Active Sleep in Cold-Exposed Infant Norway Rats and Syrian Golden Hamsters: The Role of Brown Adipose Tissue Thermogenesis

Greta Sokoloff and Mark S. Blumberg
University of Iowa

It was previously hypothesized that brown adipose tissue (BAT) thermogenesis helps to maintain high rates of myoclonic twitching during cold exposure in infant rats (M. S. Blumberg & M. A. Stolba, 1996). To test this hypothesis, the sensitivity of twitching to various levels of cold exposure was assessed in week-old rats that were untreated or whose BAT thermogenesis was inhibited using a ganglionic blocker. Because week-old golden hamsters do not exhibit BAT thermogenesis, their sleep behaviors during cold exposure also were examined. Additional investigations in infant rats were conducted in which supplemental heat was provided to the interscapular region using a thermode and in which BAT was activated pharmacologically in ganglionically blocked pups. The results support the hypothesis that myoclonic twitching is sensitive to the prevailing air temperature and the activation of BAT thermogenesis.

Brown adipose tissue (BAT) thermogenesis is a primary means of endogenous heat production in mammalian infants (Bricke, 1992; Nedergaard, Connolly, & Cannon, 1986). Week-old rats during moderate cold exposure (i.e., air temperatures between 25–34 °C; see Blumberg & Sokoloff, in press) increase BAT thermogenesis, maintain cardiac rate, and do not emit ultrasonic vocalizations. In contrast, extreme cold exposure (i.e., air temperatures below 25 °C) overwhelm the ability of BAT thermogenesis to compensate for heat loss, resulting in bradycardia and ultrasound production (Blumberg & Sokoloff, 1997; Blumberg & Sokoloff, in press; Blumberg, Sokoloff, & Kirby, 1997; Blumberg & Stolba, 1996; Kirby & Blumberg, 1998; Sokoloff & Blumberg, 1997; Sokoloff, Kirby, & Blumberg, in press). In addition, Blumberg and Stolba (1996) examined the relationship between successful BAT thermogenesis and the expression of myoclonic twitching (a primary component of active sleep in infant rats; Gramsbergen, Schwartz, & Prough, 1970; Jouvet-Mounier, Astic, & Lacote, 1970) and found that pups exposed to the moderate air temperature of 30 °C exhibited levels of myoclonic twitching that were identical to baseline levels at a thermoneutral air temperature. In contrast, during exposure to the extreme air temperature of 21 °C, myoclonic twitching decreased substantially.

Although the results of Blumberg and Stolba (1996) were suggestive of a relationship between BAT thermogenesis and myoclonic twitching, only two subthermoneutral air temperatures were tested. Therefore, in Experiment 1a, week-old rats (Rattus norvegicus) were exposed to two moderate and two extreme air temperatures to determine more precisely the relationship between BAT thermogenesis and myoclonic twitching. In Experiment 1b, to examine the effects of air temperature and BAT thermogenesis on myoclonic twitching in cold-exposed pups, BAT thermogenesis was inhibited using chlorisondamine, a ganglionic blocker. Finally, in Experiment 1c, myoclonic twitching was measured in week-old Syrian golden hamsters (Mesocricetus auratus) during cold exposure; golden hamsters were chosen for comparison with rats because they do not exhibit functional BAT thermogenesis until 2 weeks of age (Blumberg, 1997; Hissa, 1968). All together, these experiments further elucidate the protective role of BAT thermogenesis for the expression of myoclonic twitching.

Experiment 1

Method

Subjects. Twelve 6–7-day-old male rat pups from seven litters born to Harlan (Indianapolis, IN) Sprague-Dawley Norway rat females and 6–8-day-old male hamster pups from six litters born to Charles River (Wilmington, MA) Syrian golden hamster females were used. Body weights ranged from 11.7–16.5 g for the rat pups and 7.3–12.7 g for the hamster pups. For both species, litters were culled to 8 pups within 3 days after birth (day of birth = Day 0). 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. All animals were maintained on a 12-hr light–dark schedule with lights on at 6 a.m.

Test environment. Individual pups were tested in a double-walled glass chamber (height = 17 cm; i.d. = 12.5 cm; see Blumberg & Stolba, 1996). 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. Pups were tested on a round polyethylene mesh platform fitted inside the chamber.

Temperature measurements. Air temperature (T<sub>a</sub>) and interscapular temperature (T<sub>i</sub>) 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<sub>a</sub> within the metabolic chamber was determined by averaging the temperature recorded by two thermocouples situated beneath the platform. T<sub>i</sub> was measured by attaching a thermocouple to the pup’s skin in the interscapular region above the brown fat pad with the adhesive collodion.

Oxygen consumption measurements. Oxygen consumption was measured as described by Blumberg and Stolba (1996). Briefly, compressed air was passed through two lines. One line circulated air through the metabolic chamber at 300 ml/min and then was drawn into the first channel of an electrochemical oxygen analyzer. A second line passed air directly into the second channel of the oxygen analyzer. The percentage of oxygen present in each airstream was measured simultaneously and differences were computed to 0.001%. Data were then fed into the computerized data acquisition system and converted into a measure of oxygen consumption (VO<sub>2</sub>) in ml O<sub>2</sub>/kg/min.

