Ultrasonic Vocalizations by Rat Pups in the Cold: An Acoustic By-Product of Laryngeal Braking?

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Isolated rat pups respond to cold exposure physiologically by increasing metabolic heat production and behaviorally by emitting ultrasound. The relationship between these 2 responses was investigated by monitoring oxygen consumption, heat production by brown adipose tissue, respiratory rate, and ultrasound production during cold exposure in pups 10–12 days of age. All 3 physiological measures increased contemporaneously with the initiation of ultrasound. Pups also exhibited a respiratory pattern characterized by the prolongation of expiratory duration in relation to inspiratory duration. Ultrasound was often detected during these prolonged expirations, suggesting that pups were using laryngeal braking. Laryngeal braking is thought to enhance oxygen uptake in the lungs. Thus, ultrasound may be an acoustic by-product of a respiratory maneuver that increases oxygen delivery to metabolically active tissues during cold exposure.

An infant rat depends on its mother and littermates to maintain its body temperature at 37–38 °C (Alberts, 1978; Hull, 1973). This dependence results from the neonate's large surface-to-volume ratio and lack of adequate insulation, characteristics that promote heat loss. Thus, rat pups huddle together when their mother is outside the nest, a behavior that decreases heat loss by decreasing the exposed body surface area of the pups (Alberts, 1978).

When isolated from both its mother and littermates and exposed to cold temperatures, the rat pup exhibits two responses, one physiological and the other behavioral, to combat heat loss. The physiological response of the isolated pup is to increase metabolic heat production (Spiers & Adair, 1986; Taylor, 1960). The pup's capacity for significant metabolic heat production exists shortly after birth and is provided largely, if not exclusively, by nonshivering thermogenesis (NST; Hull, 1973; Taylor, 1960). Nearly all of the heat produced through NST is generated by a specialized, heat-producing organ called brown adipose tissue (BAT; Hull, 1973; Smith, 1964; Smith & Roberts, 1964; see also Hull & Segall, 1965). This tissue is concentrated at specific anatomical sites including the interscapular and superior cervical regions, in the thorax near the aorta and sympathetic chain, and in close proximity to the kidneys (Smith & Roberts, 1964). Thus, BAT is strategically positioned for localized heating of vital nervous and systemic structures during cold exposure.

The pup's behavioral response to isolation and cold exposure is the emission of ultrasonic vocalizations (Allin & Banks, 1971; Noirot, 1972; the term "ultrasonic" refers to

sound with frequencies beyond the hearing range of humans, not rats, as described later). These vocalizations span frequencies of 30–50 kHz, well within the hearing range of adult rats (Gourevitch & Hack, 1966). Indeed, auditory sensitivities of adults in a number of rodent species, including the Norway rat, have peaks of sensitivity in the cochlea and inferior colliculus that correspond to the vocal frequencies emitted by conspecific young (Brown, 1973a, 1973b). Furthermore, there is evidence that mothers respond to the vocalization by approaching the pup and retrieving it to the warm nest (Allin & Banks, 1972; Sewell, 1970; Smotherman, Bell, Starzec, & Elias, 1974). In this way, these ultrasonic emissions are thought to complement the neonate's undeveloped thermoregulatory abilities and are sometimes referred to as "distress calls."

These two responses of the isolated rat pup to cold exposure (viz., metabolic heat production via NST and ultrasound production) have each been studied in some detail. Nevertheless, we are aware of no information or postulations concerning how these responses interact with each other. Specifically, are NST and ultrasound production two independent responses to cold exposure or is there a causal connection between them? In this article, we attempt to answer this question.

Although there is no direct evidence linking ultrasound production to NST, there is some indirect support for a developmental connection between ultrasound production and thermoregulation (Okon, 1970, 1971). Specifically, during the 1st week postpartum, the rat pup's vocal response to cold exposure is weak. It is also during the 1st week postpartum that rat pups are most able to survive hypothermia and hypoxia (Adolph, 1948); that is, when cold or when oxygen deprived, newborn rats survive longer than do older rats (Barker, 1957; Seidler & Slotkin, 1985); this is due, among other reasons, to the greater affinity of neonatal hemoglobin for oxygen (Barker, 1957). Then, during the 2nd week postpartum, ultrasound production in response to cold exposure increases. At this time, the ability to maintain body tem-

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perature in a cold environment improves, and resistance to hypoxia diminishes. Finally, during the 3rd week postpartum, ultrasound production disappears as the pup's ability to maintain body temperature in the cold is improved further.

The developmental parallel between ultrasound production and thermoregulatory ability seems to fit nicely with the presumed function of ultrasound production as a means of eliciting maternal retrieval. Okon (1970) suggests the following scenario: in the 1st week postpartum when the pup is maximally resistant to hypothermia and hypoxia, calling in response to cold exposure is weak, perhaps because it is less critical that the pup call its mother to elicit retrieval. As the pup's resistance to hypoxia diminishes during the 2nd week postpartum, calling becomes more important for attracting the mother and eliciting retrieval to the nest. Finally, during the 3rd week postpartum, calling becomes unnecessary and ceases altogether as thermoregulatory competence is achieved.

