Behavioral Neuroscience
1991, Vol. 105, No. 6, 1030-1037


0735-7044/91/$3.00

# Both Hypoxia and Milk Deprivation Diminish Metabolic Heat Production and Ultrasound Emission by Rat Pups During Cold Exposure

Mark S. Blumberg and Jeffrey R. Alberts
Indiana University

Rat pups (7–9 days of age) were made cold and hypoxic simultaneously while interscapular temperature, rectal temperature, and ultrasound emission were monitored. These hypoxic pups cooled faster than control pups, which indicates decreased thermogenesis and decreased oxygen consumption, and produced less ultrasound. In a separate experiment, pups deprived of milk for 24 hr cooled faster and also produced less ultrasound than did nondeprived littermates. Further analyses revealed that those pups that cooled the slowest (and thus used the most oxygen) vocalized the most, both among control animals as well as across the two manipulated groups. This finding suggests that ultrasound emission covaries with thermogenesis. The observed pattern is opposite to that predicted by traditional communication hypotheses of rat pup vocalizations and favors understanding the sounds as symptoms of laryngeal braking.

Newborn rodents of many different species respond to cold exposure by emitting a high frequency ("ultrasonic") vocalization (e.g., rats: Okon, 1971; hamsters: Okon, 1971; mice: Okon, 1970; lemmings: Brooks & Banks, 1973). This vocalization appears to attract the attention of the mother and elicit searching and retrieval behaviors (Allin & Banks, 1972). The apparent functional benefit of ultrasound emission (i.e., retrieval to the warm nest) has led to the assumption that this vocalization is emitted to elicit maternal retrieval. As a consequence, the vocalization has come to be called a "distress call," and the vocalization itself has come to be used as a metric of "separation distress" (e.g., Kehoe & Blass, 1986). In turn, these interpretations have led to the adoption of the ultrasound-emitting pup as a model for the neuropharmacological treatment of anxiety in newborns (e.g., Hard, Engel, & Lindh, 1988).

Recently, we proposed an alternative view of the physiological basis of ultrasound emission (Blumberg & Alberts, 1990, 1991). This alternative view arose after we began to study in 10–12-day-old rat pups the relations between ultrasound emission and other physiological responses to cold exposure. Specifically, when we monitored simultaneously oxygen consumption, heat production of brown adipose tissue (BAT), respiratory rate, and ultrasound emission before and after cold exposure, we found that ultrasound emissions began contemporaneously with a suite of other responses, including increases in oxygen consumption, BAT thermogenesis, and respiratory rate.

We might have concluded that the infants' responses to cold exposure consist of two components that are activated contemporaneously. The first component would be designated as "physiological," which involves increases in oxygen consumption, nonshivering thermogenesis (NST), and respiratory rate. The second component would be designated as "behavioral," which involves the production of ultrasound as a correlate of isolation and cold and as a means of communicating distress to the mother and thus eliciting maternal retrieval.

However, we did not conclude that there exists such a division between physiological and behavioral responses. Instead, based on the respiratory mechanics underlying ultrasound emission (Blumberg & Alberts, 1990; Roberts, 1972, 1975), we suggested that the ultrasonic vocalization is an acoustic by-product of a respiratory maneuver called laryngeal braking. Laryngeal braking is ubiquitous among mammalian newborns and often accompanies stimulation of the respiratory system and increases in oxygen consumption (Mortola, 1987). It occurs when the laryngeal musculature constricts at the beginning of the expiratory cycle and results in a prolongation of expiratory duration and an increase in intrathoracic pressure. In turn, laryngeal braking prevents alveolar collapse, recruits additional alveoli, and thus increases the lung surface available for gas exchange (Davis & Bureau, 1987; England, Kent, & Stogryn, 1985; Johnson, 1985). Viewed in this way, laryngeal braking is a component of the rat pup's physiological response to cold exposure that also happens to produce sound.

The validity of this new view of ultrasound emission is enhanced when observations of other species are considered. For example, human infants, especially premature infants and those suffering from respiratory distress syndrome (RDS), exhibit laryngeal braking as a symptom of respiratory distress. Although many instances of laryngeal braking occur inaudibly (e.g., Lindroth, Johnson, Ahlstrom, & Svenningsen, 1981), RDS infants emit an audible grunt during breathing that coincides with laryngeal constriction and reduced

This research was supported by U.S. Public Health Service Grant MH-28355 from the National Institute of Mental Health to Jeffrey R. Alberts.

We thank Ann Huguenard for technical assistance.

Correspondence concerning this article should be addressed to Mark S. Blumberg, Department of Psychology, Indiana University, Bloomington, Indiana 47405.

expiratory flow (Harrison, de V. Heese, & Klein, 1968). The similarities between grunting human infants and ultrasounding infant rats suggested to us that grunting and ultrasound emission may be homologous behaviors. Furthermore, newborn lambs can also emit an audible, loud grunt during laryngeal braking (P. Johnson & D. Andrews, personal communication, October 18, 1990).

If laryngeal braking is linked in a significant way with metabolic heat production during cold exposure, then manipulations that decrease metabolic heat production should also decrease laryngeal braking and thus decrease ultrasound emission. A number of manipulations can be used to decrease the metabolic response of infants to cold exposure. For example, hypoxia and the resultant decrease in blood-oxygen levels suppresses heat production by BAT during cold exposure because of this organ's sensitivity to decreased levels of oxygen in blood (Heim & Hull, 1966). Because NST is responsible for most, if not all, of the metabolic heat produced by rat pups during cold exposure (Hull, 1973), the thermogenic response to cold exposure can be virtually eradicated by hypoxia. Moreover, this hypoxia-induced decrease in metabolic heat production is likely due to a regulated depression in ventilation (Saetta & Mortola, 1987) that is maintained by brainstem respiratory neurons (Martin-Body & Johnston, 1988).

Another manipulation that decreases the metabolic response to cold exposure is deprivation of milk (Bignall, Heggeness, & Palmer, 1974). Pups removed from maternal care and maintained in a warm incubator for 10–20 hr display a greatly reduced metabolic response to cold challenge. This decreased responsiveness to cold appears to be caused by active suppression of heat production by the central nervous system rather than by depletion of energy stores (Bignall, Heggeness, & Palmer, 1975).

