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On the Significance of Similarities Between Ultrasonic Vocalizations of Infant and Adult Rats

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BLUMBERG, M. S. AND J. R. ALBERTS. *On the significance of similarities between ultrasonic vocalizations of infant and adult rats.* NEUROSCI BIOBEHAV REV **15**(3) 383–390, 1991.—The communicatory significance of the 40 kHz vocalization of rat pups and the 22 kHz vocalization of adult rats have been topics of research for over three decades. The 40 kHz vocalization is emitted by pups during cold exposure, whereas the 22 kHz vocalization is emitted by adults following ejaculation, following defeat in aggressive encounters, as well as in other contexts. Recent research suggests that the 40 kHz vocalization is the acoustic by-product of a respiratory mechanism that enhances gas-exchange in the lungs during times of increased oxygen consumption. Furthermore, a reevaluation of research into the physiological basis of the 22 kHz vocalization suggests a similar conclusion. In the present paper, we discuss mechanistic and contextual aspects of ultrasound production. We conclude that these two vocalizations, produced by identical mechanisms and reflecting identical physiological states, are actually the same vocalization, albeit at different frequencies. This alternative view of ultrasound production has implications for our interpretation of the communicatory significance of these vocalizations.

Laryngeal braking Ultrasound Communication Metabolic heat production Oxygen consumption Rat Respiration Brown adipose tissue Nonshivering thermogenesis Fever Neonate

INDIVIDUALS of many rodent species emit vocalizations with frequencies above the range of human hearing. These "ultrasonic" vocalizations, first recognized in the mid-1950's (4,61), occur in a variety of behavioral and social contexts across the lifespan of the animal. Examples include the "distress vocalization" or "40 kHz vocalization" of infant rats exposed to cold temperatures and the "22 kHz vocalization" of adult rats emitted during copulation and aggressive encounters. These two vocalizations are the subject of this paper.

Typically, communication researchers suggest a function for a vocalization after a behavioral context with which it is correlated has been identified. As we will see, this has been the case with ultrasound research. Furthermore, for the most part, investigators have assumed that each context-related ultrasonic vocalization plays a communicatory role in regulating the social interactions among conspecifics. This assumption has helped generate a multitude of communicatory hypotheses for these vocalizations.

The present paper departs from the conventional path of understanding ultrasonic vocalizations primarily as communicatory signals. This departure arises because we have been impressed by the interrelations of these vocalizations and certain physiological changes that occur simultaneously. Based on these interrelations and the respiratory maneuver underlying production of these ultrasonic vocalizations, we hypothesize that the respiratory mechanism underlying the 40 kHz and 22 kHz vocalizations enhances gas exchange in the lungs; the production of sound by this respiratory mechanism is incidental. With this hypothesis, we suggest that these two rat vocalizations, heretofore considered separate behaviors, are, in fact, the same vocalization.

Although communication is not the focus here, it cannot and should not be ignored. Virtually all ultrasound research until now has been done with an eye towards its implications for our understanding of communication in rodents. To acknowledge this emphasis and to place our current understanding of these vocalizations into perspective, we will, in the course of this paper, review the communicatory aspects of the two vocalizations that comprise the subject of this paper. Finally, once the physiological synthesis, alluded to above, has been completed, our understanding of the possible communicatory uses of these sounds will be addressed.

COMMUNICATORY INVESTIGATIONS OF THE 22 KHZ VOCALIZATION

The 22 kHz vocalization of adult rats was the first rodent ultrasonic vocalization to be detected by human experimenters (4). It is a long call, with a duration between 1 and 3 seconds, and is associated with deep expiratory movements; it can be emitted continuously or intermittently for many minutes at a time. Although this vocalization was first detected from adult rats alone in their cages, we now know that it accompanies a variety of behavioral contexts including aggression (51), sexual behavior (9,10), and electric shock (10). Although the vocalization is emitted predominantly by male rats, it has also been detected in females when they are resisting mounting attempts by males (9); it has even been detected from sleeping lactating females (24). The vast majority of work on the possible communicatory value of the 22 kHz vocalization, however, has dealt with its emission by males during aggressive encounters and copulation, and, thus, the present review will focus on these two contexts.

The detection of the 22 kHz vocalization from submissive rats during aggressive encounters prompted the hypothesis that the vocalization inhibits the aggression of other animals (51). Early analyses seemed to support this suggestion: aggressive behaviors were rarer during encounters in which the vocalization was detected than during encounters in which the vocalization was not detected. This finding, in conjunction with the emission of the vocalization from rats displaying the crouched posture characteristic of submissive rats, led Sales (51) to conclude that "the motivation for the production of these pulses therefore appears to be a state of submission of the animal concerned."

