

Rat Behavioral Thermoregulation Integrates With Nonshivering Thermogenesis During Postnatal Development

William J. Farrell

Queens College, City University of New York

Jeffrey R. Alberts

Indiana University Bloomington

Rat pups are capable of behavioral thermoregulation, both in the nest and on a thermocline, as early as the 1st week of postnatal life, and these pups can also produce heat metabolically without shivering. The rat pup's primary source of nonshivering thermogenesis is the sympathetically mediated metabolism of brown adipose tissue (BAT). BAT is well formed in newborns and functions shortly after birth. While infant behavioral thermoregulation and BAT thermogenesis have been extensively studied, little is known about the extent to which thermoregulatory behavior can be influenced by BAT thermogenesis. In the present study, 2-, 7-, and 14-day-old pups were observed on a thermal gradient following pharmacological stimulation or inhibition of BAT thermogenesis, and their thermal preferences were quantified. The authors found that 7- and 14-day-old pups treated with norepinephrine (NE), which increases BAT thermogenesis, preferred cooler portions of the gradient than saline-treated controls, whereas 2-day-olds failed to show a similar NE-induced behavioral adjustment. These findings indicate that the ability to adjust thermoregulatory behavior to compensate for enhanced metabolic thermogenesis develops during the 1st week of postnatal life.

Keywords: thermocline, temperature regulation, norepinephrine, brown adipose tissue, rat pup

The vital importance of maintaining stable body temperatures (thermal homeostasis) is reflected in the anatomy, physiology, and behavior of all animals. Thermal homeostasis poses a particular challenge to altricial infants, such as Norway rat pups (*Rattus norvegicus*). At birth, the infant's muscular sources of thermoregulation (e.g., locomotion, piloerection, and shivering) are immature. For example, shivering does not significantly contribute to heat production until the 3rd week of postnatal life (Taylor, 1960). Infants also lack the insulation of fur and subcutaneous white adipose tissue. These factors, along with a large surface-area-to-mass ratio, lead to rapid heat loss in a cool environment (Conklin & Heggenes, 1971; Hull, 1973; Malik & Fewell, 2003). Based in part on observations such as these, and on data indicating that the core (rectal) temperature of pups declines rapidly when exposed to low ambient temperatures (e.g., 10 °C to 22 °C), it is often suggested that young rat pups are "poikilothermic" (e.g., Fowler & Kellogg, 1975; Gordon, 1993). This classification has been challenged, however, by observations indicating that when exposed to moderate thermal challenges more consistent with the temperature

fluctuations experienced in the nest environment (e.g., 25 °C to 34 °C), the thermogenic capabilities of 1-week-old rats are not overwhelmed and they are able to stabilize the temperature of the thoracic region, if not rectal temperature (Blumberg & Sokoloff, 1997, 1998).

Whereas shivering is not available as a functional source of metabolic heat during the first 2 weeks postpartum, all infant eutherian mammals, including rat pups, possess brown adipose tissue (BAT), a specialized effector for heat production. BAT is generally considered the infant mammal's sole source of nonshivering thermogenesis (Hull, 1973). It is well formed in the newborn rat and is distributed strategically near major organs and blood supplies (Blumberg, 2001; Hull, 1973; Smith, 1964). The largest deposit of BAT in the infant rat is located beneath the skin of the interscapular region of the back. BAT is thermogenic tissue; its metabolism is so intense that tissue temperature rises, warming the blood that passes through it as well as nearby organs (Smith & Roberts, 1964). The initiation and maintenance of BAT thermogenesis is under the control of the sympathetic nervous system (Girardier & Seydoux, 1986; Schneider-Picard & Girardier, 1982) and is mediated by beta-adrenergic receptors (Zau, Unelius, Cannon, & Nedergaard, 1994). Systemically administered norepinephrine (NE) causes an increase in BAT metabolism (Farrell & Alberts, 2000; Hofer & Shair, 1991; Hsieh, Emery, & Carlson, 1971), and propranolol (PROP), an adrenergic beta-blocker, inhibits the metabolism of BAT in response to a cold challenge (Farrell & Alberts, 2000; Heim & Hull 1966).

When the infant rat's thermoregulatory thermogenesis is developmentally limited, pups are nonetheless capable of behavioral thermoregulation, both as a group in the nest (Alberts, 1978, 2007) and individually on a thermal gradient (Hoffman et al., 1999b; Kleitman & Satinoff, 1982; Malik & Fewell, 2003). Huddling, which reduces the surface-to-mass ratio of the group and hence

William J. Farrell, Department of Psychology, Queens College, City University of New York; Jeffrey R. Alberts, Department of Psychological and Brain Sciences, Indiana University Bloomington.

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Correspondence concerning this article should be addressed to William J. Farrell, Psychology Department, Queens College, City University of New York, 6530 Kissena Boulevard, Flushing, NY 11367. E-mail: william.farrell@qc.cuny.edu

attenuates heat loss in a cool environment, is the most ecologically relevant form of behavioral thermoregulation for infants and possibly for adults as well. During early postnatal life, rat pups actively huddle in the nest, and systematic observations of these huddles over a range of environmental temperatures indicate that the huddle is a dynamic structure in which the behavior and physiology of individual pups contribute to the thermoregulation of the huddle as a whole (Alberts, 1978, 2007; Sokoloff & Blumberg, 2001, 2002). When exposed to cool ambient temperatures, individual members of the huddle circulate from the top to the bottom of the tightly packed huddle in a convection-like pattern (Alberts, 1978). As the ambient temperature approaches thermoneutrality (approximately 34 to 36 °C, depending on age) this pattern of movement reverses, and eventually the huddle dissipates. Consistent with these observations, it has also been demonstrated that pups as young as 1 day of age can behaviorally regulate their body temperature by moving to appropriate locations on a thermal gradient (Hoffman et al., 1999b; Kleitman & Satinoff, 1982; Malik & Fewell, 2003).