In this study, we investigated the effects of hypoxia and milk deprivation on ultrasound emission and thermogenesis during exposure of rat pups to cold. As just stated, both of these manipulations appear to effect reductions in metabolic heat production via centrally mediated inhibition. Therefore, if laryngeal braking complements the thermogenic response to cold and if the ultrasonic vocalization is the acoustic by-product of laryngeal braking, then hypoxic pups and milk-deprived pups should display decreased levels of laryngeal braking (i.e., they should vocalize less) in relation to controls, even as they are cooling rapidly. On the other hand, if the conventional wisdom is correct and ultrasound emission is primarily a behavior designed to communicate distress, then hypoxic pups and milk-deprived pups should display either increased or similar levels of ultrasound emission in relation to controls.

## Experiment 1: Effect of Hypoxia on Body Temperatures and Ultrasound Emission During Cold Exposure

Exposure of rat pups to low-oxygen conditions in the absence of cold exposure elicits a suite of responses (e.g., slowing of the heart rate, decreased blood flow to the periphery) that act in concert to conserve energy and thus increase survival time during hypoxic challenges (Lagercrantz & Slotkin, 1986; Seidler & Slotkin, 1985). In contrast, as already discussed, exposure of rat pups to cold causes an increase in oxygen consumption accompanied by physiological responses (e.g., increased respiratory rate) that supply needed oxygen to metabolically active tissues such as BAT. The combination of hypoxia and cold exposure, therefore, imposes a double stress on the infant and forces the pup to make a strategic "decision" between responses that decrease or increase oxygen consumption. The prevailing evidence indicates that, in newborn animals, cold-exposed pups made hypoxic will suppress metabolic heat production (see Rudolph, 1984).

As stated earlier, it is known that hypoxia during cold exposure diminishes metabolic heat production (Heim & Hull, 1966) and decreases ventilation (Saetta & Mortola, 1987). We have previously observed that hypoxia under thermoneutral conditions does not elicit ultrasound emission. We do not know, however, whether cold exposure elicits ultrasound emission even when thermogenesis is not activated maximally. We expect that it does not and thus predict that hypoxia during cold exposure will (a) decrease thermogenesis and (b) decrease ultrasound emission. This combination of results would not be predicted by conventional perspectives because ultrasound emission has been functionally linked to cooling.

### *Method*

*Subjects.* Sixteen Sprague-Dawley rat pups of both sexes from 13 litters were used. All the pups were born in the Indiana University colony and were descendants of a breeding population that was derived from stock originally purchased from Laboratory Supply, Incorporated, Indianapolis, Indiana. The pups were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and their mothers were housed in standard laboratory cages (48 × 20 × 26 cm) in which food and water were freely available. All animals were maintained on a 16:8-hr light–dark schedule with lights on at 7:00 a.m. On the day of testing, all pups were 7–9 days of age and weighed between 15.5 and 22.8 g.

*Temperature measurements.* Physiological temperatures were measured with chromel–constantan thermocouples attached to a digital readout device (Omega Engineering Inc., Stamford, CT) that displayed temperature to within ±0.1 °C. Thermocouple probes were calibrated against a mercury thermometer. Thereafter, calibrations were checked periodically to ensure accuracy. Rectal temperature ($T_{rectal}$) was measured by inserting the tip of a thermocouple 1.0–1.5 cm beyond the anal sphincter. To reduce movement of the thermocouple within the rectum, the thermocouple lead was fastened to the base of the tail on its ventral side. Interscapular temperature ($T_{is}$) was measured at the midline above the scapulae. A thermocouple was inserted subcutaneously through a small hole, which was created by a hypodermic needle, in the overlying skin. The thermocouple was glued in place. In addition, chamber-air temperature ($T_{air}$) was measured using an ambient temperature thermistor (Probe No. 405; Yellow Springs Instr. Co., Yellow Springs, OH). $T_{air}$ was read on a tele-thermometer (Yellow Springs) to within 0.25 °C.

*Ultrasonic vocalizations.* The rat pup's ultrasonic vocalizations were detected by an ultrasonic microphone that was placed inside the metabolic chamber. The microphone was connected to a "bat detector" (Model S100; QMC, Ltd., London, United Kingdom) that was tuned to a range centered on 40 kHz. It was sometimes necessary to vary the tuning of the bat detector to detect the maximum number

of ultrasonic pulses. To quantify the vocalization data, each minute was divided into twelve 5-s bins. The number of bins that contained at least one ultrasonic pulse was counted for each minute of the test. These data are presented as estimated percentage of time spent vocalizing.

*Procedure.* On the day of testing, a pup was removed from its cage and weighed. The thermocouples for measuring $T_{rectal}$ and $T_{is}$ were attached. The pup was then placed in a polyethylene mesh cage that confined the pup without restraint and permitted airflow all around the animal. The pup and cage were then placed inside a cylindrical Plexiglas chamber (volume = 1,460 $cm^3$). This chamber was immersed in a temperature-controlled water bath. Clean, dry, compressed air passed through the chamber and around the animal at a flow rate of approximately 400 ml/min. When first placed in the chamber, $T_{air}$ was maintained at 35 °C, which is within the thermoneutral zone of 7–9-day-old rat pups (Spiers & Adair, 1986).

The experiment began after a 60-min habituation period, during which both $T_{rectal}$ and $T_{is}$ had stabilized. At this time, a timer was started, and $T_{rectal}$, $T_{is}$, and $T_{air}$ were recorded, by hand, at the beginning of each minute. Monitoring of ultrasound also began at this time.

