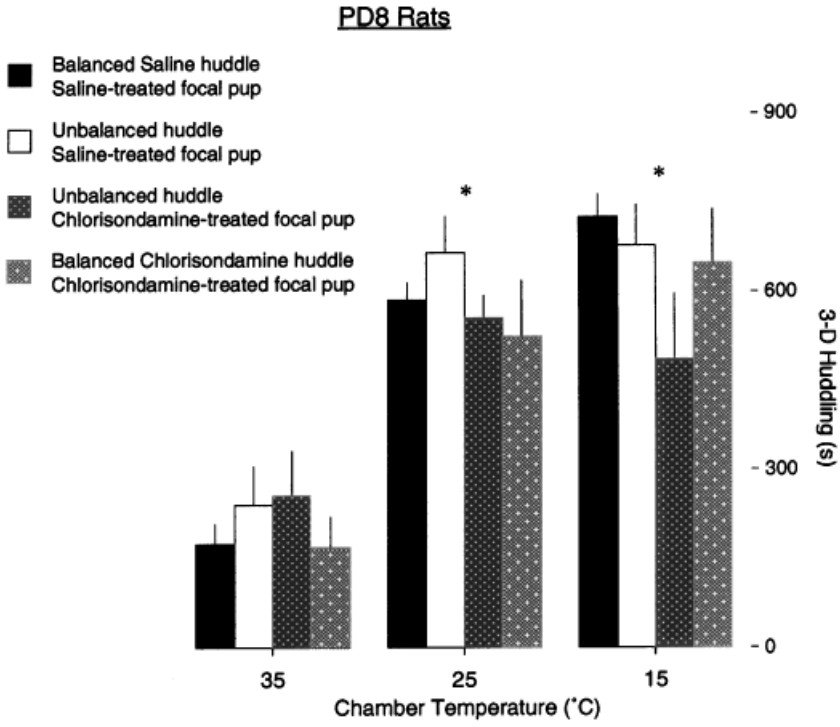

FIGURE 3 Duration of three-dimensional (3-D) huddling for PD8 focal pups. PD8 focal pups showed increases in 3-D huddling when the temperature of the chamber was decreased. There were no significant differences between groups. Mean ± *SEM*. *Significantly different from 35°C.

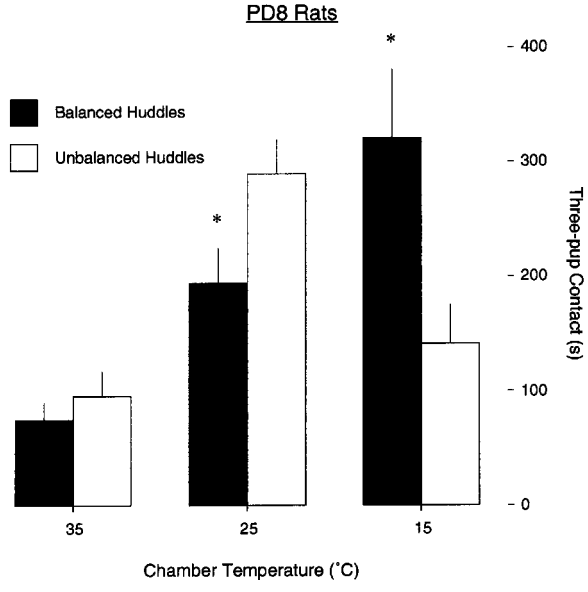

FIGURE 4 Duration of 3-pup contact for PD8 focal pups from balanced (filled bars) and unbalanced (open bars) huddles. For both huddle conditions, 3-pup contact increased as the temperature of the chamber was decreased below 35°C. At 25°C, focal pups from unbalanced huddles exhibited more 3-pup contact than focal pups from balanced huddles. At 15°C, however, focal pups from unbalanced huddles exhibited less 3-pup contact than focal pups from balanced huddles. Mean ± SEM. *Significantly different from unbalanced huddles.

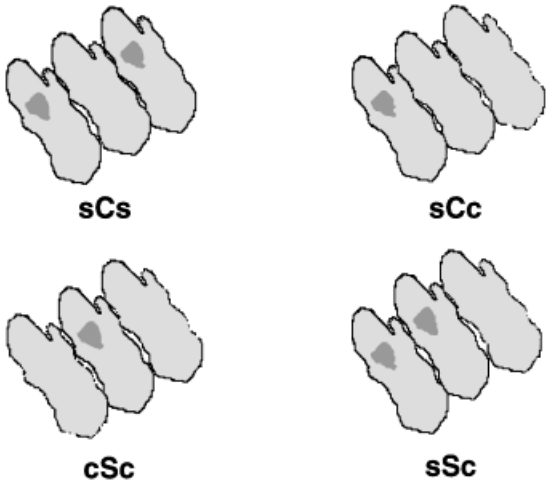

FIGURE 5 Illustration depicting the possible combinations of pairs of pups with which PD8 focal pups from unbalanced huddles could be in contact (c = chlorisondamine, s = saline). Focal pups (C = chlorisondamine-treated focal pup, S = saline-treated focal pup) could be in contact with a mixed pair of pups (i.e., s and c) or a pair of pups from the opposite treatment condition (i.e., s and s or c and c).