Data acquisition. Thermal and metabolic measures were acquired at least four times each minute throughout the test using a customized data acquisition system for the Macintosh computer (LabView, National Instruments, Austin, TX). Twitching and awake data were acquired by pressing a designated key on a computer keyboard; the specific event (i.e., twitching or awake behavior) and the time of the key press were recorded using an event recorder program written in Hypercard for the Macintosh.

Myoclonic twitching and awake behavior. An experienced observer monitored twitching and awake behavior through the glass walls of the chamber. Twitching was 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 et al., 1970). Awake behaviors included coordinated motor activities such as stretching, kicking, yawning, and locomotion. In addition, if the pup exhibited postural elevation of the head, torso, or both, the observer scored this as an awake behavior and repeatedly hit the key at a rate of at least once per second until the behavior ceased.

Drugs. The ganglionic blocker, chlorisondamine hydrochloride (Ciba-Geigy Corp., Summit, NJ), was dissolved in isotonic saline before use (Experiment 1b). Drug injections were administered subcutaneously at a volume of 1 μl/g body weight.

Procedure. On the day of testing, a pup was removed from its cage, weighed, and placed inside an incubator maintained at 35–36 °C. For pups in Experiments 1a and 1b, littermates were never used in the same experiment. Only pups that had fed recently were tested, as determined from the presence of a milk band visible through the pup’s abdominal skin. After the thermocouples were attached, the pup was placed inside the metabolic chamber (T<sub>a</sub> = 35 °C) and allowed to acclimate for at least 45 min, after which acquisition of physiological data began. After the acclimation period, a 15-min baseline period of behavioral data was acquired.

In Experiment 1a (rat pups), after the baseline period, T<sub>a</sub> was decreased from 35 °C to 30 °C, 27 °C, 23 °C, and 19 °C for each of 6 pups. For each decrease in T<sub>a</sub>, the pup was given 45 min to stabilize followed by a 15-min period of behavioral data acquisition. To ensure that the length of the experiment did not affect the responses of the pups during cold exposure, after the fourth temperature drop T<sub>a</sub> was returned to 35 °C and, after 45 min, there was a final period in which behavioral data were acquired.

In Experiment 1b (ganglionically blocked rat pups), each of 6 pups received a subcutaneous injection of chlorisondamine hydrochloride, at a dose of 5 mg/kg, 30 min after being placed in the metabolic chamber. This dose has previously been shown to be effective in blocking BAT thermogenesis in week-old rats (Blumberg et al., 1997). After the injection, the chamber was resealed and the oxygen analysis system was allowed to restabilize for 15 min, allowing for a total of 45 min for the pup to acclimate to the chamber. After acquisition of baseline behavioral data at 35 °C, T<sub>a</sub> was decreased to 30 °C, 26 °C, and 23 °C. As with the untreated pups, for each decrease in T<sub>a</sub>, the pup was allowed 45 min to stabilize followed by a 15-min period of behavioral data acquisition.

In Experiment 1c (hamster pups), after the baseline period at 35 °C, T<sub>a</sub> was decreased to 30 °C, 26 °C, and 23 °C. As with both previous experiments, for each decrease in T<sub>a</sub>, each of the 6 hamster pups was given 45 min to stabilize followed by a 15-min period of behavioral data acquisition.

For all three experiments, after the final period of behavioral data acquisition the pup was removed from the chamber and the chamber was resealed to allow the oxygen analyzer to rezero to verify minimal drift in the system. Due to inadequate rezeroing of the oxygen analyzer, VO<sub>2</sub> data were lost for a rat pup from both Experiment 1a and Experiment 1b and a hamster pup from Experiment 1c. After removal of the thermocouples, the pup was returned to its home cage.

Data analysis. The data for twitching and awake behavior were imported into a custom program written in Hypercard for the Macintosh. This program segmented each 15-min period of behavioral data into 900 1-s bins and, for each bin, determined whether a twitch or awake behavior occurred. Behavioral data were then imported into StatView 4.5 for the Macintosh. At each T<sub>a</sub>, a mean value for thermal and metabolic data was calculated from 60 data points acquired over the 15-min period corresponding to the acquisition of behavioral data. Thermal and metabolic data (i.e., T<sub>a</sub>, T<sub>i</sub>, and VO<sub>2</sub>) also were imported into StatView 4.5 for analysis.