The test began by collecting data for at least 10 min with $T_{air}$ maintained at 35 °C. After this baseline period, pups were exposed to one of two experimental conditions. Pups in the *control group* (4 males, 4 females) were exposed to a decrease in $T_{air}$ to 24–25 °C; this 10 °C reduction in $T_{air}$ was accomplished within 10 min. Pups in the *hypoxia group* (4 males, 4 females) were exposed to an identical decrease in $T_{air}$; in addition, the air supply of these pups was replaced gradually by a gas mixture composed of 10% $O_2$ in $N_2$. The introduction of this 10% $O_2$ mixture began simultaneously with the decrease in $T_{air}$. Flow rate of the hypoxic gas was also maintained at 400 ml/min, and the oxygen concentration of air leaving the animal chamber was monitored each minute using an electrochemical oxygen sensor (Ametek, Pittsburgh, PA). This measure of oxygen concentration combines the influences of the oxygen concentration of the incoming air, the oxygen consumption by the pup, and the mixing of gas within the chamber; thus, it is an estimate of the actual oxygen concentration to which the pup was exposed at any given time. For both groups, data collection continued for at least 15 min after the beginning of chamber-air cooling.

Finally, after hypoxic pups had experienced 15 min of cooling, normoxic air was reintroduced into the test chamber. After a few minutes, by which time chamber oxygen concentration surpassed 12%, the pups were monitored for another 10 min. Air temperature was not manipulated during this part of the test.

*Data analysis.* Data are presented for four time periods during the test: the 10-min baseline period, and 1–5 min, 6–10 min, and 11–15 min after the onset of cooling. Temperature data are presented for the last minute of each period of the test, as are data for oxygen concentration of chamber air. The percentage of time spent vocalizing was averaged over the entire 10-min baseline period as well as the other three 5-min periods. Values are presented as mean ± *SE*. Differences between groups were tested using the unpaired *t* test (two-tailed).

## *Results and Discussion*

Table 1 presents data on seven measured or derived variables for the two groups of pups across four measurement periods. We will review these data sequentially within each of these phases of the experiment.

*Baseline.* At the end of the 10-min baseline period, $T_{air}$ was 35.1 °C, the percentage of oxygen in the chamber air was the same for both groups (20.4 %), $T_{is}$ and $T_{rectal}$ were 38.2 °C for both groups, and ultrasound emission was negligible (only six ultrasonic pulses were detected from the 16 pups in the two groups during these 10 min).

*5 Min.* After 5 min of chamber-air cooling, when hypoxic pups were breathing air that contained 15% oxygen, control pups did not differ from hypoxic pups with respect to any of the recorded variables. Moreover, ultrasound emission rate remained low in both groups.

*10 Min.* After 10 min of chamber-air cooling, hypoxic pups were breathing air that contained only 12% oxygen. $T_{is}$ and $T_{rectal}$ were lower in the hypoxic pups than the control pups, although only $T_{is}$ was significantly different ($t = 2.674$, $df = 14$, $p < .02$). In addition, control pups were vocalizing nearly twice as much as hypoxic pups, but this difference did not achieve statistical significance.

*15 Min.* After 15 min of chamber-air cooling, hypoxic pups were breathing air that contained 11% oxygen. $T_{is}$ was 1.0 °C cooler in the hypoxic pups than in the control pups, and $T_{rectal}$ was 0.7 °C cooler. Both of these differences are significant ($T_{is}$: $t = 5.788$, $df = 14$, $p < .0001$; $T_{rectal}$: $t = 3.095$, $df = 14$, $p < .01$). Moreover, the control pups vocalized more than 33% of the time during this period, whereas the hypoxic pups vocalized only 5% of the time. This difference also is significant ($t = 2.654$, $df = 14$, $p < .02$). Thus, these data support the hypothesis that metabolic heat production is linked to ultrasound emission: Pups that cooled slowly vocalized more than pups that cooled quickly.

A relationship between metabolic heat production and ultrasound emission also is apparent when the control pups are analyzed individually. It is well known that the production of ultrasound during cold exposure is highly variable, and in fact, we found that during the 15 min of cold exposure, control pups vocalized from 0% to 64% (22.2 ± 7.6) of the time. Variability in cooling rates of $T_{is}$ was also high: specifically, after 15 min of cooling, $T_{is}$ had decreased 1.8–4.4 °C in the 8 pups. Interestingly, the pup that cooled 1.8 °C vocalized 37% of the time, and the pup that cooled 4.4 °C vocalized 2% of the time. Overall, the correlation between cooling rate and ultrasound emission was highly significant ($r = .897$, $n = 8$, $p < .005$).

In contrast to the control pups, the hypoxic pups showed little variability in either cooling rate or ultrasound emission. Specifically, during the 15 min of cold exposure, hypoxic pups vocalized from 1% to 19% (6.9 ± 2.3) of the time; after 15 min of cooling $T_{is}$ had decreased 4.0–4.5 °C. Thus, with respect to ultrasound emission and cooling rate, the hypoxic pups resembled those control pups that vocalized little and cooled fast.

We have demonstrated that over 15 min of chamber-air cooling, hypoxic pups vocalized 6.9% of the time and $T_{is}$ decreased 4.0–4.5 °C (over the 15-min test, this corresponds to a cooling rate of −0.27 to −0.30 °C/min). After these 15 min of cold exposure, hypoxic pups were followed for 10 min more as normoxic air was restored to the chamber. Over these 10 min, the pups vocalized from 16% to 58% (36.6 ± 5.5 %) of the time. In addition, during this same time period, changes in $T_{is}$ ranged from a rate of decrease of 0.07 °C/min to a rate of increase of 0.04 °C/min (−0.02 ± 0.01 °C/min).