In its primary emphasis on the function rather than the mechanism of ultrasound production and in its assumption that ultrasound production is a perfectly adapted communicatory response to cold exposure. Okon's scenario illustrates the bias of many contemporary biologists for adaptive stories (see Gould & Lewontin, 1979, for a detailed discussion of the "adaptationist programme"). Regarding the justdescribed scenario, a purely adaptationist perspective leads to some curious suggestions. For example, although it is certainly true that newborn pups (1-7 days old) are relatively resistant to hypoxia, this in no way suggests that they are less susceptible than older pups to the dangers of being outside the nest and thus could not benefit from calling their mother and being returned to the nest. Quite the contrary; pup mortality is highest in the first few days postpartum (Calhoun, 1962), suggesting that newborns can least afford to be away from maternal care and protection.

Clearly, we cannot improve our understanding of the function and development of ultrasound production without a better understanding of the relationship between ultrasound production and the other physiological responses of the rat pup to cold. To accomplish this, we have monitored ultrasound production by rat pups during cold exposure and have related it to oxygen consumption, NST, and respiratory rate. We have treated the pup's various responses to cold exposure as parts of an integrated whole and have derived from our interpretation of the mechanisms underlying the ultrasonic vocalization some useful insights into the evolution and function of ultrasound in the rat pup.

Experiment 1A: Interrelations of Oxygen Consumption and Ultrasound Production

Although it is well known that an isolated rat pup responds to cold exposure both by increasing metabolic rate (Spiers & Adair, 1986; Taylor, 1960) and emitting ultrasonic pulses (Okon, 1971), the relationship between these two responses is unknown. To determine this relationship, we monitored simultaneously oxygen consumption and ultrasound production of infant rats during cold exposure.

Method

Subjects. Five male Sprague-Dawley rat pups from three different litters were used. All rat pups that were used in these experiments were born in the Indiana University colony and were descendents of a breeding population that was derived from stock originally purchased from Laboratory Supply, Inc., Indianapolis, Indiana. On the day of testing, all pups were 10–12 days of age and weighed between 22 and 31 g. The pups were raised in litters that were culled to 8 pups within 3 days after birth. Litters and their mothers were raised in standard laboratory cages ($48 \times 20 \times 26$ cm) in which food and water were available ad libitum. All animals were maintained on a 16:8-hr light-dark schedule with lights on at 7:00 a.m.

Oxygen consumption measurements. Because configuration of systems for metabolic measures can affect the interpretation of results, particularly in behavioral applications, it is germane to describe our apparatus. Clean, dry, compressed air was passed through a twostage regulator and split into two lines, one of which entered a digital flowmeter (Digi Flow 200) for controlled delivery (310-350 ml/min flow rates were used) into the animal chamber (to be described later) where it could be respired. The effluent from the animal chamber was dried by a column of desiccant and then drawn (100-140 ml/ min) through one of the two channels of an electrochemical oxygen sensor (Ametek, Pittsburgh, PA). The second line of air traveled independently to the second channel of the oxygen sensor. The two channels of air were drawn into independent electrochemical cells where the oxygen was heated to ionization (750 °C). Each cell's sensor acted as an electrolytic conductor, the resistance of which changed with the concentration of ionized oxygen. Based on this resistance, the system's control unit displayed oxygen concentration digitally.

Air flow into the metabolic chamber was greater than that into the oxygen sensor, by virtue of flow regulation and in-line overflow valves. Because the animal chamber was under positive pressure, leakage could not introduce air into the chamber and thus dilute the sample. Furthermore, because the flow rate of air through the metabolic chamber exceeded that entering the oxygen sensor, this system was not perturbed by pressure fluctuations associated with movement, a problem associated with other methods. The low volume of this system in relation to air flow and animal size, in combination with the sensitivity of the oxygen sensor, provided a system response time estimated to be about 1-2 min. There is, however, inevitable variability in response latencies because of factors such as subject size, orientation in the chamber, internal turbulence, and momentto-moment fluctuations in flow rates.

The cylindrical metabolic chamber (volume = $1,460 \text{ cm}^3$) was immersed in a water bath. Water temperature determined air temperature within the chamber and was regulated by a heater-circulator. Because of the use of a water bath for temperature control and the glass and Plexiglas construction of the respiratory chamber, the system provided excellent visibility of the pup, which is another unusual and desirable feature of this system.

