Thermogenesis During Ultrasonic Vocalization by Rat Pups Isolated in a Warm Environment: A Thermographic Analysis

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Ultrasonic vocalizations, emitted by rat pups when separated from their mother, littermates, and home cage, have been used as a measure of isolation distress. Recently, we demonstrated that cold exposure is the primary component of isolation that induces the vocalization. We were unable, however, to suppress all ultrasound production when transferring pups to a thermoneutral (35°C) environment.

Using an infrared thermography system that allows us to estimate noninvasively heat production by brown adipose tissue, we found that pups transferred from the home nest to a 35°C test chamber exhibited sizable levels of heat production while they were vocalizing. Moreover, both heat production and ultrasound emission decreased over the 15-min test. Next, we used extreme care to minimize thermal, and therefore respiratory, stimulation of pups before, during, and after the transfer procedure. We found that such precautions prevented both heat production and ultrasound emission following transfer. These results indicate that infant rats' thermal sensitivities are far greater than previously suspected.

Appreciation of the fundamental roles played by thermal factors in the behavior and physiology of mammalian infants has grown steadily (e.g., see Leon, 1986; Hull, 1973). A variety of infantile or immature behavioral systems have been found to be very sensitive to temperature: Huddling by rat pups is temperature-modulated (Alberts, 1978); unique forms of ingestive behavior in infants can be activated in a warm surround (Johanson & Hall, 1979); species-typical behaviors are elicited

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in response to warm and cold challenges (Kleitman & Satinoff, 1982; Pfister, 1990); and several kinds of learning depend on thermal cues or are reinforced by temperature stimuli (Guenaire, Costa, & Delacour, 1982; Johanson & Hall, 1979; Pfister, 1990).

In parallel, knowledge has increased concerning the effects of thermal factors on infant physiology: Altricial infants can produce regulatory increases in metabolic heat production (Spiers & Adair, 1986); infants show bidirectional cardiac responses to warm and cool stimuli (Martin & Alberts, 1982); and mammalian infants possess a specialized thermogenic organ, that is, brown adipose tissue, that plays a key role in the warming of vital nervous and systemic structures during cold exposure (Smith, 1964).

The 40 kHz ultrasonic vocalization of infant rats is another neonatal behavior that is profoundly affected by thermal factors. Beginning more than 2 decades ago, experimenters noted that cold exposure is a highly effective stimulus for eliciting these vocalizations (Allin & Banks, 1971; Okon, 1971). In time, investigators were finding that features in the environment that are apparently devoid of thermal significance are also capable of modulating ultrasound production (e.g., Hofer & Shair, 1978, 1980). Thus, it seemed as though this vocalization, like many topics of psychobiological research, presented an analytic problem that contained a complex mixture of behavioral, physiological, psychological, and social variables.

When we focused on the rat pups' physiological responses to cold exposure and found regulatory changes in oxygen consumption, brown fat thermogenesis, and respiratory movements, we were led to hypothesize that the ultrasonic vocalization is the acoustic by-product of a respiratory maneuver called laryngeal braking (Blumberg & Alberts, 1990), similar to the audible and inadvertent grunting of human infants (Harrison, de V. Heese, & Klein, 1968)) and lambs (Johnson, Harding, McClelland, & Whyte, 1977). The apparent power of this physiological explanation encouraged us to investigate further the influence of thermal factors on ultrasound production.

Thus, in a previous article, we utilized a simple and common procedure used by many ultrasound investigators, that is, the gentle transfer of a rat pup from its nest to a warm test chamber in order to determine the extent to which thermal factors can account for "isolation-induced" ultrasound (Blumberg, Efimova, & Alberts, 1992a). As expected, it was found that pups vocalized significantly less when transferred from the nest to a warm test chamber than to a cold test chamber. Nonetheless, some pups transferred to a warm chamber did vocalize, albeit at very low levels and for only a few min.