Table 1
*Chamber-Air Temperature ($T_{air}$), Oxygen Concentration in Air, Interscapular Temperature ($T_{is}$), and Rectal Temperature ($T_{rectal}$) at the End of Baseline (BASE) and at the End of Each 5-Min Interval After the Onset of Cooling for Hypoxic (H) and Control (C) Pups*

| Period/ Group | $T_{air}$ | | % $O_2$ in air | | $T_{is}$ | | $\Delta T_{is}$ in relation to baseline | | $T_{rectal}$ | | $\Delta T_{rectal}$ in relation to baseline | | % Time spent vocalizing | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* |
| BASE | | | | | | | | | | | | | | |
| C | 35.1 | 0.1 | 20.4 | 0.0 | 38.2 | 0.3 | — | — | 38.2 | 0.4 | — | — | 0.3 | 0.2 |
| H | 35.1 | 0.1 | 20.4 | 0.0 | 38.2 | 0.1 | — | — | 38.2 | 0.2 | — | — | 0.3 | 0.2 |
| 5 Min | | | | | | | | | | | | | | |
| C | 27.0 | 0.3 | 20.5 | 0.0 | 36.8 | 0.3 | −1.4 | 0.1 | 37.4 | 0.3 | −0.8 | 0.1 | 6.5 | 3.7 |
| H | 27.4 | 0.1 | 14.9* | 0.2 | 36.6 | 0.2 | −1.6 | 0.1 | 37.2 | 0.1 | −1.0 | 0.1 | 0.8 | 0.5 |
| 10 Min | | | | | | | | | | | | | | |
| C | 25.0 | 0.1 | 20.5 | 0.1 | 35.6 | 0.1 | −2.5 | 0.2 | 35.8 | 0.2 | −2.4 | 0.2 | 26.3 | 10.5 |
| H | 25.1 | 0.1 | 12.4* | 0.1 | 35.1* | 0.2 | −3.1* | 0.1 | 35.4 | 0.2 | −2.8 | 0.1 | 14.2 | 5.7 |
| 15 Min | | | | | | | | | | | | | | |
| C | 24.5 | 0.0 | 20.5 | 0.1 | 34.9 | 0.1 | −3.3 | 0.4 | 34.7 | 0.1 | −3.5 | 0.3 | 33.3 | 10.7 |
| H | 24.5 | 0.1 | 11.1* | 0.1 | 33.9* | 0.1 | −4.3* | 0.1 | 34.0* | 0.2 | −4.2* | 0.1 | 5.0* | 1.3 |

*Note.* $\Delta T_{is}$ and $\Delta T_{rectal}$ in relation to their respective baseline temperatures are also shown. Percentage of time spent vocalizing represents average levels of vocalization over each of the four test periods.
*$p < .05$ in relation to control value.

Thus, restoring normal oxygen levels to these previously hypoxic pups resulted in increases in both metabolic heat production and ultrasound emission (and thus, we argue, laryngeal braking), once again supporting the hypothesis that laryngeal braking and metabolic heat production are complementary responses.

## Experiment 2: Effect of Milk Deprivation on Body Temperatures and Ultrasound Emission During Cold Exposure

As predicted, Experiment 1 showed that a manipulation that reduces metabolic heat production also reduces ultrasound emission. Experiment 2 was conducted to extend this prediction by imposing on rat pups a different manipulation, again designed to reduce NST and hence reduce ultrasound emission. This manipulation involved the isolation of pups from their mother, which resulted in milk deprivation. As stated earlier, this procedure causes an active suppression of heat production during cold exposure (Bignall et al., 1975).

### Method

*Subjects.* Sixteen pups from eight litters were used; they were from the same stock as the pups described in Experiment 1. All pups were reared as in Experiment 1.

*Temperature measurements.* $T_{rectal}$ and $T_{is}$ were measured as in Experiment 1.

*Ultrasonic vocalizations.* Ultrasound emission was measured as in Experiment 1.

*Procedure.* Two test groups were formed from eight pairs of siblings. When a litter was 7 days of age, it was divided into two groups of 4 pups each; the two groups were formed such that they were nearly identical in terms of both weight and sex. The first group (*control*) was returned to its mother in the home cage. These pups and their mother were returned to the colony. The second group (*milk deprived*) was placed in a plastic vessel that contained wood shavings. This vessel was placed inside an incubator that was maintained at 34 ± 1 °C by means of a temperature controller (Yellow Springs).

On the next day (the day of testing), when the pups were 8 days of age, a pup was removed either from its cage or from the incubator and weighed. The thermocouples for measuring $T_{rectal}$ and $T_{is}$ were attached, according to procedures described in Experiment 1. The pup was then placed in the polyethylene mesh cage, which was then placed in the Plexiglas chamber. Airflow through the chamber was again maintained at 400 ml/min. $T_{air}$ within the chamber was 35 °C during the 60-min habituation period. After this time, a timer was started, and $T_{rectal}$, $T_{is}$, and $T_{air}$ were recorded, by hand, at the beginning of each minute. Monitoring of ultrasound also began at this time.

The test began by collecting data for at least 10 min with $T_{air}$ maintained at 35 °C. Pups in both groups were exposed to a decrease in $T_{air}$ to 24–25 °C as in Experiment 1. Data collection continued for 20 min after the beginning of chamber-air cooling. After the completion of this test, the other pup in the pair was tested using an identical procedure. This paired pup was always the same sex as the first pup tested; the same number of males and females were used in this experiment. The order of testing was counterbalanced.

*Data analysis.* Data are presented for five time periods during the test: the 10-min baseline period, and 1–5 min, 6–10 min, 11–15 min, and 16–20 min after the onset of cooling. Temperature data are presented for the last minute of each period of the test. The percentage of time spent vocalizing was averaged over the entire 10-min baseline period as well as the four 5-min periods. Values are presented as mean ± *SE*. Because the pups were tested in pairs, differences between groups were evaluated using the paired *t* test (two-tailed).

### Results and Discussion

At the time of testing, the milk-deprived pups had been away from their mother for 22.5–26 hr. As expected, these