Individual pups were placed in a small cage made of polyethylene mesh (8.5 \times 3 \times 2.2 cm) that served to confine the pup without restraint and permitted air flow all around the animal. This arrangement also prevented contact between the animal and the inner surface of the respiratory chamber and thus prevented conductive heat exchange between the pup and the chamber wall. The cage containing the pup was slipped into the respiratory chamber, and the chamber was sealed shut. Oxygen concentration in each airstream was measured simultaneously. The difference in percentage oxygen between the animal's effluent airstream and the nonrespired airstream reflects oxygen consumption. Specifically, by knowing the air flow rates and the weight of the pup, oxygen consumption in millimeters O₂ per gram per minute can be calculated.

Temperature measurements. Physiological temperatures were

Table 1Percentage of Time Vocalizing at Three Different Phasesand Peak Percentage Increase in Oxygen Consumption forIsolated Rat Pups in Experiment 1A

	Percent	Peak percent- age increase			
Subject	Phase 1	Phase 2	Phase 3	consumption	
1	0	0	79	98	
2	0.5	0.5	79	43	
3	0	1	53	95	
4	0.5	0	2.5	103	
5	0	0	2	55	

Note. Phase 1 = Baseline (thermoneutrality) when maintained at thermoneutral air temperature (i.e., 35-36 °C); Phase 2 = Before onset of increased oxygen consumption but during the reduction of air temperature; Phase 3 = After the onset of increased oxygen consumption. All 5 rat pups were 10-12 days old. The rightmost column reports the peak percentage increase in oxygen consumption over baseline values for each subject. The percentage of time vocalizing was calculated over 10-min periods. Onset of oxygen consumption was estimated as occurring 2 min before the recorded increase in oxygen consumption to compensate for the response delay of the system.

measured using 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 in a temperature-controlled water bath. Thereafter, calibrations were checked periodically to ensure continued accuracy. Tail temperature (T_{tail}) was measured at the base of the tail on the dorsal surface. The thermocouple was fastened to the tail using collodion and porous tape. In addition, chamber-air temperature (T_{air}) was measured using an ambient temperature thermistor (Yellow Springs Probe No. 405). T_{air} was read on a tele-thermometer (Yellow Springs) to within 0.5 °C.

Ultrasonic vocalizations. The rat pup's ultrasonic vocalizations were detected using an ultrasonic microphone placed inside the metabolic chamber. The microphone was connected to a "bat detector" (QMC, Ltd., London, UK; Model S 100) 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. The rate of ultrasonic vocalization was measured by listening for the vocalization and triggering an event marker on a chart recorder every time a pulse was detected. To quantify the vocalization data, each minute was divided into twenty 3-s bins. The number of bins containing 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. A thermocouple for measuring T_{taib} as explained previously, was attached. The pup was then placed in the polyethylene cage, and both were placed inside the metabolic chamber. During habituation, air temperature within the chamber was maintained at 35 °C, which is within the thermoneutral zone of 10–12-day-old rat pups (Spiers & Adair, 1986).

The experiment began only after the pup's oxygen consumption had reached a steady state; this usually required 30-60 min of habituation. When a steady state was reached, a timer was started and oxygen consumption, T_{air} and T_{iail} were recorded, by hand, at the beginning of each minute. Monitoring of ultrasound production also began at this time.

The test began by collecting data for at least 10 min with T_{air} maintained at 35 °C. After these baseline data were taken, T_{air} was

decreased to 26–30 °C. Changes in oxygen consumption, T_{iaib} and vocalization rate were monitored continuously for at least 1 hr. After this, T_{air} was decreased further to 19–21 °C. Continuous monitoring continued for at least 10 min more.

Results and Discussion

For the first 10 min of the test when T_{air} was 35–36 °C, 3 pups were completely silent, and the 2 other pups each vocalized 0.5% of the time (see Table 1). This is consistent with other reports (e.g., Allin & Banks, 1971) showing that if a pup is maintained at a thermoneutral temperature, isolation is not an effective stimulus for ultrasound production. In addition, during these first 10 min, oxygen consumption was stable and ranged from 0.0401 to 0.0528 ml O₂/g·min⁻¹ across all 5 pups.

After T_{air} was decreased to 26–29 °C, all 5 pups began increasing ultrasound production 1–3 min before recorded oxygen consumption increased, a time lag similar to the response delay of our system (see earlier discussion of air flow and volume). Table 1 shows that 3 pups increased ultrasound production dramatically, vocalizing 53%, 79%, and 79% of the time (over 10 min). The remaining 2 pups exhibited lower levels of ultrasound production; these 2 pups vocalized only 2% and 2.5% of the time (over 10 min). Finally, for all 5 pups, oxygen consumption increased steadily until it had increased 43–103% over baseline values (range = 0.0711– 0.0952 ml O₂/g·min⁻¹).