In addition to actively adjusting their position in the huddle and on a thermal gradient, infant rats as young as 1 day of age are also able to learn to perform an operant response for a thermal reinforcer. Flory, Langley, Pfister, and Alberts (1997) conducted an experiment in which pups were positioned on a thermally regulated platform whose temperature could be adjusted rapidly and independently from the air temperature. When trained and tested in a cool, 25 °C ambient environment, 1-day-old pups rapidly (within 30 min) learned to turn their head to break a light beam for a 20-s elevation of the platform temperature from 25 °C to 36 °C. Similar operant learning for a warm reinforcer in a cool environment has also been reported for 5- and 11-day-old pups (Hoffman, Flory, & Alberts, 1999a). Importantly, however, whereas 5- and 11-day-old pups also readily learn to perform a head-turning response for substrate cooling in a hot (40 °C) environment, 1-day-old pups fail to learn this response contingency (Hoffman et al., 1999b). This failure of 1-day-olds to perform an operant for a cool reinforcer in a hot environment, despite readily learning to perform an identical response for a warm reinforcer in a cool environment, has been interpreted as an ontogenetic adaptation (Oppenheim, 1980, 1981) that prevents the thermally challenged neonate from learning contingencies that might draw it away from the nest (Hoffman et al., 1999b).

Although much is known about the physiology of BAT and the capabilities of pups with respect to group and individual thermoregulation, researchers still have little understanding regarding the extent to which infant rats are capable of coordinating their behavioral and metabolic thermoregulatory responses. The goal of the present study was to examine the relationship between BAT metabolism and behavioral thermoregulation. Specifically, we examined the thermal preferences exhibited by rat pups (2, 7, or 14 days of age) on a thermal gradient (thermocline), following pharmacological manipulations that either enhanced (NE administration) or attenuated (PROP administration; 14-day-old pups only) BAT metabolism. We hypothesized that the administration of NE would decrease pups' preferred temperatures in a dose-dependent manner and that PROP administration would lead to an increase in preferred temperature. That is, there would be an inverse relationship between BAT metabolism and the thermal preferences displayed by pups. In light of the ontogenetic shift reported for

learning for thermal reinforcers, we also sought to examine the developmental time-course of the interaction between BAT metabolism and behavioral thermoregulation as the pup's capacity for metabolic thermogenesis increases substantially over the first 2 weeks of life.

Experiment 1: Thermal Preferences of 7-Day-Old Pups Following NE Administration

As a first step in examining the extent to which metabolic thermogenesis can influence the thermoregulatory behavior of infant rats we sought to determine whether systemic injections of NE, which stimulates BAT thermogenesis, would cause dose-dependent changes in the thermoregulatory behavior of individual 7-day-old pups on a thermocline. We hypothesized that NE-treated pups would dose-dependently shift their thermal preferences to progressively cooler locations on the thermal gradient as NE-induced BAT thermogenesis increased. Such behavior would indicate that pups of this age behaviorally counteract increased physiological heat production by selecting cooler ambient environments, and thus regulate body temperature.

Method

Subjects. Sixty-seven 7-day-old Sprague-Dawley rat pups weighing an average of 16.9 ± 0.2 g were used in this experiment. Sixty pups were used in the pharmacology study. An additional 7 pups were run independently on the thermocline to assess the extent to which pups typically use BAT thermogenesis while on the thermocline. Of the pups used in the pharmacology portion of the study, 20 served as saline-injected controls, and the remaining forty received 100, 200, 400, or 800 $\mu\text{g}/\text{kg}$ NE ($n = 10$ per group).

Pups were born to animals bred at the Indiana University Animal Behavior Laboratory from stock purchased from Taconic Laboratories (Germantown, NY). Litters were born and reared in standard plastic maternity cages ($45 \times 25 \times 20$ cm) on hardwood chip bedding and were culled to a total of 8 pups (4 male, 4 female) at 3 days of age (day of birth = Day 0). The breeding colony was maintained on a 12-hr light-dark cycle, with lights on at 0800. The temperature of the colony was maintained at $22 \text{ °C} \pm 2 \text{ °C}$, and food and water were available ad libitum. Equal numbers of male and female pups were used in all pharmacology treatment conditions, and no more than 1 pup from a litter was used per treatment condition. All pups had milk-bands at the beginning of experimental sessions, signifying recent ingestion of milk.

Apparatus. A more comprehensive description and diagram of the thermocline apparatus can be found in Hoffman et al. (1999b). The thermocline consisted of a copper sheet housed in a temperature-controlled enclosure. Plexiglas barricades (10 cm tall) divided the internal surface of the thermocline into two parallel testing lanes (95.25×6.35 cm) separated by a buffer zone (2.54 cm). Lines drawn on the interior copper surface divided the length of both test lanes into eight equal zones. The lid covering the thermocline was constructed of clear Plexiglas to facilitate video recording.

Ambient temperature inside the enclosure was maintained ≈ 37 °C. A thermal gradient was established across the floor of the thermocline by heating and cooling the opposing ends of the copper sheet, which protruded through the end walls of the en-

sure. The surface temperature of the thermocline was monitored using type E (chromel-constantan) thermocouples and a thermocouple meter (Omega, Stamford, CT). Surface temperature was measured in nine different locations corresponding to the high and low temperature boundaries of each of the eight physical zones demarcated on the surface of the thermocline. Air temperature inside the enclosure was measured using a thermistor air-probe (YSI, Yellow Springs, OH) positioned at the midpoint of the thermocline 2 cm above the copper surface. All temperature measurements were obtained from the buffer zone located between the two parallel testing lanes.