Despite the success of this early correlational study, subsequent attempts to identify a causal connection between the 22 kHz vocalization and decreased aggressiveness were unsuccessful. Using an experimental design in which an intruder male is introduced into the cage of a resident male, it was found that devocalizing the intruder (by cutting bilaterally the inferior laryngeal nerve) had no measurable effect on the aggressiveness of the resident male, as measured by such parameters as number of bites, latency to bite, and incidence of a boxing posture (60). Similarly, deafening the resident male had no measurable effect on its aggressiveness (57). Takeuchi and Kawashima (58) carried out similar experiments; they, like Sales (51), found a correlation between the vocalization and aggressive behavior in their male rats, but they failed to find a causal connection when they observed deafened or muted rats. Based on these findings, they concluded that "ultrasounds emitted during aggressive encounters may have little communicative value in male rats."

The 22 kHz vocalization also accompanies sexual behavior. The copulatory sequence of rats consists of a series of mounts and intromissions (penile insertions) that culminate eventually in an ejaculation (50). After a period of quiescence, called the postejaculatory interval (PEI) or refractory period, copulation resumes. This pattern of copulation, ejaculation, and quiescence can be repeated as many as 6–12 times in a single evening.

Following ejaculation, the male often walks to the edge of the cage and either adopts a crouched posture similar to that of a defeated male (50) or lies down. It is during this period of time that Barfield and Geyer (9) noted the emission of the 22 kHz vocalization. They also found that the vocalization persists throughout 75% of the PEI, during which time the male is physiologically incapable of resuming copulation. This portion of the PEI is referred to as the absolute refractory period because no amount of exogenous stimulation can shorten its duration. When the male stops vocalizing, he typically begins walking about the cage and seeking out the female. This portion of the PEI is referred to as the relative refractory period because its duration can be shortened by exogenous stimulation.

The finding that the emission of the 22 kHz vocalization is correlated with the absolute phase of the PEI suggested to Barfield and Geyer (9,10) that the call reflects a state of social withdrawal and/or a state of behavioral inhibition. The possible communicatory value of the vocalization was suggested by the change in the female's behavior over the course of the copulatory series. Specifically, female rat sexual behavior is characterized by solicitation behaviors (consisting of approaches, hops, darts, and ear wiggles) between mounts. During the PEI, these behaviors cease. It is reasonable, therefore, that the 22 kHz vocalization was suggested as the mechanism that effects this change in female behavior; specifically, Barfield and Geyer suggested that the vocalization functions as a "desist-contact signal"(9).

Attempts to show a causal relationship between the emission of the 22 kHz vocalization and the distancing of the female during the PEI have been unsuccessful. For example, solicitation behaviors by the female during the PEI occur just as often when the male vocalizes as when he does not vocalize (7). It has also been shown that the female rat, like the male, exhibits her own refractory period following ejaculation (36). Moreover, the emission of the 22 kHz vocalization by the male is not necessary to elicit sexual quiescence by the female following ejaculation (7).

It has been suggested that, in most cases, the male rat emitting the 22 kHz vocalization is in a "state of social withdrawal"(1, 7, 9). This is apparently true of the defeated subordinate male, the postejaculatory male, as well as the animal that has experienced electric shock. Thus, an animal emitting the 22 kHz vocalization is "broadcasting" (to conspecifics or to researchers) information regarding its behavioral or physiological state. Whether conspecifics make use of this information has not yet been established. It should be stressed, however, that not all of the contexts in which the vocalization is emitted fit convincingly into this framework. For example, Francis (24), like Anderson (4), detected the vocalization from rats housed in single cages, a context that obviously has no social component. In addition, as noted earlier, the 22 kHz vocalization has been detected from sleeping rats (24).

PHYSIOLOGICAL INVESTIGATIONS OF THE 22 KHZ VOCALIZATION

It will be recalled that the male rat, following ejaculation, walks to the edge of the cage and either assumes a crouched posture or lies down. It is at this time that the male begins to vocalize. In addition, the postejaculatory male often lies down in a prone position, stretches his legs behind, puts his forepaws in front, and may also rest his head on the substrate; this behavior is known to thermoregulatory biologists as "sprawling" and is an effective means of losing body heat (49). Based on our observations of the postejaculatory rat (Blumberg and Moltz, unpublished), we inferred that the animal was lying down not only because it may be tired but also because it was overheated.

The postejaculatory sprawling of the male rat and the primary importance of hypothalamic thermosensors in the control of sprawling (48) suggested to us that the postejaculatory rat was sprawling in response to a hot brain. We tested this possibility by monitoring hypothalamic and body temperatures throughout the copulatory series (14). As expected, we found that both temperatures increased during copulation. Following ejaculation, however, the hypothalamus cooled faster than the body, a phenomenon known as "selective brain cooling" (21). Selective brain cooling is thought to be beneficial to over-heated animals due to the brain's greater susceptibility to tissue damage at high temperatures (21). Thus, it did appear as though the sprawling behavior of the postejaculatory rat was a response to increased hypothalamic temperature.