For each experiment, paired t tests were used to test for differences at each subthermoneutral T<sub>a</sub> between a dependent variable and its baseline value. For all experiments, the significance level was set at p < .05 and α was corrected for multiple comparisons using a Bonferroni procedure.
Results

Experiment 1a: Rat pups. Data from each 15-min observation period are presented in Figure 1. With each successive decrease in $T_a$, decreases in $T_{is}$ became disproportionately larger, as has been reported previously (e.g., Sokoloff & Blumberg, 1997). After exposure to a $T_a$ of 27 °C, the average decrease in $T_{is}$ from baseline was 1.9 °C. Further decreases in $T_a$ to 23 °C and 19 °C, produced average decreases in $T_{is}$ from baseline of 5.7 °C and 10.8 °C, respectively. All values of $T_{is}$ at $T_a$s of 27 °C, 23 °C, and 19 °C were significantly different from baseline, $6.5 < t(5) < 15.1, p \leq .005$.

$V_O_2$ increased significantly after the first drop in $T_a$ to 30 °C, $t(4) = 15.5, p = .0001$. This response is typical of week-old rats during moderate cold exposure (Sokoloff & Blumberg, 1997; Spiers & Adair, 1986). $V_O_2$ continued to increase until it reached a maximum level at a $T_a$ of 27 °C, which was also significantly different from baseline, $t(4) = 16.8, p < .0001$. As $T_a$ was decreased further to 23 °C and 19 °C, $V_O_2$ decreased and was no longer significantly different from baseline. This depression of $V_O_2$ reflects decreased metabolism due to falling body temperatures (Schmidt-Nielsen, 1991).

The amounts of twitching and awake behavior were quantified by determining the number of 1-s bins in which one of the above behaviors was observed. The amount of twitching at 30 °C was not significantly lower than baseline. At a $T_a$ of 27 °C, twitching did decrease significantly from baseline values, $t(5) = 4.3, p < .01$; however, the percentage decrease in twitching at this $T_a$ was only 16% in relation to baseline. In contrast, after exposure to 23 °C and 19 °C, the percentage decreases in twitching were 60% and 85%, respectively; these decreases were significant, $3.8 < t(5) < 9.4, p \leq .01$.

Figure 1 shows that awake behavior increased as $T_a$ was decreased. However, it was only at the $T_a$ of 27 °C that awake behavior differed significantly from baseline, $t(5) = 5.50, p < .005$.

After exposure to 19 °C, $T_a$ was increased to 35 °C for 45 min, after which there was a final behavioral data acquisition period. All behavioral and physiological variables returned to values similar to baseline values. Most important, the amount of twitching after the return to 35 °C did not differ significantly from twitching at baseline, $t(5) = 1.2, p > .25$. Because prolonged maternal deprivation and cold exposure did not influence the pups' behavioral responses on return to a thermoneutral $T_a$, test length was not considered a significant variable in the relatively short experiments that follow.

Figure 1. Interscapular temperature ($T_{is}$), oxygen consumption ($V_O_2$), and amounts of twitching and awake behavior for the untreated week-old rats in Experiment 1a. At moderate air temperatures ($T_a$) of 30 °C and 27 °C, brown adipose tissue (BAT) thermogenesis increased and myoclonic twitching remained near baseline levels. In contrast, at extreme air temperatures ($T_a$) of 23 °C and 19 °C, BAT thermogenesis could increase no further and twitching levels decreased ($M \pm SEM$). Asterisks refer to values significantly different from baseline.
Experiment 1b: Ganglionically blocked rat pups. Figure 2 presents the data for each 15-min observation period for week-old chlorisondamine-treated pups during cold exposure. Unlike the untreated pups in Experiment 1a, ganglionically blocked pups did not maintain T_{is} at any subthermonuclear T_{a} during the experiment. At each change in T_{a}, to 30 °C, 26 °C, and 23 °C, T_{is} differed significantly from baseline, 38.6 < t(5) < 57.2, p < .0001.

Figure 2 also shows VO_{2} for chlorisondamine-treated pups during cold exposure. As with T_{is}, VO_{2} differed
significantly from baseline, decreasing further with each decrease in $T_a$, $9.3 < t(4) < 11.3$, $p < .001$. Again, these decreases in $V_O_2$ reflect the depression of metabolism due to falling body temperatures.

The amount of twitching decreased significantly from baseline at each $T_a$, $3.8 < t(5) < 9.0$, $p < .01$. At the $T_a$ of $30^\circ C$, $26^\circ C$, and $23^\circ C$, percentage decreases in twitching from baseline levels were 37%, 59%, and 100%, respectively.

When $T_a$ was $35^\circ C$, ganglionically blocked pups unexpectedly showed a relatively high level of awake behavior compared with the untreated pups in Experiment la (see Figure 1). Then, at the $T_a$ of $30^\circ C$, awake behavior decreased significantly, $t(5) = 4.6$, $p < .01$. As $T_a$ decreased further, levels of awake behavior began to increase progressively, but it was only at the $T_a$ of $23^\circ C$ that awake behavior approached a level significantly greater than the high baseline level, $t(5) = 3.4$, $p = .02$.

Experiment lc: Hamster pups. Figure 2 also presents the data for week-old hamsters during cold challenge. Like the chlorisondamine-treated rat pups, infant hamsters did not maintain $T_is$ in the cold. At each subthermoneutral $T_a$, from $30^\circ C$ to $23^\circ C$, $T_is$ differed significantly from baseline, $40.9 < t(5) < 86.9$, $p < .0001$.