We found it most useful to examine composite profiles of temperature and ultrasound data for individual pups, rather than rely on group averages of each parameter. Figure 1 presents the individual data for one of the pups that exhibited high levels of ultrasound. It can be seen that there was a virtual absence of ultrasonic pulses at the beginning of the test, when T_{air} was 35 °C. During this period, both T_{tail} and oxygen consumption were stable. When T_{air} was decreased to 26 °C, T_{tail} began to drop. Vocalizations began within about 10 min. Within 2 min of the onset of the vocalization there was a sharp and prolonged increase in oxygen consumption; again, this 2-min delay was due largely, if not entirely, to a delay in the system's response. Figure 1 shows that at Min 45 there was a spontaneous fall-off of both oxygen consumption and vocalizing, followed closely by two peaks and troughs in oxygen consumption that were paralleled by peaks and troughs in ultrasound production.

The mirroring of ultrasound production and oxygen consumption in Figure 1 might lead us to conclude that ultrasound production is causing the increase in oxygen consumption. There are, however, reasons for suspecting that vocal production is not the major cause of the increase in oxygen consumption. First, Figure 1 shows that the pup's oxygen consumption is retained at a high level even after vocalization has nearly stopped (e.g., Min 65–95). Second, the 2 pups that only rarely emitted ultrasound after cold exposure nonetheless achieved increases in oxygen consumption that were comparable to those of the other 3 pups (see Table 1 and Figure 2). It follows, therefore, that ultrasound production is not a prerequisite for increased oxygen consumption, nor can it be responsible for the majority of



Figure 1. Oxygen consumption, percentage of time vocalizing, tail temperature (T_{iail}) , and chamberair temperature (T_{air}) for a 12-day-old rat pup.

the increase in oxygen consumption. On the other hand, we cannot rule out the possibility that ultrasound production contributes to the increase in oxygen consumption during cold exposure.

The diminution of ultrasound production with time in Figure 1 is interesting because it suggests that the pup may be fatigued. We do not accept exhaustion as an explanation: Figure 1 shows that when T_{air} was reduced a second time, to 21 °C, high levels of ultrasound production were resumed.

It seems reasonable to conclude that ultrasound production and heat production in the cold are related events. The nature of their relationship, however, is not obvious. In Experiment 1B, we investigated further the relationship between ultrasound production and the pup's physiological response to cold exposure.

Experiment 1B: Interrelations of Heat Production by Brown Adipose Tissue and Ultrasound Production

When exposed to a cold environment, a newborn mammal can produce heat through shivering, NST, or both (Alexander, 1975). Some newborn mammals, such as the human infant and rabbit, rarely shiver, and thus heat production is due largely, if not exclusively, to NST (Hull, 1973). Shivering is also rarely seen in newborn rats (Taylor, 1960). As stated earlier, NST is produced by BAT, a heat-producing organ located, among other places, in the interscapular region of the rat (Smith, 1964; Smith & Roberts, 1964). Its subcutaneous position in the interscapular region allows for convenient and fairly direct measurement of temperature changes



Figure 2. Oxygen consumption, percentage of time vocalizing, tail temperature (T_{tail}) , and chamberair temperature (T_{air}) for an 11-day-old rat pup.



Figure 3. Percentage of time vocalizing, rectal temperature (T_{rectal}) , interscapular temperature (T_{IS}) , back temperature (T_{back}) , and chamber-air temperature (T_{air}) , for a 10-day-old rat pup. (Note that the initiation of ultrasound production coincides with the point at which T_{IS} begins to decrease more slowly than T_{back} . Although both temperature measurements were taken just under the skin surface, T_{IS} was measured in close proximity to the location of brown adipose tissue. Thus, the deviation of T_{IS} from T_{back} indicates the initiation of heat production by brown adipose tissue.)

that result from heat production by BAT (Dawkins & Hull, 1964). Thus, in the present experiment, we monitored interscapular temperature and other physiological temperatures, as well as oxygen consumption and ultrasound production, during cold exposure. Because changes in interscapular temperature can be detected more rapidly than changes in oxygen consumption, we should be able to determine more accurately the temporal relationship between ultrasound production and the initiation of the pup's physiological response to cold exposure.

Method

Subjects. Twelve male pups from nine litters were used; they were from the same stock as the rats described in Experiment 1A. All pups were reared as in Experiment 1A. On the day of testing, all pups were 10-12 days of age and weighed between 19 and 29 g.

Oxygen consumption measurements. Oxygen consumption was measured as in Experiment 1A.

Temperature measurements. As many as three physiological temperature measurements were recorded from the same animal. Rectal temperature (T_{rectal}) was measured by inserting the tip of a thermocouple 1-3 cm beyond the anal sphincter. To reduce movement of the thermocouple within the rectum, the thermocouple lead was glued to the base of the tail on its ventral side. Tail temperature (T_{tail}) was measured as in Experiment 1A. Interscapular temperature (T_{Is}) was measured at the midline above the scapulae. A thermocouple was inserted subcutaneously through a small hole in the over-

lying skin, created by a hypodermic needle; hair was clipped, if necessary, to clear the region. The tip of the thermocouple was inserted approximately 3 mm beneath the skin and was secured in place using glue and collodion. Subcutaneous temperature was also taken in the lumbosacral region (T_{back}) using the same attachment procedure as for T_{IS} . T_{back} was measured on the midline approximately 1 cm rostral to the base of the tail. Chamber-air temperature (T_{air}) was measured as in Experiment 1A.