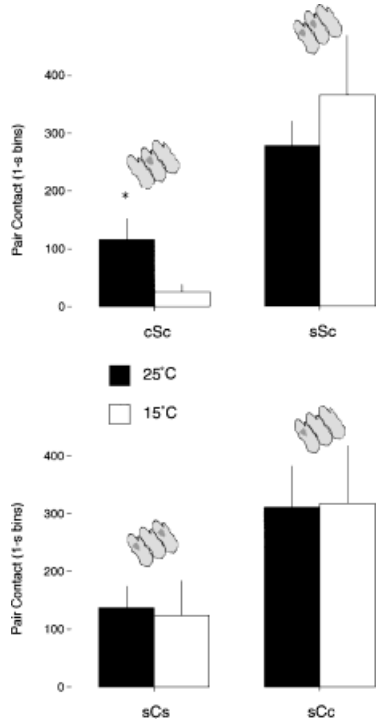

FIGURE 6 Number of 1-s bins of contact with pairs of pups for PD8 saline- and chlorisondamine-treated focal pups within unbalanced huddles at 25°C (filled bars) and 15°C (open bars). The longer duration of contact for both the sSc and the sCc combinations arise because they are twice as likely to occur by chance than are the cSc and sCs combinations. Mean ± *SEM*. *Significantly different from 15°C.

of pups (i.e., sc) or a pair of pups from the opposite treatment condition (i.e., ss or cc). Because the huddle was comprised of 4 pups, each focal pup could be in contact with 2 different mixed pairs of pups, but only one pair comprised of pups from the opposite treatment condition.

Figure 6 presents the duration of contact of PD8 focal pups with specific pairs of pups during cold exposure for each 15-min stability period. There were no significant differences in the duration of contact with a mixed pair of pups (i.e., sSc or sCc) between 25°C and 15°C. Similarly, for chlorisondamine-treated focal pups, there was no difference in the duration of contact with a pair of saline-treated pups at either temperature. For saline-treated focal pups, however, there was a significant decrease in the duration of contact with a pair of chlorisondamine-treated pups at 15°C (i.e., cSc; $F(1,6) = 7.6$, $p < .05$). This change in focal pup behavior suggests that saline-treated focal pups avoided the colder chlorisondamine-treated pups as the magnitude of cold exposure increased.

To expand our analysis beyond the focal pup, the contact behavior of the unbalanced huddle as a whole was assessed. Figure 7a shows the four possible combinations of the pups that comprised an unbalanced huddle. This huddle composition was determined from the IR images taken before and after the stability period (Mins 45 and 60). A Pearson chi-square goodness-of-fit analysis was performed to analyze the frequency of huddle composition. Figure 7b shows the observed and expected frequencies of combinations of all 4 pups in unbalanced huddles of PD8 rats at 25°C and 15°C. At 25°C (left-hand plot), the pups were found equally in each of the four possible combinations. When the temperature was decreased to 15°C (right-hand plot), however, the frequency of observed huddle combinations differed significantly from expected frequencies (right-hand plot; $\chi^2_3 = 16.5$, $p < .001$). At 15°C, the predominant huddle combination was CCSS. One conclusion that could be drawn from the increase in this particular huddle combination is that chlorisondamine-treated pups are attracted to other chlorisondamine-treated pups. This does not appear to be the case, however, because the next most frequent huddle combination was CSSC in which, once again, the 2 saline-treated pups were directly in contact, but the 2 chlorisondamine-treated pups were not. Furthermore, the combination of SCCS, in which 2 chlorisondamine-treated pups are in contact but 2 saline-treated pups are not,