As with $T_is$, $V_O_2$ was significantly lower than baseline at each subthermoneutral $T_a$, $4.9 < t(4) < 8.9$, $p < .01$. These decreases in $V_O_2$ are comparable with those seen in the ganglionically blocked pups.

Twitching exhibited a pattern similar to that observed in chlorisondamine-treated rat pups at each $T_a$. At a $T_a$ of $30^\circ C$, twitching began to decrease, although the decrease from baseline only approached significance, $t(5) = 3.3$, $p = .02$; however, the percentage decrease in twitching from baseline levels was relatively high at 31%. At $T_a$s of $26^\circ C$ and $23^\circ C$, percentage decreases from baseline were 55% and 80%, respectively; these decreases were significantly different from baseline, $4.6 < t(5) < 8.9$, $p < .01$.

During the first drop in $T_a$ to $30^\circ C$, awake behavior increased significantly from baseline levels, $t(5) = 3.8$, $p = .01$. However, even though the amount of awake behavior remained elevated at $T_a$s of $26^\circ C$ and $22^\circ C$, these values were not statistically different from baseline.

Regression analyses. Figure 3 presents linear regression analyses of myoclonic twitching versus $T_is$ for Experiments la, lb, and lc. Because all three experiments produced multiple data points for each pup and in order to ensure that statistical assumptions of independence were not violated, parallel analyses were conducted: (a) linear regressions were performed on group mean data at each $T_{is}$ and (b) t tests were used to determine whether mean slopes (calculated from linear regressions for each individual pup) deviated significantly from zero. Because these parallel analyses produced similar results to those reported below, they will not be discussed further.

Linear regression analyses were performed separately on the data for each experiment. For all three experiments, there was a significant relationship between twitching and $T_{is}$. In Experiment la, $r^2 = .78$, $F(1, 28) = 96.4$; in Experiment lb, $r^2 = .73$, $F(1, 22) = 58.2$; and in Experiment lc, $r^2 = .78$, $F(1, 22) = 76.3$, $p < .0001$. These analyses point to a strong association between twitching and $T_{is}$ regardless of whether BAT thermogenesis is activated during cold exposure, blocked pharmacologically, or not exhibited at a particular stage of development.

Discussion

The results of Experiment 1 replicate and expand on the findings of Blumberg and Stolba (1996) by showing that
week-old rats are able to maintain baseline levels of myoclonic twitching during moderate cold exposure. As $T_s$ continues to decrease, $T_a$ begins to decrease substantially and $V_O$ plateaus or is depressed, indicating that the limits of BAT thermogenesis have been reached. When this transition from moderate to extreme cold exposure occurs, myoclonic twitching also decreases substantially.

In contrast to the responses of untreated rat pups, the twitching of ganglionically blocked pups in Experiment 1b decreases in lockstep with decreasing $T_s$. Although ganglionic blockade, in addition to inhibiting BAT thermogenesis, disrupts neural control to other organs (e.g., heart; Blumberg et al., 1997), these other effects do not appear to interfere with the expression of twitching at a $T_s$ of 35 °C (see Figures 1 and 2). On the other hand, there does appear to be more awake behavior in the ganglionically blocked pups than in the untreated pups at a $T_s$ of 35 °C, although the experiments were not designed to compare these two groups directly.

Examination of twitching in infant golden hamsters in Experiment 1c provided the opportunity to generalize the results of Experiment 1b to a species that, at the age tested, does not exhibit BAT thermogenesis in the cold and thus exhibits physiological responses similar to ganglionically blocked rats (Blumberg, 1997). These physiological similarities in response to cold were mirrored by similar changes in twitching with each successive decrease in $T_s$ and $T_a$ (Figures 2 and 3). By using the comparative method to control for a pharmacological intervention, the results of Experiment 1c add a unique form of support to the hypothesis that BAT thermogenesis protects myoclonic twitching in infant rats and, furthermore, suggests that species differences in twitching are related in part to species differences in thermoregulatory capabilities.

**Experiment 2**

Although BAT deposits are found throughout the mammalian body (e.g., Foster, Depocas, & Frydman, 1980), the interscapular BAT pad is a primary source of nonshivering thermogenesis in infant rats (Nedergaard et al., 1986; Yahata & Kuroshima, 1993). In Experiment 1a, during extreme cold exposure (i.e., $T_s \leq 23$ °C), $T_a$ decreased substantially, indicating that interscapular BAT thermogenesis was no longer capable of compensating for heat loss, and the amount of twitching decreased as well. In the present experiment, week-old rat pups were observed during extreme cold exposure with local warming or cooling of the interscapular region. If decreases in myoclonic twitching during the transition from moderate to extreme cold exposure result from the inability of BAT thermogenesis to retard heat loss, then the application of extra heat to the interscapular region at an extreme air temperature should be sufficient to maintain high levels of twitching.