Ultrasonic vocalizations. The rate of ultrasound production was measured as in Experiment 1A.

Procedure. The tests were conducted as in Experiment 1A except that after the collection of baseline data for at least 10 min, T_{air} was decreased directly from 35 °C to 20–23 °C and was not changed for the remainder of the test.

Results and Discussion

Once again, we found it useful to examine the composite data for individual representative pups (group data will follow). Heat production by BAT can be visualized by plotting interscapular temperature (T_{ls}) in conjunction with another surface temperature (e.g., T_{back}) or with core temperature (e.g., T_{rectal}). This is illustrated in Figure 3, which presents the data for a 10-day-old pup. When T_{air} was maintained at 35 °C, all temperatures (as well as oxygen consumption) were stable. When T_{air} was decreased to 22 °C, T_{IS} , T_{rectal} , and T_{back} began to decrease as well. Initially, because of the greater thermal inertia of the body core, T_{rectal} decreased more slowly than either of the two surface temperatures (i.e., T_{IS} or T_{back}), and these last two temperatures fell at the same rate. At Min 20, however, BAT began producing heat, as indicated by the abrupt change in the cooling rate of T_{IS} . This occurred approximately 1 min before the initiation of ultrasound production.

BAT activity can be better visualized by plotting, for each minute, $T_{IS} - T_{rectal}$. This arithmetic correction improves the visualization of BAT activity by partially removing those thermal influences on T_{IS} that are not due to BAT-related heat production. Figure 4 shows, in a 12-day-old pup, the relationship between ultrasound production and BAT-related heat production; oxygen consumption is also shown. BAT-related heat production and ultrasound production began within the same minute, whereas oxygen consumption, as in Figure 1, showed a delay of approximately 2 min. Because we know that heat production by BAT is oxygen-dependent (see Heim & Hull, 1966), we can assume that oxygen consumption increased at the time when BAT-related heat production began.

Table 2 presents the available group data for all of the pups in the experiment. It shows mean $T_{IS} - T_{rectal}$ and mean T_{back} $- T_{rectal}$ for the 5 min before (Min -5 to -1) and the 7 min after (Min 1 to 7) the initiation of ultrasound production (Min 0). From Min -5 to -1, mean $T_{IS} - T_{rectal}$ decreased significantly from -0.5 °C to -0.8 °C (paired t = 4.039, p < .002, df = 11), and mean $T_{back} - T_{rectal}$ decreased significantly from -1.1 °C to -1.6 °C (paired t = 5.303, p < .001, df = 8); in other words, T_{IS} and T_{back} cooled faster than T_{rectal} . This is typical of any surface temperature in relation to a core temperature during exposure to a cold environment. When ultrasound production began (Min 0), however, mean $T_{IS} - T_{rectal}$ reversed its previous trend. By Min 2, mean $T_{IS} - T_{rectal}$ had increased significantly (paired t = 4.795, p < .001, df = 11), and by Min 7, mean $T_{IS} - T_{back}$ was equal to zero. In contrast, $T_{back} - T_{rectal}$ continued to decrease (the increase at Min 6 is due largely to the influence of a single outlyer).

Because the temperature values were recorded at the beginning of each experimental minute, whereas ultrasound production was recorded throughout the minute, Table 2 suggests that the initiation of BAT-related heat production precedes the initiation of ultrasound production. However, it should be stressed that the temporal resolution of our measurements in these experiments (i.e., 1 min), as well as the difficulty of pinpointing when BAT-related heat production begins, does not allow us to state with certainty the exact temporal relationship between the two events. For the moment, we can conclude only that the two events are contemporaneous.

In Experiment 1A, cold-induced ultrasound production was followed by an increase in oxygen consumption. By monitoring changes in BAT-related heat production in the present experiment, we were able to clarify the relationship between ultrasound production and the rat pup's physiological response to cold. Specifically, ultrasound production began contemporaneously with BAT-related heat production, and oxygen consumption increased at this time as well. Because heat production by BAT requires a generous supply of oxygen (Dawkins & Hull, 1964), we know that heat production by BAT can account for the increase in oxygen consumption after cold exposure. This raises the question of how the oxygen requirements of the pup are satisfied during cold exposure. We addressed this question in the next experiment.