The surface temperature of the test lanes ranged from $24.62 \pm .04$ °C to $44.02 \pm .04$ °C. The air temperature was $37.27 \pm .02$ °C. The mean boundary temperatures for the eight surface zones (nine boundaries) of the thermocline were $24.62 \pm .04$ °C, $31.58 \pm .03$ °C, $33.68 \pm .03$ °C, $35.22 \pm .04$ °C, $36.47 \pm .03$ °C, $37.64 \pm .03$ °C, $38.90 \pm .04$ °C, $40.73 \pm .04$ °C, and $44.02 \pm .04$ °C.

All experimental sessions were videotaped using a Sony color video camera (Model DXC-151A) outfitted with a wide-angle (8.5-mm) lens. The video camera was positioned 1.15 m above the top of the enclosure housing the thermocline, allowing the entire surface of the thermocline to be viewed simultaneously. Output from the video camera was sent to a time-lapse VCR (Gyrr, TLC1800, Anaheim, CA), and experimental sessions were recorded at a 1:6 record–playback ratio.

Normal levels of BAT thermogenesis were examined in 7 pups by measuring skin temperature at 20-min intervals while the pups behaviorally regulated on the thermocline for 2 hr. Skin temperature was measured using an infrared thermography system, which was able to measure skin surface temperature with 0.1 °C of resolution. This thermography system consisted of an infrared scanner (Thermovision 870, Agema Infrared Systems, Daneryd, Sweden), an IBM-compatible computer, and software (CATS-E, Version 2.0, Agema Infrared Systems) that digitized the infrared radiation emitted from the skin surface of the pup creating real-time pseudocolor video images in which different colors represented different skin temperature ranges. To facilitate use of the infrared scanner, which required an unobstructed view of the pup, a modified lid was placed on the thermocline. This lid was constructed of Plexiglas and had two series of circular holes cut above the length of each lane that allowed us to measure skin temperature at any location in either of the two lanes. The holes were covered when not in use.

Procedure. Once temperatures on the thermocline had stabilized, two 7-day-old pups were removed from their home cage, which had been previously moved into the testing room. The pups were voided of urine and were injected with NE (100, 200, 400, or 800 $\mu\text{g}/\text{kg}$; Arterenol, Sigma, St. Louis, MO) or saline vehicle. Injections were administered subcutaneously at a volume of 4 ml/kg under the loose skin on the rear flank.

Immediately following injection, individual pups were placed in each of the two test lanes on the thermocline, and behavior was videotaped for 2 hr. Pups were started in the center of the fifth zone of the thermocline. Half of the animals started facing the warm end and half the cool end of the thermocline. Starting direction and lane assignments were balanced across drug doses. Pups were removed from the apparatus after 2 hr and returned to their home cage. All testing sessions were videotaped for subsequent analysis. Following the completion of each thermal preference test, the lanes

of the thermocline were washed with detergent and air and gradient temperatures were allowed to restabilize.

The 7 pups used to assess normal BAT thermogenesis on the thermocline underwent similar procedures after receiving saline injections. In addition, the behavior of these pups was not videotaped. Instead, the position of these pups was recorded by hand every 20 min. A thermal “snapshot” of the dorsal surface of the pup was obtained at each of these time points for subsequent analysis (see below).

Data analysis. Although the entire 2-hr thermal preference tests were videotaped, only behavior from the middle hour of the session was analyzed, thus avoiding complications associated with the onset and decline of drug activity. Prior research (Farrell & Alberts, 2000) indicates that the 800- $\mu\text{g}/\text{kg}$ dose of NE causes a robust and sustained activation of BAT thermogenesis, which remains stable from 30 to 90 min following injection. Videotapes were coded using custom software (Event Coder, n.d.) that allowed the observer to record the position of the pup with regard to the eight zones marked on the surface of the thermocline. Pups were considered to have entered a zone when half of their body (tail excluded) was positioned inside the zone. Data obtained from video analyses were used to calculate the proportion of time that each pup spent in each of the eight different physical zones on the thermocline. We then converted these values to the proportion of time pups spent in 3 °C temperature regions (seven 3 °C regions ranging from 24 °C to 45 °C), using a method similar to that of Hoffman et al. (1999b). We converted from the proportion of time spent in each of the eight physical zones on the thermocline for each pup individually using the mean zone boundary temperature measurements taken before and after each pup’s testing session. When the temperature range of a physical zone on the thermocline spanned multiple 3 °C temperature regions, we divided the proportion of time spent in the physical zone equally over the encompassed 3 °C temperature regions. For example, if 1 of the NE-treated pups spent 20% of its time in a physical zone on the thermocline that ranged from 35.22 °C to 36.47 °C, we converted this value to 10% of the time spent in the 33 °C to 36 °C temperature region and 10% of the time spent in the 36 °C to 39 °C temperature region.

To further facilitate statistical analysis, the proportion of time spent in the 3 °C temperature regions was converted to a weighted thermal preference score for each pup. This preference score characterizes how the pup distributed its time over the 3 °C temperature regions during the thermal preference test. The weighted thermal preference score was calculated by multiplying the percentage of time a pup spent in each 3 °C temperature region by the ordinal position of each respective temperature region and summing these values. The coolest 3 °C temperature region was assigned the lowest ordinal position (1) and the warmest 3 °C temperature region was assigned the highest ordinal position (7). Lower weighted thermal preference scores indicate a behavioral preference for cooler regions on the gradient, whereas higher scores indicate preferences for warmer regions. Because there were seven 3 °C thermal regions for this experiment, the maximum thermal preference score that a pup could obtain was 700 and the minimum was 100. A one-way analysis of variance (ANOVA) for independent samples was performed to examine the overall effect of drug dose on weighted thermal preference scores, and post hoc comparisons

between each drug dose and control were examined using Dunnett's *t*-tests. The criterion for statistical significance for all comparisons was $p < .05$, two-tailed.