From the work of Barfield and Geyer (9), we knew that the 22 kHz vocalization is emitted predominantly during the period when the male is lying down following ejaculation. We predicted, therefore, a relationship between the emission of the vocalization and the period of hypothalamic cooling. In fact, we found a positive correlation between the rate of vocalization and the rate of hypothalamic cooling (14).

We hypothesized that the 22 kHz vocalization might be the acoustic by-product of a respiratory maneuver, characterized by deep expiratory movements against a maximally constricted larynx, that helps to cool a heat-threatened hypothalamus. As a first step towards determining whether the vocalization is a by-product of a thermoregulatory mechanism, we carried out two experiments designed to ascertain the effect of hypothalamic temperature on the emission of the vocalization (15). In one experiment, we infused prostaglandin E_2 (PGE_2) into the cerebral aqueducts of *isolated* male rats in order to raise hypothalamic temperature (Fig. 1). Following infusion, the rats adopted a crouched stationary posture and, while hypothalamic temperature

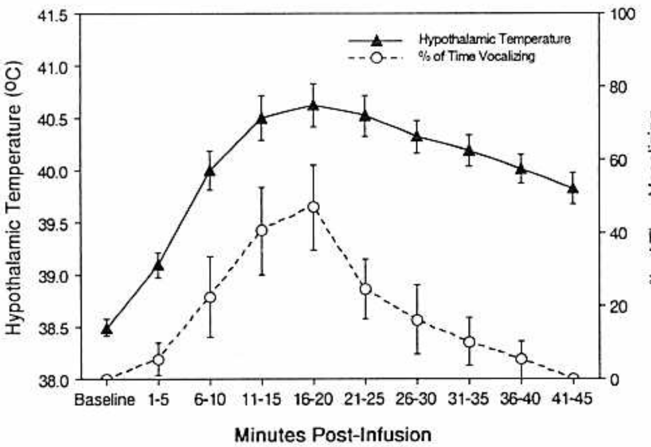

FIG. 1. Mean hypothalamic temperature and estimated percentage of time emitting the 22 kHz vocalization following the intracerebroventricular infusion of prostaglandin E_2 in adult rats. Data are for 8 animals with two tests per animal. Vertical lines indicate ±SEM. [Adapted from (15)].

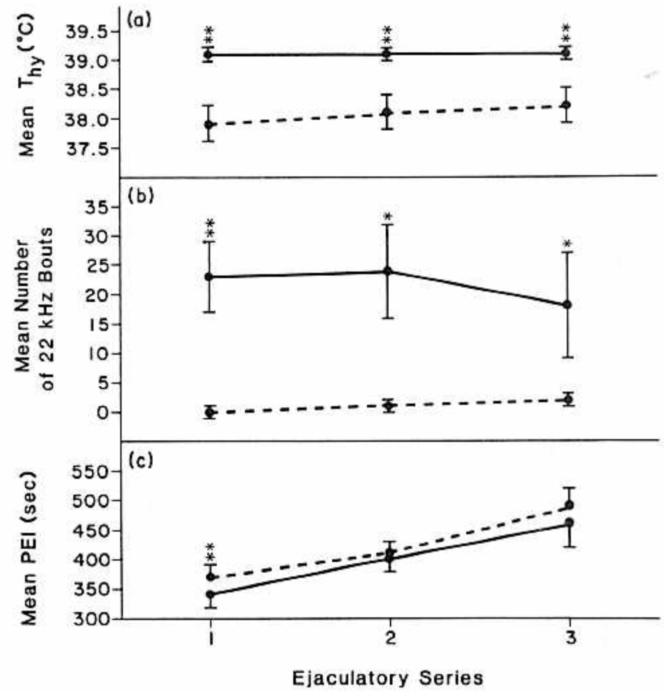

FIG. 2. (a) Mean hypothalamic temperature at ejaculation, (b) mean number of 22 kHz bouts following ejaculation, and (c) mean duration of the PEI for 3 ejaculatory series following the intraperitoneal injection of 200–300 mg/kg sodium salicylate (dashed line) and saline (solid line). Each of six males was tested under both conditions. Vertical lines indicate ±SEM. $*p<0.05$; $**p<0.005$. Paired t-test. [Adapted from (15)].

was increasing, began emitting the 22 kHz vocalization. The vocalization continued until hypothalamic temperature stopped increasing, at which time the rat stopped vocalizing and began moving about the cage. In contrast, rats infused with vehicle showed neither the temperature increase nor the emission of the vocalization.