Table 2

Mean (\pm SE) T_{IS} – T_{rectal}, Mean (\pm SE) T_{back} – T_{rectal}, and Percentage (\pm SE) of Time Vocalizing for the 5 Min Before and the 7 Min After the Initiation of Ultrasound Production (Minute 0) in the Cold for Experiment 1B

	$T_{IS} - T_{rectal}$		$T_{back} - T_{rectal}$		Percentage of time vocalizing	
Minute	M	SE	M	SE	%	SE
-5	-0.5	0.1	-1.1	0.1	0	0
-4	-0.6	0.1	-1.3	0.1	0	0
-3	-0.7	0.1	-1.4	0.1	0	0
-2	-0.8	0.1	-1.5	0.1	0	0
-1	-0.8	0.1	-1.6	0.1	0	0
0	-0.7	0.1	-1.6	0.2	8	2
1	-0.7	0.1	-1.7	0.1	18	6
2	-0.5	0.1	-1.7	0.2	22	6
3	-0.4	0.1	-1.7	0.2	25	8
4	-0.3	0.1	-1.8	0.2	24	8
5	-0.2	0.1	-1.8	0.2	15	6
6	-0.1	0.2	-1.7	0.3	18	6
7	0.0	0.2	-1.7	0.3	19	6

Note. T_{IS} = interscapular temperature; T_{rectal} = rectal temperature; T_{back} = subcutaneous temperature taken in the lumbosacral region. For $T_{IS} - T_{rectal}$, n = 12; for $T_{back} - T_{rectal}$, n = 9; for percentage of time vocalizing, n = 12.



Figure 4. Oxygen consumption, $T_{1S} - T_{rectab}$ percentage of time vocalizing, and chamber-air temperature (T_{au}) for a 12-day-old rat pup.

Experiment 2: Respiratory Adjustments of the Infant Rat During Cold Exposure in Relation to Ultrasound Production

When BAT begins producing heat, its rate of oxygen consumption increases. To supply the necessary oxygen to BAT, blood flow to the tissue is increased (Heim & Hull, 1966). In addition, oxygen delivery to BAT can be enhanced by increasing oxygen uptake in the lungs; this can be accomplished through an increase in respiratory rate, tidal volume, or both (Mortola, 1983). Oxygen uptake in the lungs can also be improved by prolonging expiratory duration in relation to inspiratory duration, a maneuver that increases end-expiratory lung volume (England, Kent, & Stogryn, 1985; Gautier, Remmers, & Bartlett, 1973). With regard to this last possibility, the respiratory movements underlying the ultrasonic vocalization are informative.

In a series of elegant studies, Roberts (1972, 1975a, 1975b, 1975c) demonstrated that ultrasound production by rat pups is produced by the expiration of air through the two plates of a constricted larynx. Roberts also showed that after inspiration, the larynx is constricted, air flow is reduced, and the ensuing expiration is prolonged because of the resistance offered by the constricted larynx; this maneuver is also likely to cause an increase in intrathoracic pressure (Roberts, 1972), similar to the Valsalva maneuver (Sharpey-Schafer, 1953).

Roberts (1972) also reported respiratory frequencies before, during, and after ultrasound production, although he did not control for ambient temperature. Thus, in Experiment 2, we monitored respiratory frequency, the pattern of respiration, and ultrasound production before and after cold exposure to determine the relationship between ultrasound production and any respiratory adjustments to increased oxygen consumption that result from heat production by BAT.



Figure 5. Respiratory frequency, oxygen consumption, and chamber-air temperature (T_{air}) for a 12-day-old rat pup. (The arrow indicates the minute in which the first cold-induced ultrasonic vocalization was detected. Only periods of regular respiration were used for calculating respiratory frequency.)

Method

Subjects. Four male rat pups from four litters and from the same source as those in Experiment 1A were used. The pups were raised as in Experiment 1A. On the day of testing, all pups were 11-12 days of age and weighed between 26 and 30 g.

Respiratory measurements. Respiratory movements were measured and monitored using strain gauge plethysmography (Alberts & May, 1980). Mercury-filled, circular strain gauges (0.5 in. [1.27 cm] diameter) were manufactured for us by Parks Electronics (Beaverton, OR). The gauges were connected to a plethysmograph (Parks Electronics), and the output of the plethysmograph was filtered, amplified, and recorded using a Grass Model 7 polygraph (Grass Instruments Co., Quincy, MA).

Ultrasonic vocalizations. Ultrasonic pulses were recorded simultaneously with the respiratory movements by connecting the output of the bat detector to a second channel on the polygraph.

Procedure. After the pup was fitted with the gauge about the midsection and a thermocouple was attached to its tail, the pup was placed in the metabolic chamber. The pup was initially habituated to a chamber-air temperature of 35 °C. After habituation, each test consisted of recording at least 10 min of respiration at the thermoneutral temperature. Chamber-air temperature was then reduced to 18-22 °C, and monitoring of respiration continued each minute for at least 10 min. Oxygen consumption was also monitored in 3 pups.