The extent to which saline-injected pups used BAT thermogenesis while on the thermocline was assessed using the thermal snapshots obtained from the independent group of 7 saline-injected pups who had been thermally imaged at 20-min intervals on the thermocline. BAT metabolism was estimated from each of these snapshots by comparing the skin temperature of a 0.25-cm² area of skin directly over the middle of the pups' interscapular region (T_{is}), an area overlying the largest BAT deposit, to an equivalent area of skin over the sacral region of the pups' back (T_b), an area that lacks BAT. The difference between the skin temperatures of these two regions ($T_{is} - T_b$) can be used as an estimate of BAT thermogenesis such that larger values indicate selective heating of the interscapular BAT deposit and lower values indicate minimal thermogenic activity. Several studies indicate that increasing $T_{is} - T_b$ values correlate well with increases in oxygen consumption exhibited by infant rats during cold challenge (Blumberg & Alberts, 1990; Blumberg & Stolba, 1996; Kirby & Blumberg, 1998).

Results and Discussion

NE administration caused a dose-dependent reduction in the thermal preferences of 7-day-old pups. The mean percentage of time spent by pups in the eight physical zones of the thermocline during the middle hour of the 2-hr thermal preference test is enumerated in Table 1. Saline-injected control pups spent the majority of their time in Zone 5 (39.9%) or Zone 4 (33.0%) of the thermocline, which had mean temperatures of 37.1 °C and 35.9 °C,

respectively. As the dose of NE was increased from 100 to 800 µg/kg, pups spent their time in progressively cooler regions of the thermocline. Pups receiving the highest dose of NE (800 µg/kg) spent over 89% of their time in the two coldest zones of the gradient (31.5% and 57.9% in Zones 1 and 2, respectively), with mean temperatures of 28.1 °C and 32.6 °C.

The behavioral shift toward cooler zones on the thermocline was also represented as a dose-dependent reduction in weighted thermal preference scores (see Figure 1). The results of a one-way ANOVA for independent samples comparing the weighted thermal preference scores of the saline and NE-treated 7-day-old pups indicated that there was a significant effect of drug dose, $F(4, 55) = 26.18$, $p < .001$. Dunnett's *t*-tests comparing the weighted thermal preference scores of each NE-treated group to the saline-treated controls indicated that the thermal preference scores exhibited by the pups receiving 400 and 800 µg/kg NE were significantly lower than the scores of control pups ($p < .001$ for both the 400- and 800-µg/kg comparisons).

It is important to note that the changes in thermal preference exhibited by the NE-treated 7-day-old pups do not appear to be a consequence of an overall change in activity level. Saline-treated pups changed physical zones an average of 13.0 ± 2.5 times during the middle hour of the 2-hr preference test. NE-treated pups were all somewhat less active, and pups receiving 100, 200, 400, or 800 µg/kg NE changed zones an average of 7.8 ± 2.7 , 6.6 ± 2.4 , 5.7 ± 1.0 and 6.8 ± 2.3 times, respectively. The results of an ANOVA comparing the number of times pups switched physical zones on the thermocline during the middle hour of the 2-hr preference test failed to yield a significant effect of dose, $F(4, 55) = 1.87$, $p = .13$.

Table 1
Mean Percentages of Time Spent in the Eight Physical Zones of the Thermocline During the Middle Hour of the 2-Hr Thermal Preference Test

| Variable | Ambient | Zone 1 | Zone 2 | Zone 3 | Zone 4 | Zone 5 | Zone 6 | Zone 7 | Zone 8 |
|--------------------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|
| 2-day-old pups | | | | | | | | | |
| Mean temperature (°C) | 37.02 | 27.84 | 31.99 | 33.84 | 35.25 | 36.54 | 37.99 | 39.56 | 42.35 |
| % time | | | | | | | | | |
| Saline | | 0.00 | 0.00 | 0.00 | 10.24 | 54.25 | 23.67 | 7.55 | 4.29 |
| 400 µg/kg norepinephrine | | 0.00 | 7.05 | 5.35 | 14.94 | 32.80 | 28.88 | 9.10 | 1.88 |
| 800 µg/kg norepinephrine | | 0.00 | 1.20 | 8.91 | 14.69 | 48.04 | 14.18 | 7.13 | 5.86 |
| 7-day-old pups | | | | | | | | | |
| Mean temperature (°C) | 37.27 | 28.10 | 32.63 | 34.45 | 35.85 | 37.05 | 38.27 | 39.82 | 42.37 |
| % time | | | | | | | | | |
| Saline | | 0.00 | 4.49 | 11.09 | 32.98 | 39.94 | 9.62 | 1.19 | 0.70 |
| 100 µg/kg norepinephrine | | 0.00 | 4.23 | 14.49 | 45.78 | 29.53 | 5.60 | 0.28 | 0.08 |
| 200 µg/kg norepinephrine | | 1.28 | 21.91 | 19.22 | 34.20 | 18.88 | 3.67 | 0.86 | 0.00 |
| 400 µg/kg norepinephrine | | 17.68 | 48.16 | 23.52 | 0.55 | 9.87 | 0.23 | 0.00 | 0.00 |
| 800 µg/kg norepinephrine | | 31.51 | 57.85 | 9.40 | 0.95 | 0.13 | 0.04 | 0.04 | 0.08 |
| 14-day-old pups | | | | | | | | | |
| Mean temperature (°C) | 26.32 | 23.43 | 27.10 | 29.93 | 32.72 | 36.11 | 40.44 | 45.71 | 54.70 |
| % time | | | | | | | | | |
| Saline | | 9.12 | 2.82 | 1.87 | 2.85 | 15.69 | 58.17 | 9.42 | 0.07 |
| 5 mg/kg propranolol | | 7.60 | 7.07 | 3.47 | 2.24 | 17.15 | 52.67 | 9.35 | 0.45 |
| 800 µg/kg norepinephrine | | 7.56 | 4.78 | 6.93 | 12.81 | 22.47 | 38.82 | 6.45 | 0.18 |