Ultrasound in the apparent absence of cold stimulation suggests that nonthermal stimuli may also modulate vocalization. Such a finding, if true, would have little bearing on the standing of the laryngeal braking hypothesis: that hypothesis concerns the functioning of the respiratory system in relation to oxygen utilization. Such a finding might, however, leave open the possibility that nonthermal factors, such as contact comfort, play a role in ultrasound emission. Thus, in the present paper, we examine the extent to which thermal stimulation can account for ultrasound production during the seemingly gentle and thermally benign procedure of transfer from the nest to a warm environment.

A novel analysis of the pups' thermal sensitivities following transfer from the nest is performed here using an entirely noninvasive technology for measuring

physiological temperature. This technology, infrared thermography, allows the visualization of skin temperatures over the entire unfurred surface of a pup. With this instrument one can readily determine whether a pup is exhibiting nonshivering thermogenesis (NST). It is known that NST is the mammalian infants' means of producing metabolic heat (Hull, 1973) and that the effector for this response is the pups' brown adipose tissue (BAT; Smith, 1964)). When heat production by BAT is occurring, the thermograph of the pups' dorsal surface contains a circumscribed zone of heat centered in the interscapular region. Thus, with this technology, one can monitor multiple sites across the pups' body surface without the interference or stimulation that is invariably associated with thermocouples and other standard temperature measuring devices.

In Experiment 1, we investigated the possibility that pups transferred from the nest to a thermoneutral environment exhibit BAT heat production. We were also interested in relating any posttransfer NST with ultrasound emission. In Experiment 2, the importance of thermal factors for ultrasound production following transfer from the nest was analyzed in finer detail.

Experiment 1: Pups Transferred From the Nest to a Thermoneutral Environment Exhibit NST and Emit Ultrasounds

Method

Subjects

Nine pups from seven litters were used. Five were male and 4 were female, with weights ranging from 15.0 to 24.8 g. Rats were bred from Sprague-Dawley stock originally obtained from Charles River (Portage, Michigan) and were born in the Animal Behavior Laboratory colony at Indiana University. On the day of testing all pups were 7–9 days of age. 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 available ad libitum. All animals were maintained on a 16 : 8 hr light/dark schedule with lights on at 7:00 a.m.

Apparatus

The test chamber consisted of a glass cylinder (8 cm inside diameter × 17 cm deep) open at the top. The cylinder was surrounded by a clear glass water jacket with two nozzles through which water could be circulated. The two nozzles were connected via tubing to a water circulator in which the water was heated and maintained at a fixed temperature; in turn, air temperature within the test chamber was regulated and remained stable throughout the experiment.

Air temperature within the chamber (T_{air}) was measured, with a resolution of 0.5°C, using an air temperature probe (Yellow Springs Probe #405) connected to a telethermometer (Yellow Springs). Air temperature was recorded by hand at the beginning of each min of the test.

The thermal responses of pups following transfer from the nest was accomplished without the use of thermal probes by using an infrared thermography

system (Thermovision 870, Agema Infrared Systems, Danderyd, Sweden). The thermography system consists of an infrared camera connected to a microprocessor that digitizes and presents thermal information on a video monitor. Each thermograph is composed of 100 elements/line and 280 lines per frame and presents thermal information with a resolution of 0.1°C. Coded, infrared images can be recorded and stored on hard disk; this allows the experimenter to analyze each image after completion of the experiment. To do this, we used an image analysis software package supplied with the thermography system.

Ultrasonic vocalizations were detected using a microphone placed inside the test chamber. The microphone, positioned within 18 cm of the pup, was connected to a "bat detector" (QMC, Ltd., London, U.K., Model S-25) tuned to a range centered on 40 kHz. Occurrence of ultrasonic vocalization was collected every sec using the event marker on a Gould TA240 Chart Recorder. When ultrasonic pulses were detected, the event marker was activated and indicated whether a pulse was detected during each sec of the test. After the test, the number of 1-s bins in which an ultrasonic pulse had been detected was counted for each min of the test. These data are presented as estimated percentage of time spent vocalizing.