In a second experiment, we injected male rats with sodium salicylate intraperitoneally in order to reduce hypothalamic (and body) temperature. After the rat's temperature was reduced sufficiently, we introduced an estrous female into the male's cage and monitored copulatory behavior, hypothalamic temperature, and the emission of the vocalization (Fig. 2). We found that following ejaculation, rats injected with sodium salicylate had lower hypothalamic temperatures than rats injected with vehicle. We also found that, unlike rats injected with vehicle, rats given sodium salicylate failed to emit the 22 kHz vocalization; the length of the PEI was unaffected. The results of these two experiments led us to conclude that the vocalization is influenced by the thermoregulatory state of the animal.

Nonetheless, we knew that postejaculatory brain cooling can occur in the absence of the 22 kHz vocalization, which suggested that another cooling mechanism must be involved. Indeed, further experimentation led us to conclude that selective brain cooling following ejaculation is effected primarily by vasomotor changes within the nasal mucosa rather than by the respiratory maneuver underlying the 22 kHz vocalization (16). This conclusion led us to consider this respiratory maneuver as, at best, an auxiliary mechanism for brain cooling. Such a conclusion, however, did not seem consistent with our findings that changes in physiological temperature (via PGE_2 and salicylate) profoundly affect the emission of the vocalization. Oddly enough, the resolution of this problem was not apparent until the physiological basis of ultrasound production in rat pups had been investigated.

COMMUNICATORY AND SENSORY INVESTIGATIONS OF THE 40 KHZ VOCALIZATION

Rat pups, like the young of many rodent species, emit ultrasound when removed from the nest and exposed to cold (41); consequently, the vocalizations are often referred to as "distress calls." The concurrence of ultrasound production and isolation from the nest prompted investigators to hypothesize that the vocalization attracts the mother and promotes the retrieval of the pup to the warm nest. This hypothesis has found support in rats (3,53) as well as in other rodent species (19,52). Moreover, ultrasound production by rat pups in a litter also affects maternal behavior by stimulating her to return more quickly to the nest (32). Pup ultrasounds may also elicit pup transport following nest disturbance, although such ultrasound is not necessary to elicit nest transport (18).

Although pup ultrasounds are often considered to result from "social isolation," the primary stimulus for ultrasound production is cold exposure. [Tactile stimulation can also elicit ultrasound production, especially in the very young rodent (42). The degree of specificity of this response is difficult to determine. For example, it has been argued that demonstrations of ultrasound production following tactile stimulation typically involve some degree of cold exposure (11); In addition, there are indications that tactile stimulation can have profound effects on the physiological state of the animal. For example, as has been demonstrated in the very young mouse during cold exposure, tactile stimulation elicits shivering (8). It should also be noted that "contact comfort" can modify ultrasound production by rat pups (30). Again, it is important to stress that these experiments invariably entail some degree of cold exposure so that one can only conclude that sensory processes may modulate the stimulatory effect of cold exposure on ultrasound production. Furthermore, the thermal and nonthermal aspects of "contact comfort" have not been disentangled adequately. In sum, we agree with Allin and Banks (2) who write that "nonthermal stimuli associated with the isolation condition are relatively ineffective in eliciting ultrasound production.''] Pups removed from the nest but maintained at a temperature typical of that in the nest (approximately 35°C) do not emit ultrasound; ultrasound is emitted, however, when ambient temperature is reduced (2,13). The ultrasonic response of a rat pup to cold exposure is related to the pup's thermoregulatory abilities; ultrasound production in response to cold diminishes as the pup develops the ability to maintain body temperature in the cold (43), which is achieved around three weeks postpartum.

The emission of ultrasound, therefore, has been interpreted as an adapted response to the pup's limited thermoregulatory abilities in the first three weeks postpartum. That is, because the rat pup is incapable of maintaining body temperature outside of the nest, it uses a behavioral mechanism (i.e., communication via ultrasound emission) to compensate for its physiological inadequacies. Once the pup's thermoregulatory abilities mature, this behavioral mechanism is no longer required and ultrasound production ceases in response to cold exposure.

This adaptive story, however, suffers from numerous inconsistencies which appear to have been overlooked. For example, maximal ultrasound production in response to cold occurs after the first week postpartum, an anomalous finding given that pup mortality is highest during the first week postpartum (20). Furthermore, prior to the attainment of homeothermy, rat pups are capable of slowing their rate of cooling in the cold as well as maintaining a steady, albeit low, body temperature [(54); Blumberg and Alberts, unpublished observations]. It is not accurate, therefore, to suggest that rat pups exposed to cold rely exclusively, or even predominantly, for their survival on ultrasound production. It seemed that we needed an understanding of the interaction of ultrasound production with the other physiological responses of the rat pup to cold exposure before our understanding of this phenomenon could be complete.