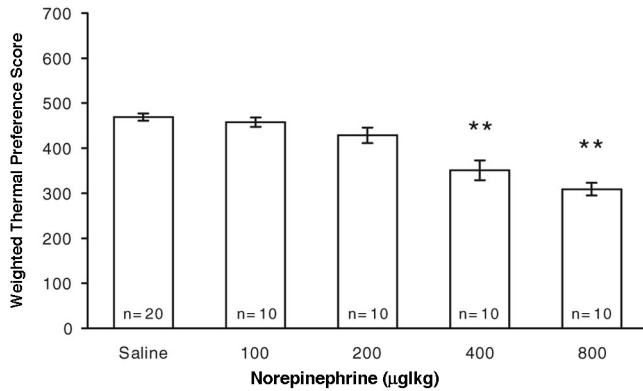


Figure 1. Mean weighted thermal preference scores exhibited by 7-day-old rat pups during the middle hour of the 2-hr thermal preference test. Error bars denote the standard error of the mean, and double asterisks indicate a statistically significant difference relative to saline-treated controls ($p < .001$).

The infrared thermal images obtained at 20-min intervals from 7 saline-injected pups during 2 hr on the thermocline indicate that 7-day-old pups are able to behaviorally regulate their body temperature and minimize BAT thermogenesis. Thermal images taken immediately after placement on the thermocline revealed mean $T_{is} - T_b$ values of 0.76 ± 0.06 °C, indicating moderate BAT thermogenesis, which commonly results from cooling associated with the handling and injection procedures. BAT thermogenesis quickly diminished while the pups navigated the thermocline. $T_{is} - T_b$ values dropped to 0.19 ± 0.06 °C after 20 min on the gradient and reached values of ≤ 0.1 °C after 40 min. These low values suggest minimal BAT activation during testing. In contrast, Farrell and Alberts (2000) found that cold-challenged (25 °C) 7-day-old pups exhibit mean $T_{is} - T_b$ values approaching 1.5 °C.

Experiment 2: Thermal Preferences of 14-Day-Old Pups Following NE or PROP Administration

The results of Experiment 1 indicate that 7-day-old pups behave on a thermocline in a manner that minimizes BAT thermogenesis. In addition, exogenous NE administration, which elevates BAT thermogenesis, caused a dose-dependent reduction in the thermal preferences of 7-day-old rat pups. Thus, by Postnatal Day 7, pups are capable of integrating their physiological responses with their thermoregulatory behavior.

To extend our analysis of behavioral and physiological integration during development, we wanted to determine whether 14-day-old pups, which have a smaller surface-to-mass ratio, enhanced insulation, and improved motor abilities, would exhibit similar compensatory behavioral responses to pharmacological manipulations designed to increase BAT thermogenesis. In addition, we also treated a separate group of pups with the beta-blocker PROP to determine whether a pharmacological manipulation that decreases BAT thermogenesis would affect thermal preferences in a manner opposite to NE.

Method

Subjects. The subjects for Experiment 2 were 36 (18 male and 18 female) 14-day-old Sprague-Dawley rat pups weighing an

average of 36.4 ± 0.6 g. Twelve pups served as saline-injected controls, while the remaining 24 received subcutaneous injections of NE (800 µg/kg) or the beta-blocker PROP (5 mg/kg; Sigma, St. Louis, MO). Twelve pups were used in each group. Animals were bred and housed under the conditions described for Experiment 1.

Apparatus. The same thermocline apparatus used for Experiment 1 was used for Experiment 2. Because we were using older pups in Experiment 2, we modified both the air temperature inside the apparatus and the surface temperatures of the gradient. Air temperature for Experiment 2 was 26.32 ± 0.16 °C and the temperature of the thermocline floor ranged from $21.03 \pm .08$ °C to 60.59 ± 0.06 °C. The mean boundary temperatures for the eight surface zones (nine boundaries) of the thermocline were $21.03 \pm .08$ °C, $25.83 \pm .10$ °C, $28.38 \pm .09$ °C, $31.48 \pm .08$ °C, $33.97 \pm .09$ °C, $38.26 \pm .09$ °C, $42.61 \pm .07$ °C, $48.81 \pm .12$ °C, and $60.59 \pm .06$ °C.

Procedure. Animals were run and thermal preference data were analyzed as in Experiment 1. Time spent in each of the eight zones on the thermocline during the middle hour of the 2-hr-long preference test was converted to proportion of time spent in 3 °C temperature regions. The wider temperature gradient used here created fourteen 3° C regions (20 °C to 62 °C), rather than the 7 in Experiment 1. Therefore, the maximum and minimum weighted thermal preference scores that a pup could display in Experiment 2 were 1,400 and 100, respectively.

Results and Discussion

NE administration (800 µg/kg) caused a reduction in the thermal preferences of 14-day-old rat pups. In contrast, PROP (5 mg/kg) failed to alter preferences. Mean percentages of time spent in the eight zones of the thermocline are enumerated in Table 1. Saline-injected control pups and PROP-treated pups spent the majority of their time in Zone 6 (58.2% and 52.7%, respectively) of the thermocline, which had a mean surface temperature of 40.4 °C. NE-treated pups also spent 38.8% of their time in Zone 6. NE-treated pups, however, spent a larger proportion of their time in Zones 2 through 5 of the thermocline than did the saline-treated animals.