Results

When at rest in a thermoneutral environment, rat pups displayed regular, sinusoidal respiratory patterns (see Alberts & May, 1980). After cold exposure, the frequency of these regular respirations increased. For the 5 min before ultrasound production began, mean respiratory frequencies for the 4 pups were 1.8, 1.9, 2.1, and 2.4 Hz; ultrasound was not detected at this time. In contrast, for the 5 min after ultrasound production began, mean respiratory frequencies were 2.2, 2.5, 5.0, and 3.3 Hz, respectively. Figure 5 depicts, for an individual pup, the sudden increase in respiratory frequency at the time when ultrasound is first emitted in the cold. These data provide further evidence that the rat pup initiates ultrasound production at the same time that it activates its physiological response to the cold.

Figure 6a is a representative sample of respiratory movements that were recorded when T_{air} was 35 °C and the pup was calm; all respiratory movements at this time were regular. Figure 6b, on the other hand, illustrates a series of respiratory movements that were recorded when T_{air} was 18.5 °C. Although many of the respiratory movements at this lower temperature were regular and, as we have seen, of higher frequency, there were also many instances of respiratory movements in which expiratory duration, in relation to inspiratory duration, was prolonged. The three tracings in Figure 6b represent some of the different forms that expiratory prolongation can take, and the black bars above the tracings indicate times when ultrasonic pulses were detected. The position of these bars indicates that ultrasound is produced during prolonged expirations.

Discussion

The results of the present experiment indicate that, during cold exposure, the frequency of regular respirations increases



Figure 6. Respiratory movements as measured by strain-gauge plethysmography in a 12-day-old rat pup (inspirations upward). (a: Regular, sinusoidal respiratory movements typical of a calm pup when $T_{air} = 35$ °C. b: Respiratory movements of the same pup after chamber-air temperature has dropped to 18.5 °C. Regular respiratory movements are now of higher frequency. In addition, respiratory movements indicative of laryngeal braking are also seen, as evidenced by the prolongation of expiratory duration in relation to inspiratory duration. Ultrasonic pulses were often detected during these prolonged expiratory movements, as indicated by the black bars.)

over baseline values. This increase in respiratory frequency is at least one of the ways that the pup can increase oxygen uptake in the lungs and, therefore, increase oxygen delivery to the metabolically active BAT. A second way that the pup can increase oxygen uptake is by increasing tidal volume; we did not measure tidal volume in the present experiment. Finally, a third way that oxygen uptake can be increased is by the prolongation of expiratory duration in relation to inspiratory duration. Instances of such expiratory prolongation were found to occur at about the same time that respiratory frequency increased and were also accompanied by the emission of ultrasonic pulses.

General Discussion

The present series of experiments was inspired by the suggestion made 2 decades ago that ultrasound production and thermoregulatory ability are related (Okon, 1970). Despite this intriguing suggestion, there has been little information since then regarding the physiological basis of ultrasound production, although there has certainly been a great deal of attention focused on the functional aspects of ultrasound. This concentration on the functional aspects of ultrasound is a reflection of the conventional wisdom that ultrasound production evolved as a communicatory behavior that reflects an attempt by the pup to elicit maternal retrieval during times of stress. Our results challenge the primacy of the functional explanations.

The results of our experiments indicate that ultrasound production is associated with the constellation of physiological responses of the rat pup to cold exposure. Specifically, ultrasound production began when the pup's BAT began producing heat; oxygen consumption and respiratory rate also increased at this time. The embedding of ultrasound production within these physiological responses raises the question of whether ultrasound production itself reflects a heretofore unrecognized physiological mechanism that benefits the cold-exposed pup.

Ultrasound Production as an Acoustic By-Product of Laryngeal Braking

Ultrasonic pulses occur in conjunction with respiratory movements of increased expiratory duration. This concurrence suggests that the pup is using a mechanism known to respiratory physiologists as *expiratory* or *laryngeal braking* (Davis & Bureau, 1987; England et al., 1985; Gautier et al., 1973). Laryngeal braking is prevalent as a mechanism for improving gas exchange in the lungs of many mammalian neonates (Davis & Bureau, 1987; Mortola, 1987); the prevalence of this mechanism is presumably due to the neonate's low oxygen stores (England et al., 1985) as well as the undeveloped state of the newborn respiratory system (Burri, Dbaly, & Weibel, 1974; Mortola, 1987). By increasing expiratory duration and intrathoracic pressure, laryngeal braking likely improves gas exchange in the lungs by elevating end-expiratory lung volume (Brancatisano, Kelly, Tully, & Engel, 1987; England et al., 1985) and by recruiting new alveoli (or preventing alveolar collapse) and thus increasing

the lung surface available for gas exchange (Davis & Bureau, 1987).

If the ultrasonic emissions are associated with laryngeal braking, then it follows that the rat pup during cold exposure is using laryngeal braking. One reason for this use of laryngeal braking may be that the decrease in body temperature during cold exposure compromises gas exchange by directly reducing the rate of gas diffusion in the lungs (see Slonim & Hamilton, 1981). The detrimental effects of this decreased gas exchange may become critical when the pup initiates NST and thus increases the need for oxygen. This would explain the initiation of ultrasound production at the time when BAT begins producing heat and the subsequent diminution of ultrasound production as the pup reaches a new physiological steady state (see Figure 1).