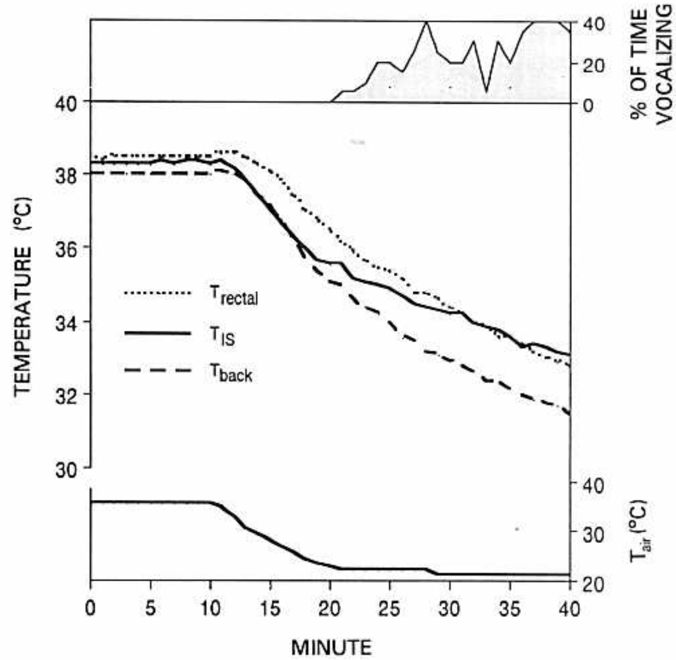

FIG. 3. Percentage of time vocalizing, rectal temperature (T_{rectal}), interscapular temperature (T_{IS}), back temperature (T_{back}), and chamber-air temperature (T_{air}), for a 10-day-old rat pup. Note that the deviation of T_{IS} from T_{back} indicates the initiation of heat production by brown adipose tissue. This deviation occurs contemporaneously with the initiation of ultrasound production. [Adapted from (13)].

PHYSIOLOGICAL INVESTIGATIONS OF THE 40 KHZ VOCALIZATION

Isolated rat pups respond to cold exposure physiologically by increasing metabolic heat production and behaviorally by emitting ultrasound. Although both of these phenomena have been well studied, each phenomenon has been investigated separately by researchers in different fields and with different interests. We suspected that metabolic heat production and ultrasound production were not autonomous responses and we sought, therefore, to determine the relationship between them.

To do this, we monitored oxygen consumption, respiration, and ultrasound production before and after cold exposure in rats 10–12 days of age (13). In addition, we measured heat production by brown adipose tissue (BAT), a thermogenic organ located, among other places, just beneath the skin in the interscapular region of the rat. BAT is responsible for a large proportion of nonshivering thermogenesis (NST) during cold exposure; in turn, NST is responsible for most, if not all, of the increase in oxygen consumption during cold exposure (31).

We found that when a pup was isolated from the nest but maintained at nest temperature (i.e., approximately 35°C), it exhibited a low metabolic rate and emitted virtually no ultrasounds. This was consistent with the findings of others [e.g., (2)]. When exposed to cold temperatures, however, the pup initiated ultrasound production contemporaneously with NST; oxygen consumption and respiratory rate also increased at this time.

Figure 3 depicts the interrelation between NST and ultrasound production. It shows rectal temperature, interscapular temperature, back temperature, air temperature, and ultrasound production for a 10-day-old pup. When air temperature was stable at 35°C, all physiological temperatures (as well as oxygen consumption) were stable. As air temperature was decreased to 22°C, all three physiological temperatures began to decrease as well. Initially, these three temperatures decreased at about the same rate; then, at Minute 20, BAT began producing heat, as indicated by the abrupt change in the cooling rate of the interscapular region. This initiation of NST began contemporaneously with the onset of ultrasound production.

Observations such as these led us to conclude that ultrasound production by rat pups is but one component of an integrated response to cold exposure. As discussed above, ultrasound production is widely considered to be a communicatory behavior that elicits maternal retrieval to the warm nest. We were led, however, to consider an alternative explanation for ultrasound production: specifically, we wondered *whether ultrasound might be the acoustic by-product of a respiratory maneuver that enhances oxygen diffusion in the lungs during times of increased oxygen consumption*. Making clear the rationale for this hypothesis, however, requires a description of the ultrasound production mechanism.

THE ULTRASOUND PRODUCTION MECHANISM

The hypothesis that both the 40 kHz and 22 kHz vocalizations are by-products of a respiratory maneuver requires that the two vocalizations be produced by identical mechanisms. This appears to be the case, although the reader may immediately be struck by the different characteristic frequencies of the two vocalizations. The frequency difference can be accounted for by the sizes of the adult and pup laryngeal and tracheal apparatuses: larger instruments produce lower frequencies, as anyone who has seen and heard a pipe organ will recognize. (One reviewer aptly noted that adult rats can vocalize at frequencies higher than