The behavioral shift towards cooler zones on the thermocline was reflected in the lower weighted thermal preference scores for NE-treated pups (see Figure 2). On average, control pups exhibited weighted thermal preference scores of 649.9 ± 51.4 , while NE-treated pups had a mean preference score of 482.4 ± 28.7 . The mean preference score of PROP-treated pups (630.0 ± 42.9) was very similar to that of the saline-treated animals. A one-way ANOVA for independent samples comparing the weighted thermal preferences scores of the saline-, PROP- and NE-treated groups indicated that there was a significant effect of drug dose, $F(2, 33) = 4.73$, $p = .016$. Dunnett's *t*-tests comparing the weighted thermal preference scores of the PROP- and NE-treated animals to the saline-treated controls indicated that the thermal preference scores exhibited by the pups receiving 800 µg/kg NE were significantly lower than those of control pups ($p = .015$). The preference scores of the PROP-treated pups were not statistically different from those of the control animals ($p = .922$).

The change in thermal preference exhibited by the NE-treated pups cannot be explained as a consequence of an overall change in

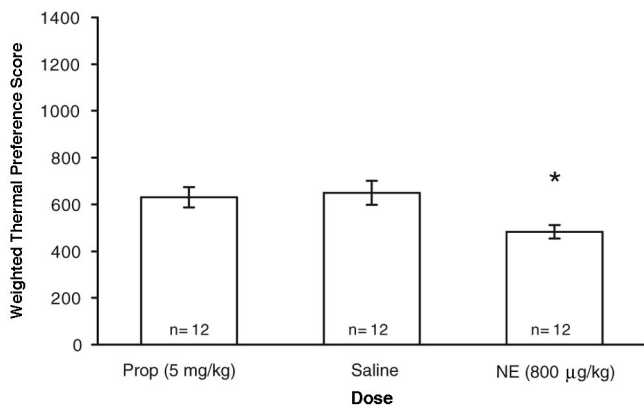


Figure 2. Mean weighted thermal preference scores exhibited by 14-day-old rat pups during the middle hour of the 2-hr thermal preference test. Error bars denote the standard error of the mean, and the asterisk indicates a statistically significant difference relative to saline-treated controls ($p < .05$).

activity level. Saline-treated pups changed physical zones an average of 24.9 ± 14.6 times during the middle hour of the 2-hr preference test. NE-treated pups were equally active, changing zones an average of 23.2 ± 6.7 times during the same period. PROP-treated animals, however, changed physical zones nearly three times more than saline-treated controls (73.0 ± 12.5). The results of an ANOVA comparing these data on switching between physical zones on the thermocline indicated a significant effect of drug, $F(2, 33) = 5.78$, $p = .007$. Post-hoc comparisons indicated that PROP-treated pups changed zones more often than did the saline-treated pups ($p = .013$). NE did not significantly alter zone-changing behavior ($p = .992$).

The design of Experiment 2 allowed for the possibility that pharmacological blockade of BAT thermogenesis would be associated with a compensatory shift to warmer regions of the thermocline, which did not occur. We believe that the most parsimonious explanation for the lack of a behavioral effect of PROP is that pups regulate body temperature on the thermocline, minimizing the energetic demands of BAT thermogenesis. Thus, blocking BAT thermogenesis would have minimal behavioral effects.

Experiment 3: Thermal Preferences of 2-Day-Old Pups Following NE Administration

The results of Experiments 1 and 2 indicate that 7- and 14-day-old pups are both capable of integrating their physiological responses (nonshivering thermogenesis) with behavioral responses (temperature selection). At these ages, behavioral responses appear privileged. That is, pups behaviorally selected substrate temperatures that precluded the need for BAT thermogenesis. If BAT metabolism was pharmacologically stimulated, however, they were capable of readily adjusting substrate temperatures to counteract the excessive metabolic heat production.

To further extend the developmental analysis, we next wanted to determine whether 2-day-old pups would exhibit a similar compensatory behavioral response to pharmacological manipulations designed to increase BAT thermogenesis. To this end, we examined the behavioral responses of 2-day-old pups on a thermocline

following saline administration (controls) or following the administration of 400 or 800 µg/kg of NE.

Method

Subjects. The subjects for Experiment 2 were 32 (16 male and 16 female) 2-day-old Sprague–Dawley rat pups weighing an average of 7.6 ± 0.1 g. Eight pups served as saline-treated controls while 16 pups received subcutaneous injections of 400 or 800 µg/kg NE ($n = 8$ per group). An additional 8 pups were used to assure that the 400 µg/kg dose of NE elicited a robust thermogenic response. Animals were bred and housed under the conditions described for Experiment 1.

Apparatus. Because we were using younger pups that have lower critical temperatures than 14-day-olds, we again modified the thermal parameters of the thermocline to make them similar to those used in Experiment 1. The air temperature was 37.02 ± 0.04 °C and the surface temperature of the thermocline floor ranged from $24.68 \pm .07$ °C to $44.27 \pm .07$ °C. The mean boundary temperatures for the eight surface zones (nine boundaries) of the thermocline for Experiment 3 were $24.68 \pm .07$ °C, $30.92 \pm .05$ °C, $32.97 \pm .06$ °C, $34.62 \pm .05$ °C, $35.79 \pm .05$ °C, $37.20 \pm .08$ °C, $38.71 \pm .05$ °C, $40.40 \pm .09$ °C, and $44.27 \pm .07$ °C.

Procedure. The testing and data analysis procedures used in Experiment 1 were again used here, but in Experiment 3 the pups were placed in the middle of the fourth physical zone on the thermocline at the beginning of each testing session. Because the dimensions of the thermal gradient in Experiment 3 were similar to those used with 7-day-old pups in Experiment 1, there were once again seven 3 °C temperature regions (24 to 45 °C), and weighted thermal preference scores could range from 100 to 700.

In addition to examining the behavior of 2-day-old pups on the thermocline following saline and NE administration, we also used the infrared thermal scanner to measure T_{is} and T_b for 8 pups isolated in an incubator maintained at 34 to 35 °C. All animals were allowed to habituate to the incubator for 30 min during which time BAT thermogenesis, estimated by examining $T_{is} - T_b$, became minimal or ceased. Following this habituation period, 4 of the pups received subcutaneous injections of 400 µg/kg of NE, while the remaining 4 animals were injected with saline. T_{is} and T_b were then monitored over the course of the next 90 min. These procedures were carried out to ensure that the minimal NE dose used in Experiment 3 elicited a robust thermogenic response that was maintained throughout the 1-hr period of data collection, which began 30 min after injection.