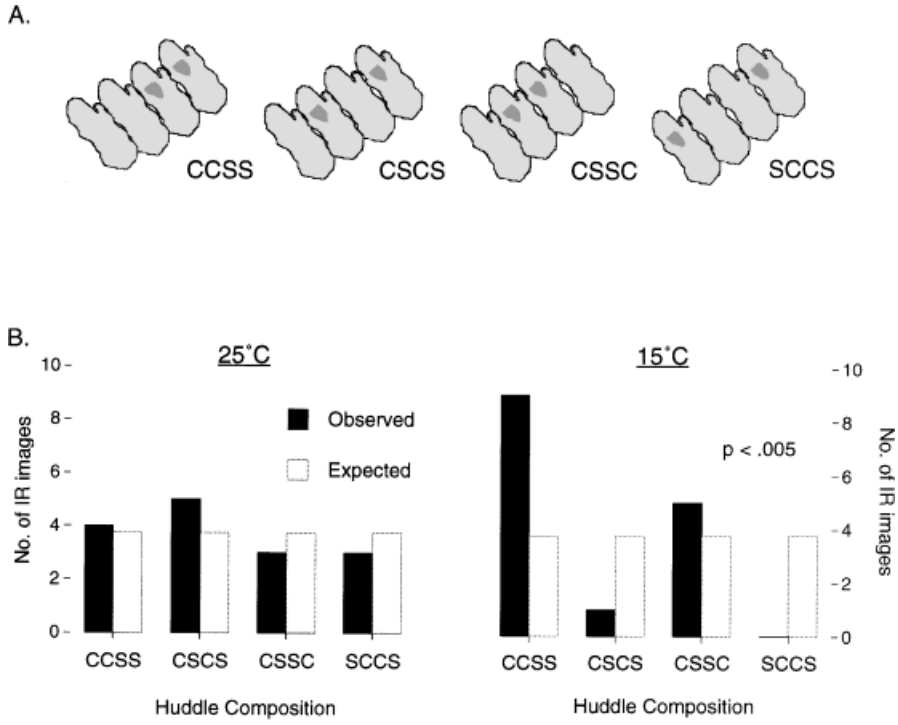

FIGURE 7 (A) The four possible combinations of contiguous pups in unbalanced huddles (C = chlorisondamine-treated pup, S = saline-treated pup). (B) The observed and expected frequencies of the four possible combinations of PD8 rats obtained from IR images taken at the beginning and the end of the stability period (Mins 45 and 60, respectively). There was no difference in the observed frequency of any combination at 25°C (left-hand plot). During exposure to 15°C (right-hand plot), however, combinations in which both saline-treated pups were in contact with each other occurred more frequently than expected, indicating that saline-treated pups were actively initiating contact with one another.

was never observed. Therefore, it appears as though saline-treated pups are preferentially aggregating with one another.

The results presented in Figures 6 and 7 suggest that, at 15°C, the inequity of the endothermic capabilities of the pups that comprise unbalanced huddles elicits competitive behaviors that are driven by each pup's individual needs. In this competition, the saline-treated pups appear to have an advantage in gaining access to each other, perhaps due to their higher body temperatures and, therefore, superior locomotor abilities. This possibility is supported by the data presented earlier showing that saline-treated pups showed more translational awake behavior (e.g., locomotion) than chlorisondamine-treated pups at 15°C.

DISCUSSION

The present results support previous findings in showing that BAT thermogenesis is a necessary thermal resource for huddling (Sokoloff et al., 2000). Specifically, huddles comprised of infant rats in which all or half of the pups were treated with chlorisondamine, thereby inhibiting BAT thermogenesis, had significantly lower huddle temperatures than huddles comprised solely of saline-treated pups (Figures 1 and 2). In other words, without heat production, huddle temperature is compromised during cold exposure.

While it is true that huddles of heat-producing pups do supplement physiological heat production with huddling to expand their thermal niche (Sokoloff et al., 2000), it is not the case that huddles of pups that cannot produce heat show enhanced huddling behavior. At no time during cold exposure did huddles comprised of 2 or more chlorisondamine-treated pups compensate for a lack of heat production with enhanced huddling. If huddling were simply a compensatory mechanism for inadequate physiological thermoregulation (Leon, 1986; Satinoff, 1996), then balanced chlorisondamine and unbalanced

huddles should have shown greater reductions in huddle surface area as well as enhanced huddling behaviors (e.g., three-dimensional huddling and 3-pup contact).

The present results replicate previous findings showing that 2-day-old rats do not form organized huddles during cold exposure even though these infants are capable of and show endogenous heat production (Sokoloff et al., 2000). This suggests that huddling is not utilized effectively to complement physiological thermoregulation early in the postpartum period. In the present experiment, huddles of 2-day-old rats, regardless of huddle condition, did not increase contact behavior or three-dimensional huddling until they were exposed to 20°C. In contrast, the substantially larger 8-day-old rats began to huddle effectively as soon as air temperature was lower than thermoneutral by decreasing huddle surface area as well as increasing contact behavior and three-dimensional huddling during moderate levels of cold exposure (30–24°C; Alberts, 1978a; Sokoloff et al., 2000).