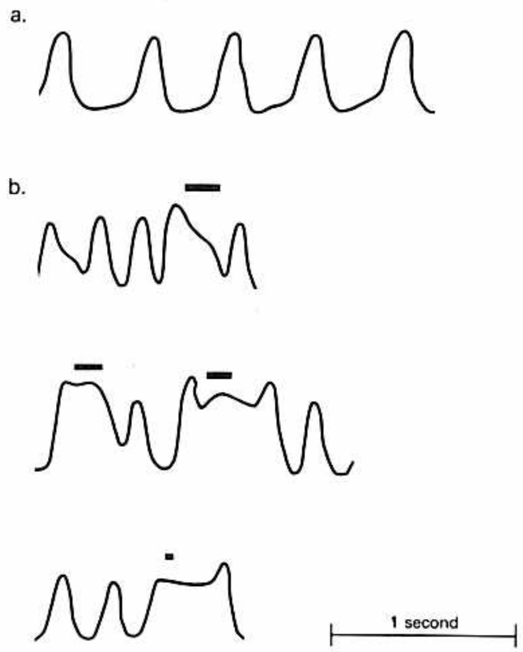

FIG. 4. Respiratory movements as measured by strain gauge plethysmography in a 12-day-old rat pup (inspirations upward). (a) Regular, sinusoidal respiratory movements typical of a calm pup when $T_{air} = 35°C$. (b) Respiratory movements of the same pup after chamber-air temperature has dropped to 18.5°C. Respiratory movements indicative of laryngeal braking are 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. [Adapted from (13)].

22 kHz [i.e., 50–70 kHz; e.g., (51, 57, 59)]. These vocalizations have been detected during [rather than after] painful electric shock, during overt aggressive encounters with conspecifics, and during precopulatory activities. They are emitted as short pulses and often occur in synchrony with strenuous locomotor activity. In contrast, the adult vocalizations we are addressing in this paper are "long calls" produced by relatively immobile animals and which, we are arguing, are linked to a specific respiratory maneuver [i.e., laryngeal braking]. We suspect, therefore, that the 50–70 kHz "short call" has a different production mechanism than either the 22 kHz "long call" or the 40 kHz "distress call.")

Nearly all of our understanding of sound production by rodents (both ultrasonic and audible emissions) is due to the work of L. H. Roberts (44–47). Roberts first showed, by monitoring air flow from the nostrils of rodent pups, that ultrasonic pulses occur during the initial phase of expiration (44). Expiratory duration is prolonged because of laryngeal constriction, air flow is reduced, and intrathoracic pressure increases [Fig. 4; (13,44)]. Sound emission occurs as air passes through the two constricted plates of the larynx, similar to a bird whistle (46). By sectioning the laryngeal nerves of pups, Roberts also showed that the emission of ultrasonic pulses requires the integrity of the larynx (45).

Roberts also investigated the 22 kHz vocalization of adult rats. As with pup ultrasound, the 22 kHz vocalization was found to be associated with expiration (44). The 22 kHz vocalization also resembles pup ultrasound in that it is characterized by prolonged expiratory duration (Blumberg, unpublished observations). Furthermore, sectioning the laryngeal nerves eradicated the adult vocalization, just as it had pup ultrasound. Based on these observations, it seems reasonable to conclude that the 22 kHz vocalization of adult rats and the 40 kHz vocalization of rat pups are produced by identical mechanisms.

IS THE 40 KHZ VOCALIZATION AN ACOUSTIC BY-PRODUCT OF LARYNGEAL BRAKING?

As we have seen, ultrasonic pulses are produced by the forced expulsion of air through a constricted larynx, resulting in the generation of high pressures within the thorax and the prolongation of expiratory duration. Interestingly, these features of ultrasound production are also the distinguishing characteristics of a mechanism known to respiratory physiologists as *expiratory* or *laryngeal braking* (22, 23, 25). Laryngeal braking, ubiquitous among mammalian neonates (39), improves gas exchange in the lungs by elevating end-expiratory lung volume and by preventing the collapse of respiratory pathways (22,23). Laryngeal braking has also been found in adult animals [e.g., dogs (17)].

Possible connections among ultrasound production, laryngeal braking, and the physiological responses of the rat pup to cold exposure emerge when one considers the human infant suffering from respiratory distress syndrome (RDS). One symptom of this disease involves the emission of an audible "grunt," a behavior that is initiated or exacerbated by the exposure of these infants to cold. Investigations of this grunting behavior show that it is caused by laryngeal braking; interference with laryngeal braking causes the infant to become cyanotic (29). It should be noted that nondiseased human infants also exhibit laryngeal braking, with or without the grunting sound (34, 35, 38).

The similarities between ultrasound production by rat pups and grunting by human infants are striking. Both are produced by the expulsion of air through a constricted larynx, resulting in prolonged expiratory duration and an increase in intrathoracic pressure. Both occur under conditions of cold exposure, when oxygen consumption increases and the oxygen stores of the rat pup and infant decrease. Thus, we hypothesized that pup ultrasound, like infant grunting, is the acoustic by-product of laryngeal braking (13). We have since gathered further support for this hypothesis (12).