Results and Discussion

NE caused a strong thermogenic response within 30 min of injection that was sustained over the course of the next hour. Pups that received 400 µg/kg NE 30 min earlier exhibited an average $T_{is} - T_b$ value of 1.93 ± 0.11 °C, indicative of NE-induced BAT thermogenesis. In contrast, 30 min following saline injection, 2-day-old pups in the 34 to 35 °C incubator exhibited $T_{is} - T_b$ values $0.35 \pm .22$ °C. This small thermogenic response likely resulted from the handling and injection procedures 30 min earlier. One hour later (90 min after injection), NE-treated pups continued to exhibit enhanced BAT thermogenesis ($T_{is} - T_b = 1.18 \pm 0.28$ °C) relative to saline-treated control pups ($T_{is} - T_b = 0.10 \pm 0.04$

°C). These findings indicate that the lower dose of NE used in Experiment 3 (400 $\mu\text{g}/\text{kg}$) was sufficient to augment and maintain increased BAT thermogenesis throughout the middle hour of the 2-hr thermal preference test.

Although NE administration elicited a strong thermogenic response from 2-day-old pups, it did not alter the behavioral responses of these pups on the thermocline. Mean percentages of time spent in the eight zones of the thermocline are enumerated in Table 1. Zone 5, which had a mean temperature of 36.5 °C, was the preferred zone on the thermocline for the pups in all treatment conditions. Saline-treated pups and pups receiving 400 or 800 $\mu\text{g}/\text{kg}$ of NE spent 54.3, 32.8, and 48.0% of their time in this physical zone respectively. The weighted thermal preference scores for the saline- and NE-treated 2-day-old pups were also very similar (see Figure 3). Saline-treated pups exhibited a mean preference score of 485.6 ± 14.9 while pups treated with 400 and 800 $\mu\text{g}/\text{kg}$ NE displayed mean weighted thermal preference scores of 466.0 ± 19.8 and 474.5 ± 21.2 respectively. The results of a one-way ANOVA for independent samples comparing these means failed to yield significant results, $F(2, 21) = .273, p = .764$.

The failure of NE to alter the thermal preferences of 2-day-old pups does not appear to have resulted from an inability to ambulate on the thermocline. Saline-treated pups changed physical zones an average of 18.4 ± 2.1 times during the middle hour of the 2-hr preference test, while pups receiving 400 and 800 $\mu\text{g}/\text{kg}$ NE exhibited an average of 17.3 ± 3.0 and 21.4 ± 5.0 respectively. Pups were demonstrably capable of changing zones, but the results of an ANOVA indicated that the number of times pups switched physical zones on the thermocline did not differ significantly, $F(2, 21) = .356, p = .705$. Thus, under the present condition, NE-treated 2-day-olds exhibited the physiological response without a compensatory behavioral change.

General Discussion

The present results add to an existing body of evidence (e.g., Kleitman & Satinoff, 1982; Malik & Fewell, 2003) indicating effective behavioral thermoregulation by infant rats on a thermal gradient. Other investigations have also demonstrated behavioral responses to substrate temperature, including rapid learning of

lateral head movements for warmth (Flory et al., 1997; Hoffman et al., 1999a, 1999b) and locomotor escape from a cool segment of a chamber floor (Sokoloff, Blumberg, Bolino, Johnson, & Streper, 2002). Furthermore, the present data represent, to our knowledge, the first demonstration that rat pups as young as 1 week respond to potentiated thermogenesis with a compensatory shift in thermoregulatory behavior. NE-induced nonshivering thermogenesis led to downward shifts in temperature selection by 7- and 14-day-old pups relative to saline-treated controls.

To obtain a clear picture of the role played by behavior in infant thermoregulation it is helpful to combine observations of isolated animals under precisely controlled conditions, such as those on the thermocline, with observations of aggregations of pups (huddles) under more natural and consequently more complex circumstances. Alberts (1978) demonstrated that 5-, 10-, 15-, and 20-day-old rat pups exhibit "group regulatory behavior"—i.e., the group's surface area varies as a function of ambient temperature, which confers a significant metabolic advantage (by reducing oxygen consumption) to aggregating pups during exposure to cool ambient temperatures. Furthermore, Alberts (1978) also found that the self-regulatory movements of 10- to 12-day-old pups underlie the group behavior. Pups at these ages actively reduce their exposed surface area at cool ambient temperatures by moving toward each other and increase their individual exposures under warm ambient conditions. Younger ages were not analyzed. Given our current findings indicating that 2-day-olds do not actively move to cooler regions of a thermal gradient when BAT thermogenesis is artificially enhanced, additional knowledge regarding the huddles of newborns (1- to 2-day-olds) and the underlying individual behaviors that regulate these huddles as ambient temperature is increased would prove helpful for interpreting the distinct response patterns of the youngest pups in the present study.

PROP, a beta-receptor blocker, is the complementary pharmacological manipulation to NE administration, but unlike NE, PROP (5 mg/kg) did not alter the behavioral thermoregulation of 14-day-old pups, even though it blocked BAT thermogenesis. We suggest that the most likely explanation for the lack of a behavioral shift to warmer regions of the thermocline in PROP-treated 14-day-olds lies in the efficacy of their behavior. If, as has been reported for 1- to 11-day-old pups (Malik & Fewell, 2003), 14-day-old pups have a primary preference for regions of the thermocline that minimize metabolic thermogenesis, then blocking BAT thermogenesis would not have an observable effect on temperature selection. In essence, the PROP manipulation blocked a thermogenic effector that was never pressed into action.