Procedure

On the day of testing, a cage containing a litter and its mother was transferred from the colony room to the testing room. The air temperature in the test room was 17–21°C. The mother and litter remained undisturbed in the test room for at least 1 hr. The test began when the experimenter removed a pup at random from the cage and placed it as quickly as possible (within 5 s) into the temperature-controlled test chamber, maintained at an air temperature of 35–36°C. The pup was carried over a distance no more than 1 m through room air. Conductive heat exchange between the pup and its surroundings was minimized by grasping the pup while wearing rubber gloves and by placing the pup inside a polyethylene mesh cage (7.5 × 6 × 9 cm) inside the glass chamber.

After a pup was transferred from the nest to the chamber, the experimenter noted the time and began recording the occurrence of ultrasound production. In addition, during each min of the test, a thermograph of the pup was recorded and stored. Each trial lasted 15 min, after which the pup was removed from the chamber, weighed, and its sex was determined. The data from any given pup were included only if the pup had been fed recently, as evidenced by the presence of a milk band. No more than 2 pups from any litter were tested; if 2 pups from the same litter were used, they were tested on different days.

Thermographic Analysis

Nonshivering thermogenesis was estimated by comparing skin temperature overlying the interscapular brown fat depot with skin temperature at a distal area on the back that does not overlie brown fat. Measurement at these sites was accomplished by first constructing, using the thermography system's software, a square area of analysis composed of 25 pixels. The computer's cursor was used to move this square over the area whose temperature was to be measured; the computer then calculated the average temperature among the 25 pixels defined by

the square. In this way, interscapular temperature (T_{is}) and back skin temperature (T_{back}; defined as the point on the midline at the anterior level of the sacral spinal cord) could be measured for each thermograph. Finally, the level of BAT activity was estimated by subtracting T_{back} from T_{is}.

Statistical Analysis

Data are presented as Mean ± *SEM*. Differences in BAT activation and ultrasound production were tested using analysis of variance and a one-group *t* test. Post-hoc analyses are considered significant when $p < 0.05$. Statistical calculations were performed using Statview II on the Macintosh computer.

Results

As expected, and as shown elsewhere (Blumberg et al., 1992a; Kehoe & Blass, 1986), ultrasound emission following transfer from the nest is high at first and then subsides during the ensuing minutes (Fig. 1a). Specifically, pups vocalized 10.7 ± 3.1% of the time during the 1st min of the test, 0.9 ± 0.3% of the time during the 5th min of the test, 0.6 ± 0.4% of the time during the 10th min, and 0.2 ± 0.2% of the time during the 15th min of the test. In addition, 8 of the 9 pups did not emit a single pulse during Min 11–15; the remaining pup emitted a single pulse during each of the last 3 min of the test.

Within 30 s of transfer of the pup from the nest and placement in the test chamber, T_{is} was 35.0 ± 0.2°C. This value is much higher than the ambient temperature at the nest and reflects the positive thermal contributions of the huddle and mother (Alberts, 1978). Within 5 min, T_{is} had increased to 35.8 ± 0.1°C, and by the end of the 15-min test T_{is} was 36.2 ± 0.1°C.

Pups transferred to the chamber initially exhibited high levels of BAT activity (Fig. 1b). This activity was readily apparent on the monitor of the thermography system as a region of high temperature located above the interscapular region. This visual identification of BAT activity was quantified by subtracting back skin temperature from interscapular temperature; the greater the difference, the greater the BAT activation. Thus, for Min 1, T_{is} was 0.9 ± 0.1°C warmer than was T_{back}, significantly different from 0; $t = 7.587$, $df = 8$, $p < 0.0001$, indicating local heat production in the interscapular region. At Min 5, T_{is} was 0.7 ± 0.1°C warmer than T_{back}, significantly different from 0; $t = 8.758$, $df = 8$, $p < 0.0001$. By Min 10, the difference had decreased to only 0.2 ± 0.1°C, although this was still statistically significant, $t = 2.813$, $df = 7$, $p < 0.05$. By Min 15, however, $T_{is} - T_{back}$ was no longer significantly different from 0, $t = 1.484$, $df = 8$, $p > 0.15$).