The behavior of 8-day-old focal pups from balanced chlorisondamine huddles (Figures 3 and 4) is consistent with the notion stated earlier that huddling behavior matures over the 1st week postpartum. In fact, focal pups from balanced chlorisondamine and balanced saline huddles exhibited similar huddling behaviors, suggesting that 8-day-olds do not depend on endothermy for successful aggregation. These data also suggest that chlorisondamine treatment did not interfere with the expression of huddling behavior through a nonselective action of the drug.

The change in huddling that appears to occur between 2 and 8 days of age could be the result of changes in the infants' behavior, changes in the dam's behavior, or both. For example, it has been shown that aggregation in thermoneutral environments increases with age in infant rats (Schank & Alberts, 1997a, 1997b). At the same time, the dam is spending more time away from the nest by the end of the 1st week postpartum (Grota & Ader, 1969; Leon, Croskerry, & Smith, 1978). These changes in infant and maternal behavior could act independently or in concert to produce the changes in huddling behavior reported here.

Although balanced chlorisondamine huddles of 8-day-old rats showed similar huddling behaviors as balanced saline huddles, a disintegration of huddling behavior emerged in unbalanced huddles at 15°C. As the magnitude of cold exposure increased, saline-treated pups in unbalanced huddles changed their behavior to maximize their individual thermoregulatory success; the result was preferential aggregation of both saline-treated pups and the exclusion of 1 or both of the chlorisondamine-treated pups (Figures 6 and 7). In fact, the blockade of BAT thermogenesis in 2 of the 4 pups in the huddle resulted in an imbalance that became problematic for the heat-producing pups when they were challenged beyond their individual capabilities. This emerging competition in unbalanced huddles is consistent with the results of Alberts (1978a), showing that an anesthetized rat pup in a huddle is actively pushed to the outside of the huddle by its unanesthetized littermates in a cold environment and allowed to sink to the bottom of the huddle in a warm environment.

The importance of thermal stimuli for the initiation of huddling behavior in infant rats has been well documented (Alberts & Brunjes, 1978; Alberts & May, 1984). Huddling, however, can be initiated by other sensory stimuli (Sullivan, Brake, Hofer, & Williams, 1986) and, as infant rats mature, these nonthermal stimuli become increasingly important for the expression of this behavior (Alberts & Brunjes, 1978; Brunjes & Alberts, 1979; Schank & Alberts, 1997a, 1997b). BAT thermogenesis may contribute to the transition from thermally to nonthermally mediated huddling through the formation of associations between the heat that is produced by a littermate and nonthermal stimuli (e.g., olfactory and/or tactile; Alberts & May, 1984).

While nonthermal stimuli increase in importance over the first 2 weeks postpartum, the preferential aggregation of saline-treated pups from unbalanced huddles suggests that the heat produced by BAT still plays an important role in huddling at 8 days of age. In contrast, as stated earlier, the behaviors exhibited by balanced chlorisondamine huddles at 8 days of age indicate that huddling can occur even in the absence of heat production. This difference between unbalanced and balanced chlorisondamine huddles suggests that at the end of the 1st week postpartum, thermal stimuli maintain some control over the expression of huddling behavior, but that they are no longer necessary for huddling to occur.

NOTES

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REFERENCES

Adair, E. R. (1977). Skin, preoptic, and core temperatures influence behavioral thermoregulation. Journal of

Applied Physiology: Respiratory, Environmental, and Exercise Physiology, 42, 559–564.

Alberts, J. R. (1978a). Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. Journal of Comparative and Physiological Psychology 92, 231–245.

Alberts, J. R. (1978b). Huddling by rat pups: Multisensory control of contact behavior. Journal of Comparative and Physiological Psychology, 92, 220–230.

Alberts, J. R., & Brunjes, P. C. (1978). Ontogeny of thermal and olfactory determinants of huddling in the rat. Journal of Comparative and Physiological Psychology, 92, 897–906.

Alberts, J. R., & May, B. (1984). Nonnutritive, thermotactile induction of filial huddling in rat pups. Developmental Psychobiology, 17, 161–181.

Blumberg, M. S. (1997). Ontogeny of cardiac rate regulation and brown fat thermogenesis in golden hamsters (Mesocricetus auratus). Journal of Comparative Physiology B, 167, 552–557.

Blumberg, M. S., Efimova, I. V., & Alberts, J. R. (1992). Thermogenesis during ultrasonic vocalization by rat pups isolated in a warm environment: A thermographic analysis. Developmental Psychobiology, 25, 497–510.