Similarities Between Ultrasound Production in Rat Pups and Grunting in Human Infants

There is an interesting parallel between the present findings in the rat pup and the clinical treatment of human infants with respiratory distress syndrome (RDS, or hyaline membrane disease). Body temperature in RDS infants is monitored very carefully to prevent any increase in oxygen consumption (Slonim & Hamilton, 1981). Should body temperature decrease and oxygen consumption increase, an effective treatment is the administration of positive end-expiratory pressure (PEEP). PEEP helps to prevent lung collapse and to improve alveolar ventilation and is, therefore, mimicking the effects of laryngeal braking. Significantly, the discovery of the usefulnes of PEEP in the treatment of RDS infants arose from earlier observations that RDS infants emit an audible grunt. Grunting in RDS infants is a by-product of laryngeal braking that has been shown experimentally to aid in the maintenance of arterial oxygen tension; when grunting is prevented by intubating the infants, they rapidly become cyanotic (Harrison, de V. Heese, & Klein, 1968). It has since been demonstrated that healthy preterm infants also exhibit laryngeal braking, although without the audible grunting sound (Lindroth, Johnston, Ahlstrom, & Svenningsen, 1981). Grunting has also been detected in healthy, fullterm babies after experimentally induced changes in thoracic gas volume (Milner, Saunders, & Hopkin, 1977, 1978). One can conclude, therefore, that laryngeal braking and even grunting are not exhibited only by diseased infants.

Distinguishing the Message From Its Meaning

If ultrasonic vocalization is a by-product of a mechanism for maintaining adequate gas exchange during cold exposure, where does this leave the vocalization itself? This question can be answered by again considering the grunting infant. No one to our knowledge has suggested that grunting is a communicatory behavior, although it is clear that grunting does communicate a state of physiological distress. Nonetheless, the message was meaningless until it was appreciated what these infants were communicating and how best to respond to their message. It should be stressed, however, that although clinicians can detect these grunts and thus treat the infants appropriately, the infants themselves are not grunting to communicate to the doctors and nurses of the maternity ward that they are in distress. Rather, they are responding to internal physiological cues (e.g., low arterial oxygen tension) with a respiratory mechanism that happens to have as its by-product the emission of sound. To draw the now obvious conclusion, infant rats are not emitting ultrasounds to elicit maternal retrieval. Rather, they are invoking a respiratory mechanism to maintain lung inflation and to maximize oxygenation of arterial blood; this respiratory mechanism also happens to produce a sound. In sum, the respiratory mechanism underlying the vocalization is primarily a response to facilitate oxygen delivery to tissues, not pup delivery to the nest.

Developmental Aspects of Ultrasound

We began this article by noting the parallel between the development of ultrasound production and the development of thermoregulation. We suggested that explaining this parallel solely from a functional perspective was not adequate. Given that we now have a better understanding of the mechanisms of ultrasound production, do we also have a better understanding of its development? Although the experiments in this article were not designed to answer this question, they do suggest that the development of ultrasound is likely to be associated with developmental characteristics of the rat pup relating, among other things, to the postnatal differentiation of BAT (Skala, Barnard, & Lindberg, 1970) as well as to allometric influences on lung development (Burri et al., 1974). The developmental questions, however, remain for further research.

Evolution and Function of Ultrasound: Adaptation Versus Exaptation

If pup ultrasound is the audible by-product of laryngeal braking, then the belief that the ultrasonic vocalization evolved as a communicatory signal for maternal retrieval would be an example of the "conflation of historical genesis with current utility" (Gould & Vrba, 1982). Gould and Vrba argue that this conflation has led many researchers to designate structures as adapted when they are more likely nonadapted. They suggest that the term "exapted" be reserved for such nonadapted structures (or behaviors in this case) that nonetheless come to play significant roles in the survival of an animal. Using their terminology for the present situation, we see that the larynx was adapted for its role in regulating respiration. When the larynx is used for laryngeal braking, sound is produced accidentally, as a by-product. The mother, in turn, can increase her reproductive fitness by using the sound to detect and locate her lost pup and return it to the warm nest. When used in this way, the ultrasonic vocalization can be viewed as an exaptation for maternal retrieval.

Once the basic structure of pup vocalization and maternal response existed, secondary adaptations could then arise and lead to a more efficacious system. For example, the auditory sensitivity of many rodents appears to have been modified so that it now has two sensitivity peaks, one of which corresponds to the vocalization frequency of conspecific young (Brown, 1973a, 1973b). Such a modification in auditory sensitivity represents a secondary adaptation. Similarly, pups may have also undergone secondary adaptations that increased the likelihood of sound production during laryngeal braking.

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