Discussion

The results of Experiment 1 indicate that pups transferred from the nest to even a thermoneutral test chamber exhibit robust BAT activity and produce ultrasonic vocalization. This transfer procedure is similar to that used by many investigators to study the "isolation" responses of neonatal rats. In fact, extra precautions were taken here to prevent or minimize cold stimulation that might accompany the transfer procedure. Specifically, we waited at least 1 hr after the

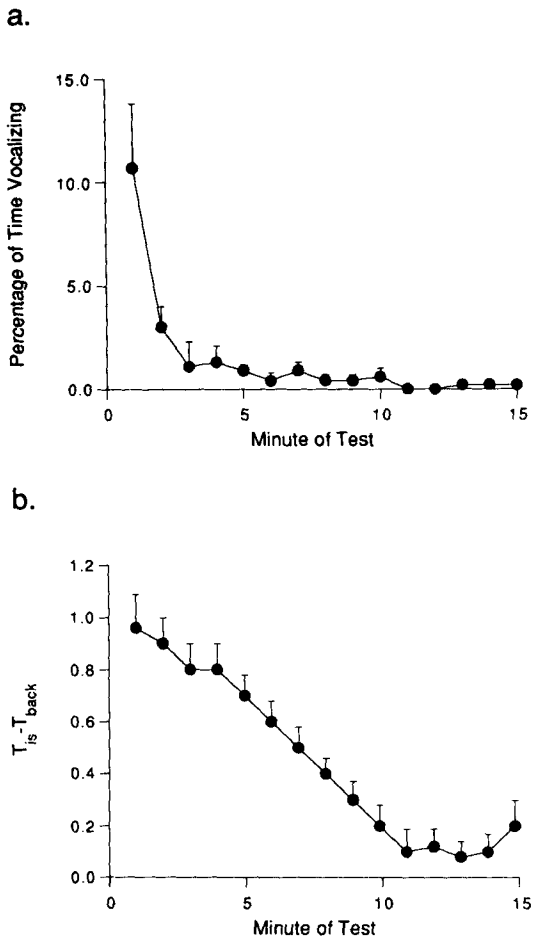

Fig. 1. (a) Percentage of time vocalizing and (b) nonshivering thermogenesis, as measured by $T_{is} - T_{back}$, for each of the 15 min following transfer of pups ($N = 9$) from the home cage and nest to a thermoneutral environment (35°C). Mean ± *SEM*.

mother and her litter were transferred to the test room to allow the animals to stabilize, a rubber glove was worn when grasping the pup to minimize conductive heat exchange between the pup and experimenter, the pup was placed in the experimental chamber within 5 s of removing it from the nest, polyethylene mesh lined the experimental chamber to minimize conductive heat exchange, and the experimental chamber was maintained at 35–36°C. Nonetheless, within the 30 s following the transfer, pups were exhibiting sizable levels of BAT activation. Moreover, more than 10 min were required before this BAT activation was no longer detectable. Therefore, it appears that these precautions were not sufficient to eliminate all sources of thermal stimulation.

It is not clear which aspect of our procedure introduced or failed to eliminate sources of cold stimulation. It is possible that carrying a small furless pup through room air is sufficient to cause significant convective heat loss. It is also possible that pups within a huddle in a nest at room temperature are very close to, if not

over, the threshold for BAT activation and that transferring the pup presents a strong enough thermal stimulus to provoke a significant BAT response. In Experiment 2, we attempted to remove these aspects of cold stimulation before and during the transfer procedure.

Experiment 2: Transfer and Isolation Without Cold Exposure Does Not Stimulate NST or Ultrasound Production in Rat Pups

Experiment 1 demonstrated that rat pups are much more sensitive to their thermal environment than generally assumed. A pup removed from its nest and placed quickly into a thermoneutral environment displays brown fat thermogenesis for many minutes after the transfer. In the present experiment, we wanted to remove even further the contributions of temperature to isolation-induced ultrasound. To do this, we placed whole litters inside an incubator maintained at either 35°C or 22°C. After at least 1 hr in the incubator, an individual pup was transferred to a chamber maintained at either 35°C or 22°C and ultrasound production and heat production (as measured using the thermovision system) were monitored. We expected that pups habituated with littermates at 35°C and then isolated in a chamber at 35°C would be virtually silent. In contrast, we expected that pups in the other three conditions (35°C–22°C; 22°C–35°C; 22°C–22°C) would emit ultrasounds in relation to their heat production responses.