**Method**

**Subjects.** Twelve 6-7-day-old male rat pups from six litters were used. Body weights ranged from 15.1-17.3 g. Rats were raised and housed as in Experiment 1.

**Thermode.** The temperature of the interscapular region was manipulated using a custom-built thermode. The conductive surface of the thermode was fashioned from the head of a brass flat-head screw (o.d. = 0.6 cm). A piece of plastic tubing (length = 1 cm; i.d. = 0.6 cm) was fitted over the head of the screw and sealed in place with cyanocrylate adhesive (Surehold, Chicago, IL). Two small pieces of silicone tubing (o.d. = 0.2 cm) were fit into the top of the body of the thermode, which then was sealed with cyanocrylate. Two longer pieces of silicone tubing (length = 7 cm; i.d. = 0.2 cm) were sealed in place over the tubes emerging from the top of the thermode, preventing leakage from the body of the thermode to the intake and outlet tubes.

**Procedures.** The test environment was the same as that used in Experiment 1. The interscapular thermocouple was anchored to the skin with collodion and secured to the skin with collodion (for the remainder of this experiment, the temperature measured by this thermocouple is referred to as $T_{thermode}$ to distinguish it from the unmanipulated measure of $T_s$ in the other experiments). In addition, a second thermocouple was attached approximately 1 cm rostral to the base of the tail to provide a measure of skin temperature ($T_{skn}$) distant from the interscapular region and thermode. Finally, physiological and behavioral measures were acquired as in Experiment 1.

**Data analysis.** Data were analyzed using a two-factor repeated measures analysis of variance (ANOVA). For post hoc analyses, paired t tests were used for within-subject comparisons and unpaired t tests were used for between-groups comparisons.
significance level was set at $p < .05$ and $\alpha$ was corrected for two comparisons using a Bonferroni procedure.

Results and Discussion

Figure 4 presents the data for the high and low conditions at the two $T_a$s tested. For all of the variables examined (i.e., $T_{thermoe}$, $T_{back}$, oxygen consumption, and twitching and awake behaviors), repeated measures ANOVAs indicated that all tests for main effects (i.e., condition and air temperature) and interactions (i.e., Condition $\times$ Air Temperature) were statistically significant, $7.3 \leq F(1, 10) \leq 2313.5$, $p < .05$. The results of the post hoc comparisons are detailed below.

At the $T_a$ of 35 °C, the groups did not differ with regard to any variable. In the low condition but not the high condition, $T_{thermoe}$ decreased significantly when $T_a$ was lowered to 23 °C, $t(5) = 33.4$, $p < .0001$. In addition, $T_{thermoe}$ was significantly different between the two groups at the lower $T_a$, $t(10) = 26.1$, $p < .0001$.

In contrast to $T_{thermoe}$, $T_{back}$ for both groups fell significantly as $T_a$ decreased from 35 °C to 23 °C; in the high condition, $t(5) = 28.5$, $p < .0001$; and in the low condition, $t(5) = 39.1$, $p < .0001$. In addition, at 23 °C, $T_{back}$ in the high condition was greater than $T_{back}$ in the low condition, $t(10) = 7.2$, $p < .0001$.

$\text{VO}_2$ increased significantly for both groups after the decrease in air temperature, for the high condition, $t(5) = 19.1$, $p < .0001$; and in the low condition, $t(5) = 13.2$, $p < .0001$. The small difference between the two groups at 23 °C was also significant, $t(10) = 3.1$, $p = .01$. The increase in $\text{VO}_2$ for the pups in the high condition indicates that the pups continued to activate BAT thermogenesis even though the thermode was providing additional heat.

After the decrease in $T_a$ from 35 °C to 23 °C, rates of twitching decreased significantly in the low condition, $t(5) = 6.0$, $p < .005$, as well as in the high condition, $t(5) = 3.7$, $p < .02$. The percentage decreases in twitching for the low and high conditions were 62% and 23%, respectively. Finally, pups in the high condition exhibited significantly higher levels of twitching than pups in the low condition, $t(10) = 3.8$, $p < .005$.

Pups in the low condition showed a significant increase in awake behavior at the $T_a$ of 23 °C, $t(5) = 3.6$, $p < .02$, as well as in the high condition, $t(5) = 3.3$, $p < .025$. The difference between the two groups at 23 °C was also significant, $t(10) = 3.0$, $p < .02$.