EXTENSION OF THE "LARYNGEAL BRAKING HYPOTHESIS" TO THE 22 KHZ VOCALIZATION

The similarities between infant grunting and pup ultrasound production, and the similarities between adult and pup ultrasound, lead to the further question of whether the 22 kHz vocalization can be given a similar physiological interpretation as the 40 kHz vocalization. To answer this question we return to the experiment, described earlier, in which PGE_2 was administered to solitary rats in order to raise hypothalamic temperature (15). It will be recalled that the increase in temperature was accompanied by the emission of the 22 kHz vocalization, a result that seemed to support our brain-cooling hypothesis. But, in many respects, the response of the adult rat to the administration of PGE_2 is similar to the response of the rat pup to cold exposure.

To make this argument clear, it is first necessary to review the physiological effects of drugs, such as PGE_2, that increase physiological temperature by inducing a febrile state. The induction of a febrile state is characterized by the coordinated and regulated activation of *heat-gain* mechanisms; in contrast, nonfebrile hyperthermias (e.g., exercise) are accompanied by the activation of *heat-loss* mechanisms (55). Thus, the increase in physiological temperature that follows the administration of PGE_2 is due to the coordinated activation of heat-gain mechanisms such as peripheral vasoconstriction (to promote heat retention),

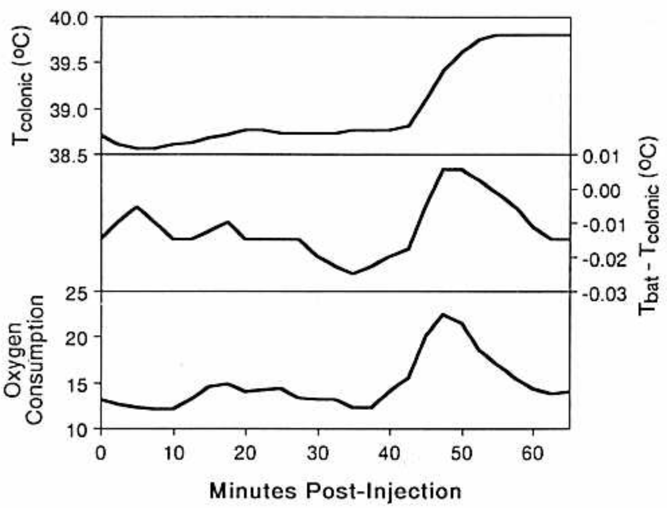

FIG. 5. Colonic temperature ($T_{colonic}$), brown adipose tissue temperature minus colonic temperature ($T_{bat}-T_{colonic}$), and oxygen consumption (ml/dm^2/min) following an intraperitoneal injection of 20 μg/kg endotoxin in a single adult rat. $T_{bat}-T_{colonic}$ provides an indication of the magnitude of nonshivering thermogenesis. [Adapted from (56)].

shivering, and nonshivering thermogenesis. Because shivering and nonshivering thermogenesis are oxygen-dependent, it is not surprising that oxygen consumption also increases during the induction of fever (37,56).

We can now reconsider the experiment in which PGE_2 was administered to the isolated rat, depicted in Fig. 1. Following the administration of PGE_2, the rat's hypothalamic temperature began to increase. Soon after, the rat began emitting the 22 kHz vocalization, and vocalizing continued until hypothalamic temperature reached its peak value. At that time, ultrasound production decreased rapidly and hypothalamic temperature decreased very slowly.

When the temporal pattern of ultrasound production following PGE_2 administration is compared with that of heat-gain mechanisms following the administration of fever-causing substances, striking parallels are found. For example, Szekely and Szelenyi (56) injected adult rats with endotoxin (a fever-causing substance) and monitored simultaneously body temperature, oxygen consumption and NST by brown fat (Fig. 5). They found that both brown fat thermogenesis and oxygen consumption increased as body temperature increased; once body temperature reached its peak value, brown fat thermogenesis and oxygen consumption decreased rapidly as body temperature decreased slowly. Similarly, Malkinson and colleagues (37) infused adult rats with PGE_1 (a drug similar in structure and effect to PGE_2) and found that shivering activity and oxygen consumption increased as body temperature increased; once body temperature reached its peak value, shivering activity declined rapidly as body temperature and oxygen consumption decreased more slowly. Finally, fever induction in young rabbits was accompanied by increased blood flow to brown adipose tissue and to skeletal muscles (which are responsible for shivering); once the fever reached its peak, blood flow returned to normal levels (28).