Method

Subjects

Thirty-two pups from 21 litters were used. All pups were 7–8 days of age at the time of testing. Twenty were male and 12 were female, with weights ranging from 12.1 to 20.9 g. All animals were bred and raised as in Experiment 1.

Apparatus

Two types of temperature-controlled chambers were used. Whole litters in their home cages were placed inside a human infant incubator (74 × 35 × 30 cm) with a Plexiglas cover. Temperature within the incubator was controlled precisely using a temperature controller (Yellow Springs Model 73A). The second chamber was a glass double-walled cylinder (12 cm inside diameter × 23 cm deep), open at the top, and connected via tubing to a heater/circulator. This second chamber is hereafter referred to as the test chamber.

The pups' thermal responses were measured using the infrared thermography system as in Experiment 1. Ultrasonic vocalizations were detected as in Experiment 1; the ultrasonic microphone was 25 cm from the pup. In this experiment, we measured the quantity of ultrasound production using a computerized data acquisition system (OmegaLog, Omega Engineering, Inc., Stamford, CT). To quantify the vocalization data, the observer pressed a button every time a vocalization was detected. Pressing the button activated a counter in the computer which indicated that a vocalization had been detected during that second. After the test, the number of 1-s bins in which an ultrasonic pulse had been detected was counted

for each min of the test. These data are presented as estimated percentage of time spent vocalizing.

Procedure

On the day of testing, the mother of the litter to be tested was removed from the home cage. The entire cage was then carried to the test room and placed within the incubator maintained at an air temperature of either 35°C or 22°C. After a period no less than 1 hr and no more than 1.75 hr, the lid of the incubator was gently raised and a pup was grasped with two gloved fingers and placed inside a bare plastic bowl (12 × 10 × 8 cm). With a hand covering the bowl's top, the bowl and pup were removed from the incubator and placed inside the test chamber whose temperature was maintained at either 35°C or 22°C. Pups were transferred over a distance no more than 0.5 m.

As expected, litters maintained at different temperatures huddled differentially (Alberts, 1978). Pups at 22°C usually maintained a tight huddle; pups chosen for isolation generally came from the top of the pile. In contrast, pups at 35°C were more dispersed; pups chosen for isolation from this condition were those that were maintaining some contact with at least one other littermate.

After the pup was placed inside the test chamber, the experimenter noted the time and began recording the occurrence of ultrasound production. In addition, a thermograph of the pup was recorded midway through each min of the test. Air temperature within the test chamber was also recorded throughout the test. Each trial lasted 15 min, after which the pup was removed from the chamber and weighed, and its sex was determined. The data from any given pup were included only if the pup had been fed recently, as evidenced by the presence of a milk band. The ordering of test conditions was randomized. No litter contributed more than 2 pups to the experiment; if 2 test pups did come from the same litter, they were used on different days and in different conditions.

Statistical Analysis

Data are presented as Mean ± *SEM*. Two-factor analyses of variance were performed using habituation temperature and test temperature as the factors. Statistical calculations were performed using Statview II on the Macintosh computer.

Results

Table 1 presents mean T_{is} approximately 30 s after pups were placed inside the test chamber for the four conditions of the experiment. It can be seen that mean T_{is} was lowest when pups were incubated at 22°C and tested at 22°C; placing pups in the test chamber at 35°C was associated with a mean increase of 0.6°C within the short 30-s interval. Similarly, mean T_{is} was highest when pups were incubated at 35°C and tested at 35°C; placing pups in the test chamber at 22°C was associated with a mean decrease of 0.6°C. A two-factor ANOVA indicated significant effects of both incubator temperature, $F(1, 28) = 70.516$, $p < 0.0001$,

Table 1
Mean (SEM) interscapular temperature approximately 30 s after transfer of pups to the test chamber from the nest for the four conditions in Experiment 2. N = 8 in each group

		Incubator Temperature (°C)	
		22	35
Test Temperature (°C)	22	34.6 (0.2)	36.7 (0.4)
	35	35.2 (0.2)	37.3 (0.2)

and test temperature, $F(1, 28) = 6.576$, $p < 0.02$. The interaction was not significant.