Table 2
*Chamber-Air Temperature ($T_{air}$), Interscapular Temperature ($T_{is}$), and Rectal Temperature ($T_{rectal}$) at the End of Baseline (BASE) and at the End of Each 5-Min Interval After the Onset of Cooling for Milk-Deprived (D) and Control (C) Pups*

| Period/ Group | $T_{air}$ | | $T_{is}$ | | $\Delta T_{is}$ in relation to baseline | | $T_{rectal}$ | | $\Delta T_{rectal}$ in relation to baseline | | % Time spent vocalizing | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* | *M* | *SE* |
| BASE | | | | | | | | | | | | |
| C | 35.5 | 0.1 | 38.5 | 0.1 | – | – | 38.4 | 0.1 | – | – | 0.7 | 0.3 |
| D | 35.5 | 0.0 | 37.4* | 0.3 | – | – | 37.2* | 0.4 | – | – | 1.7 | 0.7 |
| 5 Min | | | | | | | | | | | | |
| C | 28.5 | 0.3 | 37.1 | 0.2 | −1.5 | 0.1 | 37.6 | 0.2 | −0.8 | 0.1 | 0.2 | 0.2 |
| D | 28.2 | 0.3 | 36.1 | 0.3 | −1.3 | 0.1 | 36.2* | 0.5 | −1.0 | 0.2 | 2.3 | 1.7 |
| 10 Min | | | | | | | | | | | | |
| C | 25.5 | 0.1 | 35.7 | 0.2 | −2.9 | 0.2 | 36.0 | 0.2 | −2.5 | 0.1 | 24.7 | 9.0 |
| D | 25.1 | 0.2 | 34.2* | 0.4 | −3.2 | 0.2 | 34.2* | 0.6 | −2.9 | 0.2 | 6.0 | 3.7 |
| 15 Min | | | | | | | | | | | | |
| C | 24.9 | 0.0 | 34.8 | 0.2 | −3.7 | 0.2 | 34.8 | 0.2 | −3.7 | 0.2 | 37.5 | 10.5 |
| D | 24.5* | 0.1 | 32.8* | 0.4 | −4.5* | 0.2 | 32.8* | 0.5 | −4.4* | 0.2 | 6.0* | 1.7 |
| 20 Min | | | | | | | | | | | | |
| C | 24.6 | 0.0 | 34.4 | 0.2 | −4.2 | 0.3 | 34.0 | 0.2 | −4.4 | 0.2 | 41.0 | 7.2 |
| D | 24.5* | 0.0 | 31.7* | 0.4 | −5.7* | 0.2 | 31.6* | 0.4 | −5.6* | 0.1 | 8.5* | 3.2 |

*Note.* $\Delta T_{is}$ and $\Delta T_{rectal}$ in relation to their respective baseline temperatures are also shown. Percentage of time spent vocalizing represents average levels of vocalization over each of the five test periods.
*$p < .05$ in relation to control value.

pups lacked milk bands, whereas all control pups selected for testing clearly had milk bands. These differences in nutritive state were also reflected in the body weights of the pups. Just before testing, milk-deprived pups weighed 16.3 ± 0.5 g, whereas control pups weighed 19.5 ± 0.7 g. This difference is significant ($t = 9.985$, $p < .0001$).

Table 2 presents the data for control and milk-deprived pups, with the same measures (other than $O_2$%) used in Experiment 1. Again, we will consider the results for each of the five sequential measurement periods.

*Baseline.* At the end of the 10-min baseline period, both $T_{is}$ and $T_{rectal}$ were significantly reduced in the milk-deprived pups. This was surprising given previous reports that oxygen consumption does not differ between milk-deprived and control pups at a thermoneutral temperature (Bignall et al., 1974). These investigators, however, did not report rectal or skin temperatures; furthermore, the pups in our experiment were deprived of maternal care for 4–6 hr longer than were those in the experiment of Bignall et al. Although it is possible that the reduced body weight of the milk-deprived pups contributed to lower body temperatures, there was no correlation between body weight and baseline $T_{is}$ or $T_{rectal}$ (body weight vs. $T_{is}$: $r = .412$, $n = 16$, $p > .10$; body weight vs. $T_{rectal}$: $r = .402$, $n = 16$, $p > .10$). In light of differences in temperatures between milk-deprived and control pups, the following discussion will focus on decreases in $T_{is}$ and $T_{rectal}$ in relation to individual baseline temperatures ($\Delta T_{is}$ and $\Delta T_{rectal}$, respectively).

*5 Min.* After 5 min of chamber-air cooling, control pups did not differ from milk-deprived pups with respect to $\Delta T_{is}$, $\Delta T_{rectal}$, or ultrasound emission.

*10 Min.* After 10 min of chamber-air cooling, control pups still did not differ from milk-deprived pups with respect to $\Delta T_{is}$, $\Delta T_{rectal}$, or ultrasound emission. Differences in $T_{is}$ and $T_{rectal}$ continue to be due in large part to differences already existing during the baseline period.

*15 Min.* After 15 min of chamber-air cooling, $T_{is}$ in the milk-deprived pups had decreased 0.8 °C more than in the control pups (−3.7 °C vs. −4.5 °C), $T_{rectal}$ had decreased 0.7 °C more (−3.7 °C vs. −4.4 °C), and the control pups had vocalized six times as much as the milk-deprived pups during this period (37.5% vs. 6.0%). All these differences are statistically significant ($\Delta T_{is}$: $t = 2.656$, $df = 7$, $p < .05$; $\Delta T_{rectal}$: $t = 3.234$, $df = 7$, $p < .05$; % of time vocalizing: $t = 2.792$, $df = 7$, $p < .05$).

*20 Min.* After 20 min of chamber-air cooling, $T_{is}$ had decreased 1.5 °C more in the milk-deprived pups than in the control pups (−4.2 °C vs. −5.7 °C), $T_{rectal}$ had decreased 1.2 °C more (−4.4 °C vs. −5.6 °C), and the control pups had vocalized five times as much as the milk-deprived pups (41.0% vs. 8.5%). All these differences are statistically significant ($\Delta T_{is}$: $t = 4.499$, $df = 7$, $p < .003$; $\Delta T_{rectal}$: $t = 4.266$, $df = 7$, $p < .04$; % of time vocalizing: $t = 4.795$, $df = 7$, $p < .002$). Once again, we found that ultrasound emission and cooling rates varied together. These data also confirm a recent study (Hofer & Shair, 1991) in which milk deprivation was found to reduce ultrasound emission by 80% (although this difference barely missed statistical significance in their study).

As shown in Table 2, at Min 15 and 20, $T_{air}$ differed for the two groups. Specifically, mean $T_{air}$ for the milk-deprived pups was, respectively, 0.4 °C and 0.1 °C lower than for control pups. We cannot explain these temperature differences because identical cooling procedures were used for both groups. It is possible, however, that the relatively crude, 0.25 °C resolution used for the measurement of $T_{air}$ spuriously exaggerated the averaged values.