Figure 4. Thermode temperature ($T_{thermoe}$), back temperature ($T_{back}$), oxygen consumption ($\text{VO}_2$), and amounts of twitching and awake behavior for the week-old rats in Experiment 2. In the high condition (solid circles), $T_{thermoe}$ was maintained at 37.5 °C when $T_a$ was 35 °C and 23 °C. In the low condition (open circles), $T_{thermoe}$ was maintained at 37.5 °C when $T_a$ was 35 °C and at 32 °C when $T_a$ was 23 °C. When $T_a$ was 23 °C, pups in the high condition exhibited more twitching behavior and less awake behavior than pups in the low condition ($M \pm \text{SEM}$). Asterisks refer to values significantly different from the previous period. Crosses show significant differences between groups.
In the present experiment, the interscapular region of the pups in the low condition at the Ta of 23 °C was cooled to a temperature identical to that exhibited by the pups in Experiment 1a (see Figure 1) and, as in that experiment, amount of twitching decreased approximately 60% compared with baseline levels at a Ta of 35 °C. For those pups in the high condition for which BAT thermogenesis was supplemented by applying heat locally to the interscapular region, the decrease in twitching that normally accompanies extreme cold exposure was ameliorated, decreasing only 23%. It should be stressed that these results should not be interpreted to mean that the application of heat to other areas of the body could not have produced similar results. Rather, the present experiment was designed only to determine whether the application of supplemental heat to the region that overlies the interscapular BAT pad is sufficient to influence the amount of twitching. Whether heating of this region is necessary for this effect, or whether heating of other regions is sufficient, is a separate question that remains for further study.

Experiment 3

Ganglionic blockade of BAT thermogenesis in Experiment 1b resulted in pronounced decreases in myoclonic twitching with each successive decrease in Ta. This result suggested a causal relationship between the absence of BAT thermogenesis and the inability to protect myoclonic twitching as air temperature decreases, even at air temperatures designated as moderate in untreated rat pups. However, as discussed earlier, ganglionic blockade does not selectively inhibit BAT thermogenesis but rather blocks all sympathetic and parasympathetic outflow. Although the comparative data on golden hamsters in Experiment 1c provided additional evidence that absence of BAT thermogenesis is associated with decreasing levels of myoclonic twitching, these data did not directly address the possibility that ganglionic blockade in rats reduced twitching in the cold through a mechanism unrelated to BAT thermogenesis. Therefore, in the present experiment, twitching was monitored in ganglionically blocked rat pups in which BAT thermogenesis was activated pharmacologically. Selective activation of BAT thermogenesis is now possible with the recent availability of agonists with high affinity for the β3 adrenoceptor, a receptor complex that is concentrated in, and largely localized to, white and brown adipose tissue (Bloom et al., 1992; Takahashi et al., 1994; Zhao, Unelius, Bengtsson, Cannon, & Nedergaard, 1994). If the effects of ganglionic blockade on twitching during cold exposure are due primarily to the inhibition of BAT thermogenesis, then the selective activation of BAT in gangionically blocked pups during cold exposure should increase the amount of twitching.

Method

Subjects. Sixteen 6-7-day-old male rat pups from eight litters were used. Body weights ranged between 13.1-18.9 g. Rats were raised and housed as in Experiment 1.

Physiological and behavioral measures. The test environment was the same as was used in Experiment 1. Temperature measurements (Ta, Tis, and Tback) and VO2 were acquired in the same manner as in Experiments 1 and 2, and behavioral measures also were scored as in the previous experiments.

Drugs. CL-316243 (Wyeth-Ayerst Research, Pearl River, NY) and chlorisondamine hydrochloride (Ciba-Geigy Corp., Summit, NJ) were dissolved in isotonic saline before use. Drug injections were administered subcutaneously at a volume of 1 μg body weight.

Procedure. On the day of testing, a pup was removed from its cage, weighed, and placed inside an incubator maintained at 35–36 °C. Only pups that had fed recently were tested. As soon as the pup was placed in the incubator, it was injected subcutaneously with chlorisondamine hydrochloride (5 μg/kg). After the injection, the thermocouples were attached and the pup was placed inside the metabolic chamber at a Ta of 30 °C; recall that at this Ta, chlorisondamine-treated pups in Experiment 1b showed significant decreases in twitching from baseline. After a 45-min acclimation period, sleep and awake behaviors were scored for 15 min.

After the first period of behavioral data acquisition, the pup was then injected subcutaneously with either isotonic saline or the β3 agonist, CL-316243 (100 μg/kg). The pup again was given 45 min to stabilize followed by a second 15-min period of behavioral data acquisition. Littermates were tested on the same day, the order of the second injection was counterbalanced, and no two littermates were tested in the same condition.

After the test, the pup was removed from the chamber and the chamber was resealed to allow the oxygen analyzer to rezero. After removal of the thermocouples, the pup was returned to its home cage.

Data analysis. Data were analyzed using a two-factor repeated measures ANOVA. For post hoc analyses, paired t tests were used for within-subject comparisons and unpaired t tests were used for between-groups comparisons. The significance level was set at p < .05 and α was corrected for two comparisons using a Bonferroni procedure.

Results and Discussion

Figure 5 presents the data for ganglionically blocked pups subsequently treated either with the β3 agonist or saline. For Tis, Tis - Tback, and oxygen consumption, repeated-measures ANOVAs indicated that all tests for main effects (i.e., treatment group, time) and interactions (i.e., Group × Time) were statistically significant, 5.4 ≤ F(1, 14) ≤ 209.7, p < .05. For twitching behavior, repeated measures ANOVA indicated that only the main effects were significant, 8.8 ≤ F(1, 14) ≤ 22.9, p < .05. Finally, for awake behavior, the main effects and the interaction were not significant. The results of the post hoc comparisons are detailed below.