Blumberg, M. S., Sokoloff, G., & Kirby, R. F. (1997). Brown fat thermogenesis and cardiac rate regulation during cold challenge in infant rats. American Journal of Physiology, 272, R1308–R1313.

Blumberg, M. S., & Stolba, M. A. (1996). Thermogenesis, myoclonic twitching, and ultrasonic vocalization in neonatal rats during moderate and extreme cold exposure. Behavioral Neuroscience, 110, 305–314.

Brunjes, P. C., & Alberts, J. R. (1979). Olfactory stimulation induces filial huddling preferences in pups. Journal of Comparative and Physiological Psychology, 93, 548–555.

Carlisle, H. J. (1968). Peripheral thermal stimulation and thermoregulatory behavior. Journal of Comparative and Physiological Psychology, 66, 507–510.

Corbit, J. D. (1970). Behavioral regulation of body temperature. In J. D. Hardy, A. P. Gagge, & J. A. J. Stolwijk (Eds.), Physiological and behavioral temperature regulation (pp. 777–801). Springfield, IL: Charles C. Thomas.

Grota, L. J., & Ader, R. (1969). Continuous recording of maternal behavior in Rattus norvegicus. Animal Behaviour, 17, 78–82.

Hissa, R. (1968). Postnatal development of thermoregulation in the Norwegian lemming and the golden hamster. Annoles Zoologici Fennici, 5, 345–383.

Leon, M. (1986). Development of thermoregulation. In E. M. Blass (Ed.), Handbook of behavioral neurobiology, Vol. 8: Developmental psychobiology and developmental neurobiology (pp. 297–322). New York: Plenum Press.

Leon, M., Croskerry, P. G., & Smith, G. K. (1978). Thermal control of mother–young contact in rats. Physiology & Behavior, 21, 793–811.

Leonard, C. M. (1974). Thermotaxis in golden hamster pups. Journal of Comparative and Physiological Psychology, 86, 458–469.

Richter, C. P. (1943). Total self-regulatory functions in animals and human beings. Harvey Lectures, 38, 63–103.

Satinoff, E. (1996). Behavioral thermoregulation in the cold. In M. J. Fregly & C. M. Blatteis (Eds.), Handbook of physiology (pp. 481–505). Oxford: Oxford University Press.

Satinoff, E., & Rutstein, J. (1970). Behavioral thermoregulation in rats with anterior hypothalamic lesions. Journal of Comparative and Physiological Psychology, 71, 77–82.

Schank, J. C., & Alberts, J. R. (1997a). Aggregation and the emergence of social behavior in rat pups modeled by simple rules of individual behavior. In Y. Bar-Yam (Ed.), Proceedings of the International Conference on Complex Systems (pp. 11–25). Nashua, NH: New England Complex Systems Institute.

Schank, J. C., & Alberts, J. R. (1997b). Self-organized huddles of rat pups modeled by simple rules of individual behavior. Journal of Theoretical Biology, 189, 11–25.

Schmidt, I., Barone, A., & Carlisle, H. J. (1986). Diurnal cycle of core temperature in huddling, week-old rat pups. Physiology & Behavior, 37, 105–109.

Sokoloff, G., & Blumberg, M. S. (1998). Active sleep in cold-exposed infant Norway rats and Syrian golden hamsters: The role of brown adipose tissue thermogenesis. Behavioral Neuroscience, 112, 695–706.

Sokoloff, G., & Blumberg, M. S. (1997). Thermogenic, respiratory, and ultrasonic responses of week-old rats across the transition from moderate to extreme cold exposure. Developmental Psychobiology, 30, 181–194.

Sokoloff, G., Blumberg, M. S., & Adams, M. M. (2000). A comparative analysis of huddling in infant Norway rats and Syrian golden hamsters: Does endothermy modulate behavior? Behavioral Neuroscience, 114, 585–593.

Sokoloff, G., Kirby, R. F., & Blumberg, M. S. (1998). Further evidence that BAT thermogenesis modulates cardiac rate in infant rats. American Journal of Physiology, 274, R1712–R1717.

Stanier, M. W. (1975). Effect of body weight, ambient temperature, and huddling on oxygen consumption and body temperature of young mice. Comparative Biochemistry and Physiology, 51A, 79–82.

Sullivan, R. M., Brake, S. C., Hofer, M. A., & Williams, C. L. (1986). Huddling and independent feeding of neonatal rats can be facilitated by a conditioned change in behavioral state. Developmental Psychobiology, 19, 625–635.