Analysis of the individual control pups indicated once again that ultrasound emission covaried with cooling rates. This

relationship is illustrated by correlating ultrasound emission during the first 15 min of the test with $\Delta T_{is}$ at Min 15. With these 8 pups, however, there is only a trend for these two variables to be correlated ($r = .617$, $p = .10$). To improve the size of the sample, the data from control pups in Experiments 1 and 2 were combined. Combining these two data sets is justified because $\Delta T_{is}$ at Min 15 does not differ significantly between the two control groups ($t = 0.957$, $p > .35$), nor does ultrasound emission over those 15 min differ significantly ($t = 0.106$, $p > .95$).

With the control groups from the two experiments combined in this way, the correlation between $\Delta T_{is}$ and ultrasound emission is highly significant ($r = .775$, $p < .0005$, $n = 16$); this relationship is shown in Figure 1, which illustrates clearly the variability in both cooling rate and ultrasound emission among control pups. Figure 1 also depicts the data for the hypoxic pups in Experiment 1. It is readily seen that hypoxic pups cooled quickly and emitted few ultrasounds. Finally, Figure 1 depicts the data for the milk-deprived pups in this experiment. Once again, we see that milk-deprived pups cooled quickly and emitted few ultrasounds, as had the hypoxic pups and the nonresponding control pups.

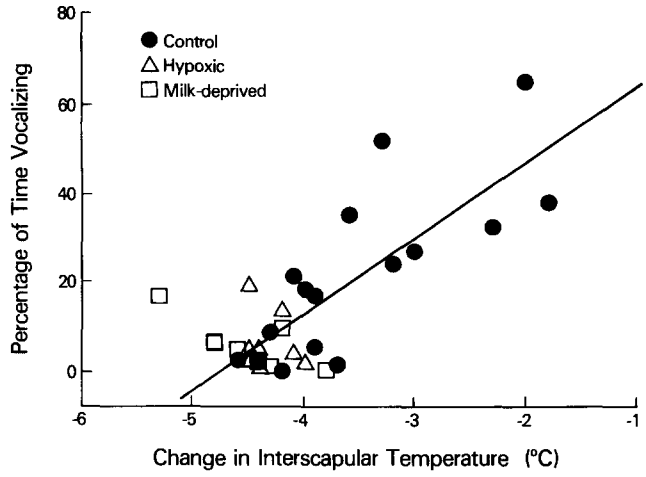


*Figure 1.* Change in interscapular temperature (°C) versus ultrasound emission during 15 min of chamber-air cooling for the 16 control pups in Experiments 1 and 2 (filled circles), the 8 hypoxic pups in Experiment 1 (open triangles), and the 8 milk-deprived pups in Experiment 2 (open squares). (The diagonal line is the best-fit regression line for the 16 control pups [$r = .775$, $p < .0005$].)

## *General Discussion*

The results of the present experiments demonstrate that ultrasound emission covaries with metabolic heat production in rat pups during cold exposure. Hypoxic pups and milk-deprived pups cooled faster and vocalized less than did their respective controls. Furthermore, those control pups that cooled the slowest vocalized the most. These results suggest an explanation for variability in ultrasound emission; specifically, variability in ultrasound emission appears to reflect a more fundamental variability in metabolic heat production. The slower cooling rates of the more vocal animals further support the hypothesis that ultrasound emission reflects the use of laryngeal braking, which in turn complements the activation of metabolic heat production (Blumberg & Alberts, 1990). These results are inconsistent with the conventional wisdom that pup ultrasound is primarily a communicatory behavior that reflects a state of emotional distress; if this were so, then one would expect the pups that cooled faster to have vocalized more, rather than less.

We used cooling rate as a measure of thermogenic effort, although other measures could have been used. For example, the difference between $T_{is}$ and $T_{rectal}$ (i.e., $T_{is} - T_{rectal}$) is a measure of thermogenic effort because, as heat is produced by BAT, the temperature in the interscapular region increases in relation to the body core. We have found, however, that $T_{is} - T_{rectal}$ is best used to illustrate the onset of BAT thermogenesis within a single pup (see Blumberg & Alberts, 1990). When comparing the magnitude of thermogenesis between pups, however, $T_{is} - T_{rectal}$ is a relatively insensitive measure. This insensitivity is illustrated in Table 1, which presents $T_{is}$ and $T_{rectal}$ for control and hypoxic pups. Fifteen minutes after the onset of cooling, $T_{is} - T_{rectal}$ is 0.2 °C for the control pups and −0.1 °C for the hypoxic pups, an absolute difference of only 0.3 °C. In contrast, $T_{is}$ is 1.0 °C higher for the control pups than for the hypoxic pups, and $T_{rectal}$ is 0.7 °C higher. Thus, cooling rate is a more sensitive measure of metabolic heat production than is $T_{is} - T_{rectal}$.

The insensitivity of $T_{is} - T_{rectal}$ as a measure of metabolic heat production between animals arises from the fact that a number of factors that have little bearing on metabolic heat production can affect the absolute value of $T_{is} - T_{rectal}$ (e.g., placement of thermocouples, transient changes in blood flow, transfer of heat from BAT to the body core). In contrast, for same-aged pups with roughly the same body weight and insulation, cooling rate must provide an accurate reflection of thermogenic effort. Nonetheless, we did find a correlation in control pups between $T_{is} - T_{rectal}$ and ultrasound emission ($r = .612$, $p < .02$, $n = 16$) as well as between $T_{is} - T_{rectal}$ and cooling rate ($\Delta T_{is}$ vs. $T_{is} - T_{rectal}$: $r = .840$, $p < .0001$, $n = 16$; $\Delta T_{rectal}$ vs. $T_{is} - T_{rectal}$: $r = .621$, $p < .02$, $n = 16$).

Recently, Hofer and Shair (1991) investigated the possibility that BAT thermogenesis evokes ultrasound emission directly because of the depletion of oxygen stores during cold exposure and increased metabolic heat production. They reported that complete removal of the interscapular BAT pad did not eliminate ultrasound emission during cold exposure. Based on this and other experiments, they concluded that interscapular BAT activation, per se, is neither necessary nor sufficient for ultrasound emission. But, as they pointed out, this conclusion does not bear on the laryngeal braking hypothesis. Specifically, it is possible that (a) laryngeal braking complements BAT activation, but (b) interscapular BAT activation is not necessary to elicit laryngeal braking.