Interestingly, though examinations of behavioral thermoregulation following pharmacological manipulations that block BAT thermogenesis are rare, Malik and Fewell (2003) examined both cold-induced oxygen consumption and behavioral thermoregulation on a thermocline following the administration of N^G -nitro-L-arginine methyl ester (L-Name) at a dose of 100 mg/kg. They found that this compound, which is a nonselective inhibitor of nitric oxide synthase and appears to inhibit BAT thermogenesis, attenuated the cold-induced increases in oxygen consumption exhibited by 1- to 11-day-old rats (when behavioral thermoregulation was not possible) without altering preferences on a thermocline.

The present findings of an NE-induced behavioral shift toward cooler regions of a thermal gradient for 7- and 14-day-old pups indicates that pups of these ages can integrate both internal (met-

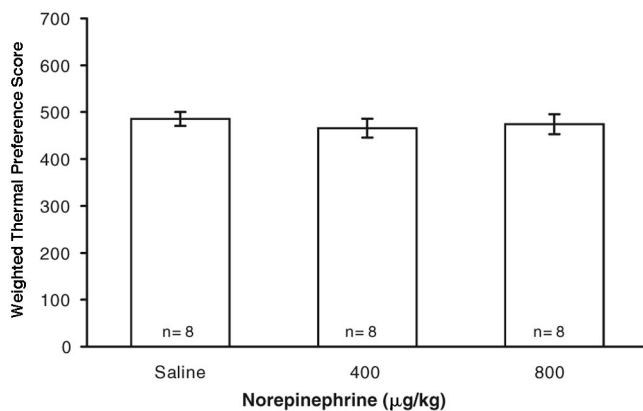


Figure 3. Mean weighted thermal preference scores exhibited by 2-day-old rat pups during the middle hour of the 2-hr thermal preference test. Error bars denote the standard error of the mean.

abolic) and external (environmental) thermal stimuli while engaging in behavioral thermoregulation. The story for 2-day-old pups, however, appears to be different. These younger pups failed to alter their behavioral thermoregulatory response following NE administration, despite evidence that the doses of NE used induced intense and sustained metabolic thermogenesis. The present age-related findings fit an emerging pattern of results from several different studies suggesting that very young pups respond differently to thermal stimuli than do older pups.

When placed on a metal platform, the temperature of which can be rapidly changed, 1-, 5- and 11-day-old rat pups readily learn to turn their head to one side (breaking a photo-beam) to warm the platform in a constantly cool (25 °C) environment (Flory et al., 1997; Hoffman et al., 1999a). Five- and 11-day-old pups also learn to perform a head-turning operant to cool the platform in a hot environment (40 °C), but 1-day-old pups fail to learn this response contingency (Hoffman et al., 1999b). This ontogenetic shift in learning for a cool thermal reinforcer in a warm environment was interpreted as an ontogenetic adaptation, which presumably prevents infants from learning thermal reinforcement contingencies that might cause them to leave the mother, siblings, or the nest environment. Hoffman et al. (1999b) suggested that this ontogenetic shift in learning may be tied to the strong "thermotaxic" response of young pups that diminishes during the 1st week of postnatal life. For the purpose of this discussion, the term thermotaxis refers to the tendency of an organism to move toward a source of warmth (Fraenkel & Gunn, 1942; Johanson, 1979; Leonard, 1974); head turning in the operant studies cited above can be conceptualized as a form of thermotaxis. Support for a developmental shift in the rat pup's thermotaxic response comes from studies indicating that young pups move farther, more rapidly, and more consistently towards sources of warmth than do older pups (Johanson, 1979; Kleitman & Satinoff, 1982; see also Sokoloff et al., 2002). Hoffman et al. (1999b) interpreted this ontogenetic shift in thermotaxic behavior as indicating that warm stimuli have a privileged status for young pups that renders them a strong reinforcer. In contrast, the reinforcing value of cool stimuli in a hot environment increases progressively with age.

Although it is compelling to link changes in infants' behavioral thermoregulatory responses to induced thermogenesis to the developmental mechanisms responsible for the shift in thermotaxic behavior, we must recognize other possible explanations. For example, while we were able to stimulate BAT thermogenesis in the youngest infants tested, their increased surface-to-mass ratio may have ameliorated the effect of our pharmacological manipulation. That is, the larger surface-to-mass ratio of the 2-day-old pups may have allowed them to dissipate heat faster than the older and larger pups. Therefore, the 2-day-olds may not have experienced a rise in body temperature sufficient to alter their behavior. Additionally, the ontogenetic differences in thermoregulatory behavior exhibited by 2-day-olds may have resulted from basic sensory biases or associations learned in the nest environment linking warmth to other appetitive stimuli such as the mother or littermates. Of course, these last possibilities may also explain the ontogenetic change in thermotaxis described above.

It also seems unlikely that developmental status of thermotaxis alone can explain the shift in the pups' behavior. The 2-day-old pups in the present study did not simply move up the thermal gradient and stop once they encountered a preferred substrate

temperature. Rather, they changed zones on the thermocline frequently, often exploring parts of the gradient that were both warmer and cooler than the zone they most preferred. These observations suggest that even 2-day-olds were actively regulating some thermal variable(s). One possible explanation for the ontogenetic shift in the infant rat's ability to compensate for enhanced physiological heat production with behavioral thermoregulation may be that the thermal variables being regulated change over developmental time. For example, perhaps the youngest pups regulate according to body surface variables such as snout or ventrum temperatures, whereas older pups regulate according to more internal thermal variables that are influenced both by the environment in which the pup is located and by the metabolic heat generated by the pup internally. Additional research examining pups' responses to different types of thermal stimulation may provide insights into the mechanisms underlying ontogenetic shifts in thermally mediated behaviors.

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