Figure 2 shows NST, as measured by $T_{is} - T_{back}$, for each of the four conditions of the experiment over the 15 min of the test. It can be seen that when habituation temperature was 22°C, NST was high initially and then either decreased if the test temperature was 35°C or remained high if the test temperature was 22°C. In contrast, when the habituation temperature was 35°C, NST was low initially and then either remained low if the test temperature was 35°C or increased if the test temperature was 22°C. As the figure suggests, habituation temperature alone significantly affected $T_{is} - T_{back}$ at Min 1 (habituation temperature: $F(1, 28) = 206$, $p < 0.0001$; test temperature: $F(1, 28) = 0.311$, ns; interaction: $F(1, 28) = 1.942$, ns) whereas, by min 15, test temperature alone significantly affected $T_{is} - T_{back}$ (habituation temperature: $F(1, 28) = 0.001$, ns; test temperature: $F(1, 28) = 9.29$, $p < 0.005$; interaction: $F(1, 28) = 0.005$, ns).

The primary goal of this experiment was to minimize the thermal perturbations during the transfer procedure in order to determine the necessity of thermal stimulation for ultrasound production. Figure 2 shows that pups habituated at an air

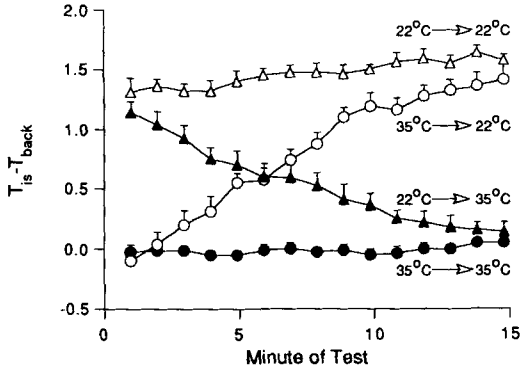

Fig. 2. Nonshivering thermogenesis, as measured by $T_{is} - T_{back}$, for each of the 15 min following transfer of pups from 35°C to either 22°C or 35°C and the transfer of pups from 22°C to either 22°C or 35°C. *N = 8 in each group. Mean ± SEM.*

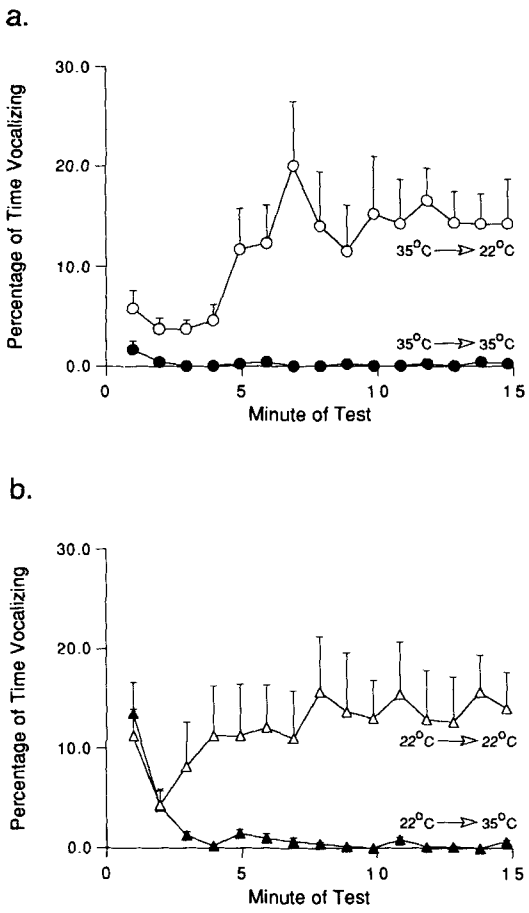

Fig. 3. Percentage of time vocalizing for each of the 15 min following (a) the transfer of pups from 35°C to either 22°C or 35°C and (b) the transfer of pups from 22°C to either 22°C or 35°C. N = 8 in each group. Mean ± *SEM*.