After the administration of the β3 agonist, Tis increased significantly, t(7) = 16.3, p < .0001. Tis did not increase in the saline-treated pups, and Tis for the two groups differed significantly after the second injection, t(14) = 9.6, p < .0001.

An increased difference between Tis and Tback in conjunction with an increase in VO2, is necessary to conclude that interscapular BAT thermogenesis was activated by the β3 agonist (Blumberg & Stolba, 1996). First, as shown in Figure 5, Tis - Tback increased significantly after injection with the β3 agonist, t(7) = 10.4, p < .0001, but not after injection with saline. Moreover, the difference between Tis - Tback for the two groups after the second injection was significant, t(14) = 4.9, p < .0005. Second, VO2 increased only for pups injected with the β3 agonist, t(7) = 16.8, p < .0001, and the
difference in VO₂ between the two groups differed significantly after the second injection, t(14) = 11.4, p < .0001. Therefore, it is apparent from these data that interscapular BAT thermogenesis was stimulated by the β₃ agonist, although it is not possible to determine whether other BAT pads were stimulated as well.

The amount of twitching increased significantly over baseline in the pups injected with the β₃ agonist, t(7) = 4.8, p < .005, but did not in the pups injected with saline. In addition, the amount of twitching differed significantly between the two groups after the second injection, t(14) = 3.7, p < .005.

There were no differences in the amount of awake behavior either over time or between conditions, despite there being significant changes in twitching. The failure to find an effect on awake behavior was due, in part, to the fact that each pup injected with the β₃ agonist exhibited stereotyped motor responses during the second observation period that were not seen during the first observation period and were not exhibited by saline-treated pups; these motor responses were scored as awake behaviors. The observed motor pattern consisted of a lateral-S motion of the pelvic girdle, scratching of the back with either hind limb, or both; the scratching did not appear to be directed toward the site of the injection. Although the cause of this stereotypy is not clear at this time, it is important to stress that the amount of twitching increased significantly in these pups despite the occurrence of these awake behaviors.

**General Discussion**

This series of experiments, using descriptive, comparative, and experimental methods, expands on the work of Blumberg and Stolba (1996) in supporting the hypothesis that BAT thermogenesis protects myoclonic twitching during cold exposure. In Experiment 1a, by exposing untreated rat pups to multiple subthermoneutral air temperatures, the relationship between BAT thermogenesis and myoclonic twitching was further defined. Specifically, at moderate air temperatures, baseline levels of myoclonic twitching were maintained. In contrast, at extreme air temperatures, myoclonic twitching fell dramatically. Therefore, the transition between moderate and extreme cold exposure is characterized by a suite of physiological and behavioral changes, including decreases in interscapular temperature, oxygen consumption, and respiratory rate (Sokoloff & Blumberg, 1997), bradycardia (Blumberg et al., 1997), increased ultra-

---

**Figure 5.** Interscapular temperature (Tᵢₛ), interscapular minus back-skin temperature (Tᵢₛ - Tᵢ₉ₑₛ), oxygen consumption (VO₂), twitching, and awake behavior for week-old rats in Experiment 3. All pups were pretreated with chlorisondamine to effect ganglionic blockade. Baseline data were then collected at a Tₑ of 30 °C, after which they were injected either with saline (open circles) or β₃ agonist (CL-316243; solid circles). After administration of β₃ agonist, pups exhibited increases in Tᵢₛ, Tᵢₛ - Tᵢ₉ₑₛ, VO₂, and myoclonic twitching (M ± SEM). Asterisks refer to values significantly different from the previous period. Crosses show significant differences between groups.
sound of myoclonic twitching (Sokoloff & Blumberg, 1997), and, as shown here, decreased myoclonic twitching.

Whether a particular air temperature qualifies as moderate or extreme depends on a number of factors including age, size, insulation, and nutritional status (Blumberg & Sokoloff, in press). Experimental manipulations also can modify the nature of a thermal stimulus. This was made clear in Experiment 1b, in which ganglionic blockade inhibited BAT thermogenesis in week-old rats, causing myoclonic twitching to decrease in lockstep with decreasing interscapular temperature. Analogously, in Experiment 1c, in which week-old golden hamsters were used because they do not yet exhibit functional BAT thermogenesis, myoclonic twitching again fell in lockstep with decreasing interscapular temperature. Therefore, an inability to activate BAT thermogenesis, whatever the cause, blurs the distinction between moderate and extreme cold exposure and is associated with the disruption of the infant’s sleep behavior in a cold environment.

At the transition from moderate to extreme cold exposure, as described above, pups exhibit large decreases in twitching behavior as both interscapular temperature and oxygen consumption decrease. In Experiment 2, it was found that additional heat provided to the interscapular region of a rat pup during extreme cold exposure was sufficient to increase the amount of twitching. Because it was possible, in effect, to extend the lower range of moderate air temperatures by applying supplemental heat to the interscapular region, this experiment supports the idea that the decrease in twitching that occurs at extreme air temperatures results when the limits of BAT thermogenesis are reached and the pup can no longer compensate for increasing heat loss.