To illustrate this point, consider the response of newborn mammals to hypoxia (Lagercrantz & Slotkin, 1986). When hypoxic, the newborn's adrenal glands secrete catecholamines, which results in decreased blood flow to the periphery, increased blood flow to the brain and heart, increased blood pressure, slowing of the heart rate, and decreased ventilation (Lagercrantz & Slotkin, 1986; Saetta & Mortola, 1987).

All of these responses are complementary and adaptive to the extent that they conserve energy and thus help the newborn rat survive periods of hypoxia. On the other hand, many of these responses are mechanistically independent; for example, slowing of the heart rate does not cause increased blood flow to the brain. Using this analogy as a guide, we suggest that laryngeal braking complements BAT thermogenesis but that the two responses are mechanistically independent.

Some readers may be confused by the finding of Experiment 1 that hypoxia diminishes ultrasound emission. These readers may wonder why a mechanism such as laryngeal braking that increases gas exchange would apparently be diminished at a time when gas exchange is compromised due to falling oxygen concentration. To resolve this apparent paradox, consider that, in some species, hypoxic newborns display a variety of physiological responses that, in combination, seem directed toward energy conservation. For example, investigations in kittens indicate that there is a "tight coupling" between metabolism and ventilation during hypoxia such that both are depressed during hypoxia as a means of conserving energy (Mortola & Rezzonico, 1988). Thus, one would not expect a pup that is trying to conserve energy to use a mechanism such as laryngeal braking that enhances gas exchange and thus oxygen consumption. In other words, laryngeal braking would be expected to accompany respiratory stimulation, not depression.

There are, however, considerable differences in the responses of newborns to hypoxia that depend on the species under consideration and the age of the subject. For example, in the precocial newborn lamb, hypoxia can be accompanied by laryngeal braking and hyperventilation (Davis & Bureau, 1987), which thus supports the notion that laryngeal braking accompanies respiratory stimulation. In contrast, in the fetal lamb, hypoxia elicits respiratory depression, not stimulation (Boddy, Dawes, Fisher, Pinter, & Robinson, 1974). However, newborns typically display a biphasic response to hypoxia, which is characterized by a brief period of hyperventilation followed by prolonged ventilatory depression. This biphasic response has been demonstrated in rats (Saetta & Mortola, 1987) as well as other species (e.g., kittens: Mortola & Rezzonico, 1988; rabbits: Martin-Body & Johnston, 1988).

In addition to species and age, and directly relevant to the results presented in this article, the response of newborns to hypoxic challenge also appears to be modified by ambient temperature (Rudolph, 1984). For example, hypoxic kittens at room temperature appear to have greater respiratory depression than do hypoxic kittens near thermoneutral temperature (Blanco, Hanson, Johnson, & Rigatto, 1984; McCooke & Hanson, 1985). These results in the kitten are consistent with the results of Experiment 1, in which newborn rats decreased ultrasound emission in response to cold and hypoxia. Ventilation in these pups was likely depressed by the combination of cold and hypoxia, which resulted in the decreased incidence of ultrasound emission and, thus, laryngeal braking.

In the present experiments, we tested pups only after they had been given 60 min to habituate to the metabolic chamber and reach a steady state. This is a standard physiological procedure (e.g., Spiers & Adair, 1986) and has been used by us in a previous study (Blumberg & Alberts, 1990). On the other hand, the majority of work on pup ultrasound has used a different procedure in which ultrasound production during the initial minutes after isolation from the nest is of primary interest (e.g., Hofer & Shair, 1978; Kehoe & Blass, 1986). It has been argued that these "isolation-induced" ultrasounds are emitted in response to isolation *per se*, especially when pups are 10 days of age and older (Hofer & Shair, 1986), and that thermal cues become less important for ultrasound production as pups get older. Although we challenge this view in another article (Blumberg et al., 1991), we should note that the present formulation of the laryngeal braking hypothesis is directed primarily at ultrasounds emitted in response to cold exposure alone. It remains an open question whether the hypothesis can meaningfully explain ultrasounds emitted in response to nonthermal stimuli.

Although we chose hypoxia and milk deprivation as manipulations for diminishing the metabolic response of pups to cold, at present we cannot rule out the possibility that these manipulations caused specific or nonspecific sensory or motor impairments that diminished ultrasound production through channels independent of a pup's metabolic effort. However, as we noted in the introduction to this article, the depressive effect of milk deprivation on the metabolic response to cold exposure can be reversed by decerebration at the pontine level (Bignall et al., 1975), which indicates that central inhibition, not depleted energy stores or some correlated form of debilitation, is responsible for metabolic depression after milk deprivation. Similarly, respiratory depression in hypoxic newborn rabbits and fetal lambs can also be reversed by a pontine cut (Boddy et al., 1974; Martin-Body & Johnston, 1988), which indicates again that central inhibition, not depleted oxygen levels, is responsible for this depression. That brain lesions can disinhibit these metabolic responses of milk-deprived or hypoxic animals suggests that the diminution of ultrasound production in the present experiments is not due to general debilitation of the pups. In sum, we consider both milk-deprived and hypoxic pups to be in an actively regulated state of respiratory quiescence, rather than suffering debilitation.

In conclusion, it is apparent that understanding the relations between metabolism, ventilation, laryngeal braking, and ultrasound emission in infant rats requires that greater emphasis be placed on understanding respiratory system mechanics during these manipulations. Although the present experiments provide us with a correlation between thermogenic effort and ultrasound emission, they do not address directly the underlying mechanisms of ultrasound emission. The prevailing evidence does indicate, however, that ultrasound emission reflects the occurrence of laryngeal braking during periods of respiratory stimulation and increased metabolism. Moreover, these increases in ventilation and metabolism and the accompanying elicitation of laryngeal braking appear to be under central, not peripheral, control.