temperature of 35°C and then transferred to the test chamber at 35°C manifested no signs of NST. And, as expected, very few ultrasounds were detected under these conditions (Fig. 3a). In fact, of the 8 pups in this condition, 4 did not emit a single pulse throughout the 15-min test, 2 pups emitted only 2 pulses, and 1 pup emitted 3 pulses. The last pup emitted 10 pulses in the first 3 min of the test and 2 pulses during the remainder of the test. Thus, excluding this one relatively vocal pup, only 7 ultrasonic pulses were detected from 7 pups over the course of 105 total min of isolation. [We observed that many of these pulses occurred during the jerking movements that often accompany REM sleep in infant rats (Whishaw, Schallert, & Kolb, 1979).] To illustrate the different levels of ultrasound production more clearly, consider that while ultrasounds were detected during a total of 19 s following transfer from 35°C to 35°C, ultrasounds were detected during 819 s following transfer from 35°C to 22°C.

Overall, levels of ultrasound emission mirrored levels of metabolic heat production, as can be seen by comparing levels of NST in Figure 2 with ultrasound

emission rates in Figures 3a and 3b. As discussed above, habituation temperature, but not test temperature, significantly affected $T_{is} - T_{back}$ during Min 1, and this pattern was reversed by Min 15. Similarly, habituation temperature, but not test temperature, significantly affected ultrasound production during Min 1 (habituation temperature: $F(1, 28) = 5.30$, $p < 0.05$; test temperature: $F(1, 28) = 0.06$, ns; interaction: $F(1, 28) = 0.74$, ns); conversely, test temperature, but not habituation temperature, significantly affected ultrasound production during min 15 (habituation temperature: $F(1, 28) = 0.001$, ns; test temperature: $F(1, 28) = 9.29$, $p < 0.005$; interaction: $F(1, 28) = 0.005$, ns).

Discussion

In Experiment 2 we attempted to remove as much of the thermal component of pup isolation as we could in order to determine the necessity of thermal stimulation for ultrasound production. In 7 of the 8 pups that were transferred from 35°C to 35°C, only seven ultrasonic pulses were detected over the entire 15-min test. Moreover, many of these pulses occurred as the pups were sleeping, an observation that is consistent with the detection of ultrasound from unconscious pups (Hofer & Shair, 1991). The one pup that exhibited higher levels of ultrasound (1.3% over 15 min) vocalized predominantly during the first min following isolation, suggesting that some mechanical aspect of the transfer procedure (e.g., tactile stimulation) was responsible. In any event, it is clear that extreme care in minimizing thermal stimulation of pups during transfer virtually eradicates the emission of ultrasound.

General Discussion

Week-old rat pups are furless and have a high surface-to-volume ratio. Thus, any preconceptions that we, as large animals, may have about the significance to rat pups of various thermal stimuli are affected by our large size and low surface-to-volume ratio and, thus, necessary insensitivity to their thermal experiences. The results of Experiment 1 show clearly that our preconceptions regarding the thermal comfort of rat pups following transfer, even to a thermoneutral environment, are misleading. Even pups transferred from the "comfort" of the nest to the "comfort" of a warm environment exhibited nonshivering thermogenesis that required some time to decrease. When extreme care was taken to remove all thermal stimulation before and during the transfer procedure, as was done in Experiment 2, nonshivering thermogenesis was prevented; moreover, pups did not emit ultrasound even though they were isolated from their mother, littermates, and nest bedding.

Thus, using the method of other investigators, it has been shown that thermal stimulation can account for the vast majority of ultrasound induced by isolation. Nonetheless, it behooves us to address the issue of ultrasound production in the nest. In the present experiments, pups were monitored only after they were transferred to the test chamber. It has been reported (Hofer & Shair, 1978) that pups do not emit many ultrasounds when in the nest with their littermates at room temperature. Does this indicate that ultrasound production is modulated by nonthermal social factors?