As described above, the marked similarities in the sleep behaviors of rat pups during ganglionic blockade and infant hamsters (see Figure 2) provided converging evidence in support of the hypothesis that BAT thermogenesis contributes importantly to the expression of twitching. This hypothesis was submitted to a final test in Experiment 3 in which BAT thermogenesis was selectively activated in rat pups that were pretreated with a ganglionic blocker and exposed to cold. Selective BAT activation resulted in sizable increases in twitching, supporting the idea that the modulation of twitching by ganglionic blockade was effected primarily through the inhibition of BAT thermogenesis.

In general, the awake data were more variable than the twitching data, making it difficult to arrive at firm conclusions regarding their significance. For the most part, awake behaviors did become more prevalent at the lower air temperatures as twitching decreased, although their variability prevented some of these increases from being statistically significant, especially with the relatively small sample sizes used here. It is not clear why awake behavior was more variable, but perhaps the relative diversity of criteria used to define awake behavior was a factor. For example, both overt motor behaviors as well as static elevations of the head were scored as awake behaviors, but the latter was often more difficult to discern and, moreover, sometimes comprised the majority of a pup’s awake behavior in the cold. Perhaps the use of more narrowly defined categories of awake behaviors would have clarified these results.

The relationship between ambient temperature, thermoregulation, and the organization of sleep states has been well documented in adult mammals (Heller, Gotzbach, Grahn, & Radeke, 1988; Parmeggiani, 1977, 1987). Active sleep is sensitive to ambient temperature (Schmidek, Hoshino, Schmidek, & Tino-Iaria, 1972; Szynusiak & Satinoff, 1981) and, in subthermoneutral air temperatures, warming of skin temperature alone can trigger entry into active sleep (Szynusiak, Satinoff, Schallert, & Whishaw, 1980). Moreover, thermoregulatory mechanisms (e.g., shivering, sweating, vasomotor activity) are inhibited during active sleep and body temperature will increase or decrease passively in a cool or warm ambient environment, respectively (Parmeggiani, 1977; Walker, Harris, & Berger, 1983). An investigation of BAT thermogenesis in adult rats showed that BAT thermogenesis also is inhibited during active sleep (Calasso, Zantedeschi, & Parmeggiani, 1993). In contrast to this last finding, however, neither in the present study nor in a previous study (Blumberg & Stolba, 1996) have we seen any indication that BAT thermogenesis is inhibited during active sleep. This lack of inhibition may generally be the case for young mammals (e.g., Francini et al., 1986), in which many of the defining features of active sleep in adults are not yet exhibited (Blumberg & Lucas, 1996; Mirmiran, 1995).

Ambient temperature may influence the expression of myoclonic twitching in a number of ways. First, it is possible that the spontaneous firing rates of the spinal and brainstem motor units that produce myoclonic twitching are temperature dependent, like many aspects of neuronal function (Jasper, Shacter, & Montplaisir, 1970; Montgomery & Macdonald, 1990); similarly, ambient temperature also can modulate the contractile properties of muscle fibers (Bennett, 1984, 1990). Second, as is known in adult rats (Schmidek et al., 1972), cold air temperatures may induce postural adjustments that either compete with the expression of twitching or make it difficult to detect twitching when it occurs; in the present study, we observed cold-exposed pups in a prone position with their limbs pulled in close to their body. Finally, the expression of sleep behaviors may be influenced by temperature-dependent changes in the cardiovascular or other physiological systems. For example, bradycardia during extreme cold exposure (Blumberg et al., 1997) may elicit a cascade of cardiovascular adjustments that interfere with the expression of myoclonic twitching or that arouse the rat. Any or all of these mechanisms may contribute to the effect of air temperature on twitching found in the present study and, in addition, they may differ in their influence at different extremes of cold exposure.

Many investigators have noted that infant rats readily exhibit active sleep behaviors in a warm environment (Kleitman & Satinoff, 1982; Whishaw, Schallert, & Kolb, 1979). The arousing effects of a cold environment also have been noted, but, because rat pups often have been considered poor thermoregulators, their ability to respond adaptively to a moderately cold environment has been overlooked (Blumberg & Sokoloff, in press). The present study clearly indicates that BAT thermogenesis contributes to the modula-
tion of behavioral state in infant rats and that the absence of BAT thermogenesis in infant hamsters likely accounts for the species differences observed in sleep behaviors during cold exposure. This study also suggests that a pup’s physiological and behavioral thermoregulatory capabilities influence the expression of myoclonic twitching, a predominant perinatal behavior that has been hypothesized to play a central role in the development of the nervous system (Blumberg & Lucas, 1996; Marks, Shaffery, Oksenberg, Speciale, & Roffwarg, 1995; Roffwarg, Muzio, & Dement, 1966).

References


Received August 22, 1997
Revision received November 25, 1997
Accepted November 25, 1997