## References

Allin, J. T., & Banks, E. M. (1972). Functional aspects of ultrasound production by infant albino rats (*Rattus norvegicus*). *Animal Behaviour, 20,* 175–185.

Bignall, K. E., Heggeness, F. W., & Palmer, J. E. (1974). Effects of acute starvation on cold-induced thermogenesis in the preweanling rat. *American Journal of Physiology, 227,* 1088–1093.

Bignall, K. E., Heggeness, F. W., & Palmer, J. E. (1975). Effect of neonatal decerebration on thermogenesis during starvation and cold exposure in the rat. *Experimental Neurology, 49,* 174–188.

Blanco, C. E., Hanson, M. A., Johnson, P., & Rigatto, H. (1984). Breathing patterns of kittens during hypoxia. *Journal of Applied Physiology, 56,* 12–17.

Blumberg, M. S., & Alberts, J. R. (1990). Ultrasonic vocalization by rat pups in the cold: An acoustic by-product of laryngeal braking? *Behavioral Neuroscience, 104,* 808–817.

Blumberg, M. S., & Alberts, J. R. (1991). On the significance of similarities between ultrasonic vocalizations of infant and adult rats. *Neuroscience & Biobehavioral Reviews, 15,* 383–390.

Blumberg, M. S., Efimova, I. V., & Alberts, J. R. (1991). *Ultrasonic vocalizations by rat pups: The primary importance of ambient temperature and the thermal significance of contact comfort.* Manuscript submitted for publication.

Boddy, K., Dawes, G. S., Fisher, R., Pinter, S., & Robinson, J. S. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia in sheep. *Journal of Physiology (London), 243,* 599–618.

Brooks, R. J., & Banks, E. M. (1973). Behavioral biology of the collared lemming [*Dicrostonyx groenlandicus* (Traill)]: An analysis of acoustic communication. *Animal Behaviour Monographs, 6,* 1–83.

Davis, G. M., & Bureau, M. A. (1987). Pulmonary and chest wall mechanics in the control of respiration in the newborn. *Clinics in Perinatology, 14,* 551–579.

England, S. J., Kent, G., & Stogryn, H. A. F. (1985). Laryngeal muscle and diaphragmatic activities in conscious dog pups. *Respiration Physiology, 60,* 95–108.

Hard, E., Engel, J., & Lindh, A. (1988). Effect of clonidine on ultrasonic vocalization in preweaning rats. *Journal of Neural Transmission, 73,* 217–237.

Harrison, V. C., de V. Heese, H., & Klein, M. (1968). The significance of grunting in hyaline membrane disease. *Pediatrics, 41,* 549–559.

Heim, T., & Hull, D. (1966). The blood flow and oxygen consumption of brown adipose tissue in the new-born rabbit. *Journal of Physiology, 186,* 42–55.

Hofer, M. S., & Shair, H. (1978). Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. *Developmental Psychobiology, 11,* 495–504.

Hofer, M. A., & Shair, H. (1991). Independence of ultrasonic vocalization and thermogenic responses in infant rats. *Behavioral Neuroscience, 105,* 41–48.

Hull, D. (1973). Thermoregulation in young mammals. In G. C. Whittow (Ed.), *Comparative physiology of thermoregulation* (Vol. 3, pp. 167–200). San Diego, CA: Academic Press.

Johnson, P. (1985). The development of breathing. In C. T. Jones & P. W. Nathanielsz (Eds.), *The physiological development of the fetus and newborn* (pp. 201–210). San Diego, CA: Academic Press.

Kehoe, P., & Blass, E. M. (1986). Opioid-mediation of separation distress in 10-day-old rats: Reversal of stress with maternal stimuli. *Developmental Psychobiology, 19,* 385–398.

Lagercrantz, H., & Slotkin, T. A. (1986). The "stress" of being born. *Scientific American, 254,* 100–107.

Lindroth, M., Johnson, B., Ahlstrom, H., & Svenningsen, N. W. (1981). Pulmonary mechanics in early infancy: Subclinical grunting in low-birth-weight infants. *Pediatric Research, 15,* 979–984.

Martin-Body, R. L., & Johnston, B. M. (1988). Central origin of the hypoxic depression of breathing in the newborn. *Respiration Physiology, 71,* 25–32.

McCooke, H. B., & Hanson, M. A. (1985). Respiration of conscious kittens in acute hypoxia and effect of almitrine bimesylate. *Journal of Applied Physiology, 59,* 18–23.

Mortola, J. P. (1987). Dynamics of breathing in newborn mammals. *Physiological Reviews, 67,* 187–243.

Mortola, J. P., & Rezzonico, R. (1988). Metabolic and ventilatory rates in newborn kittens during acute hypoxia. *Respiration Physiology, 73,* 55–68.

Okon, E. E. (1970). The effect of environmental temperature on the production of ultrasounds by isolated non-handled albino mouse pups. *Journal of Zoology (London), 162,* 71–83.

Okon, E. E. (1971). The temperature relations of vocalization in infant golden hamsters and Wistar rats. *Journal of Zoology (London), 164,* 227–237.

Roberts, L. H. (1972). Correlation of respiration and ultrasound production in rodents and bats. *Journal of Zoology (London), 168,* 439–449.

Roberts, L. H. (1975). Evidence for the laryngeal source of ultrasonic and audible cries of rodents. *Journal of Zoology (London), 175,* 243–257.

Rudolph, A. M. (1984). Oxygenation in the fetus and neonate—A perspective. *Seminars in Perinatology, 8,* 158–167.

Saetta, M., & Mortola, J. P. (1987). Interaction of hypoxic and hypercapnic stimuli on breathing pattern in the newborn rat. *Journal of Applied Physiology, 62,* 506–512.

Seidler, F. J., & Slotkin, T. A. (1985). Adrenomedullary function in the neonatal rat: Responses to acute hypoxia. *Journal of Physiology, 358,* 1–16.

Spiers, D. E., & Adair, E. R. (1986). Ontogeny of homeothermy in the immature rat: Metabolic and thermal responses. *Journal of Applied Physiology, 60,* 1190–1197.

Received February 20, 1991
Revision received June 26, 1991
Accepted June 26, 1991 ■