In considering this question, it should be noted that litters of pups at room temperature do emit some ultrasound, albeit few vocalizations are emitted (Hofer & Shair, 1978; Blumberg, Efimova, & Alberts, 1992b). Such low levels are expected given that individual pups within large huddles, through the mechanics of group regulation, maintain a high degree of thermal comfort (Alberts, 1978). Removing and isolating a pup from such a litter (or, alternatively, removing all but one pup from the nest; Hofer & Shair, 1978) has the effect of stripping the individual of the thermal insulation previously provided by the huddle, thus profoundly increasing the effective cold exposure even though air temperature has not changed.

It follows from this argument that huddles of pups should produce high levels of ultrasound if the thermal benefits of huddling can be overcome by decreasing air temperature. In fact, this has been demonstrated by cooling groups of 4 pups below room temperature (Blumberg et al., 1992a). More recently, a litter of 8 pups was placed in a chamber at 10°C. One hr later, ultrasound production was monitored for 15 min. During this period, the litter vocalized more than 50% of the time, as compared with 7% when the same litter was observed at 20°C (Blumberg et al., 1992b).

Thus, thermal stimulation is necessary for significant levels of ultrasound production both inside and outside the nest. Nonetheless, as discussed above, the laryngeal braking hypothesis does not require that cold be the only physiological stimulus for laryngeal braking. On the contrary, any stimulus or drug that activates the respiratory system, independently of its effect on heat production, could potentially elicit laryngeal braking and ultrasound (see Blumberg et al., 1992a, for discussion). The present results, however, demonstrate that the tactile, vestibular, proprioceptive, and social isolation cues involved in the transfer procedure are not sufficient elicitors of ultrasound, as long as cold stimulation is carefully avoided.

Although the importance of cold temperature as a stimulus for ultrasound has been recognized for many years, the cold stimulus has been subsumed under the seemingly broad concept of "isolation." The use of such terminology has had the effect of directing attention away from the physiological significance of cold air temperature for the vocalizing pup. Moreover, because ultrasound has been interpreted as the acoustic indicator of emotional distress, cold exposure has come to be conceptualized as a cause of that distress.

Accumulating empirical evidence suggests that the concept of distress as an explanation of ultrasound production is no longer useful. For example, when physiological responses to cold exposure are inhibited by making pups simultaneously hypoxic or by starving them before cold exposure, ultrasound production is also inhibited (Blumberg & Alberts, 1991). Even more surprising, high levels of ultrasound production have been detected from unconscious pups in deep hypothermic coma (Hofer & Shair, 1991). If ultrasounds are indeed signals of emotional distress, one would expect increased ultrasound production from pups in the former conditions (hypoxia and starvation) and no ultrasound production from pups in the latter condition (hypothermic coma).

Determining if and to what extent emotional distress plays a role in ultrasound production is beset with definitional, conceptual, and empirical difficulties. Disproving a role for emotional distress is impossible. Similarly, it is not possible to disprove that some stimuli (e.g., contact with littermates) modulate ultrasound

production independently of their effects on a pup's thermal state. On the other hand, it is possible to determine the extent to which thermal factors *suffice* to explain this phenomenon, as the experiments in this paper illustrate. And, given the results of these and previous experiments, we believe that allowing the concept of emotional distress to play a causal role in the elicitation of ultrasound introduces an intervening variable that serves only to obscure relatively clear, measurable mechanisms.

We would like to comment on the role of terminology in this research. Phrases such as "isolation call" and "distress call" convey special implications that phrases such as "cold-induced vocalization" or "vocalization associated with respiratory activation" do not. What is gained by brevity and functional description is lost by inaccuracy and inherent assumptions. Thus, we believe that isolation-induced vocalizations, as studied by previous investigators, are more aptly and objectively called cold-induced vocalizations. This distinction is important because it compels us to differentiate between a behavior that is defined by its presumed communicatory function and a behavior that is defined by its proximate stimulus (see also Blumberg & Alberts, 1992, for a discussion of interpretive issues in communication). We must stress again, however, that cold is only a stimulus for ultrasound to the extent that it results in respiratory activation (Blumberg & Alberts, 1991). Clearly, more work is required to delineate more precisely the relations between nonshivering thermogenesis, laryngeal braking, and ultrasound.

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