It is clear, therefore, that during the induction of fever, the 22 kHz vocalization is emitted in exactly the same pattern as are heat-gain mechanisms such as shivering and nonshivering thermogenesis. There is, however, more direct evidence in support of the hypothesis that the 22 kHz vocalization is the acoustic by-product of laryngeal braking. It was recently reported (33) that newborn and 30-day-old lambs exhibit laryngeal braking during fever. Specifically, laryngeal braking occurs as the lamb's brain temperature is increasing and ceases when brain temperature reaches its peak, a pattern with which we are now familiar. Moreover, audible grunts have been detected from lambs during laryngeal braking (Paul Johnson and David Andrews, personal communication). We suggest, therefore, that just as laryngeal braking may aid the rat pup during cold-induced thermogenesis and increased oxygen consumption (and incidentally result in sound production), so may laryngeal braking aid the adult rat during fever-induced thermogenesis and increased oxygen consumption (and also incidentally result in sound production).

If the 22 kHz vocalization, like the 40 kHz vocalization, is a by-product of a respiratory mechanism (i.e., laryngeal braking) that improves gas-exchange in the lungs, then it follows that the social contexts in which the 22 kHz vocalization is detected might entail some degree of increased oxygen need; such a need could arise from physical or behavioral arousal and the increases in body temperature, heat production, and oxygen consumption that result from them. We have already seen that copulating male rats experience a significant increase in body temperature and, presumably, an increase in oxygen consumption as well (14). Similarly, Andrews (5,6) found that brief agonistic encounters between montane voles (*Microtus montanus*) or deer mice (*Peromyscus maniculatus*) result in increases in core temperature of 1°C or more. Andrews has further shown that the hyperthermic response of voles is due to adrenergic activation that results in both heat conservation and metabolic heat production. Results such as these make it clear that social encounters have pronounced physiological effects, including metabolic heat production, increased oxygen consumption, and stimulation of the respiratory system. Determining the exact relationship between vocalization rates (or laryngeal braking) and autonomic arousal during social encounters remains for further research.

Finally, it will be recalled that we performed an experiment complementary to that showing that PGE_2 stimulates ultrasound production [Fig. 2; (15)]. Specifically, we injected a male rat with sodium salicylate and, after the expected decrease in hypothalamic temperature was attained, we introduced an estrous female, observed copulatory behavior, and monitored the emission of the 22 kHz vocalization. We found that, in addition to reducing hypothalamic temperature, pretreatment with sodium salicylate virtually eradicated the postejaculatory 22 kHz vocalization without affecting the length of the postejaculatory interval. Given the likelihood that sodium salicylate reduces temperature at least partly by reducing metabolic rate (27), it is possible that the animals given sodium salicylate had lower metabolic rates at ejaculation than the animals given the control injection. Therefore, the eradication of the postejaculatory 22 kHz vocalization seen with sodium salicylate is consistent with the hypothesis that the vocalization is the acoustic by-product of a respiratory mechanism that enhances oxygen uptake during times of increased oxygen consumption.

CONCLUSIONS

From a purely contextual standpoint, the 22 kHz vocalization of adult rats seems to have little in common with the 40 kHz vocalization of rat pups. The 22 kHz vocalization is primarily associated with the postejaculatory or subordinate male while the 40 kHz vocalization is emitted by an isolated pup during cold exposure. It is not surprising, therefore, that the two vocalizations have been considered separate entities with dramatically different communication functions.

On the other hand, it has been known for 15 years that the production mechanisms underlying both vocalizations are similar, and we suggest that they may be homologous. Both vocalizations require an intact larynx, both are produced by expiring against a closed glottis, and both result in an increase in expiratory duration. To these similarities can now be added the association of both vocalizations with the increases in oxygen consumption that result from activation of heat-gain mechanisms (during cold exposure in pups, during fever induction in adults).

In an early critique of research into rodent ultrasound, Bell (11) argued that explanations of the meanings of these vocalizations "appear to be overstated in terms of the available behavioral data." Since 1974, the behavioral data have failed to yield more conclusive results. Nevertheless, conventional wisdom continues to maintain that these vocalizations are emitted in order to communicate.

Bell (11) suggested a reinterpretation of infant ultrasounds as reflections of general arousal in the sender and a similar, elicited arousal in the receiver. He concluded by calling for a research program "involving the study of the interactive consequences of ultrasonic signaling in combination with other organismic, stimulus, and experiential factors." As Bell might have predicted, research into the physiological bases of rat ultrasound production has uncovered relationships that do not follow from a purely functional perspective. Furthermore, the 22 kHz and 40 kHz vocalizations can be incorporated into a single framework: the emission of both vocalizations may reflect an animal that is in the process of enhancing gas-exchange in the lungs via laryngeal braking.

Because the 22 kHz and 40 kHz vocalizations may reflect a common physiological state and self-regulatory maneuver, it may be inferred that these vocalizations communicate similar kinds of information. This inference raises the following question: is the information being signalled by the animals of use to conspecifics? In the case of the pup, the answer is yes; the pup's mother, upon detecting the 40 kHz vocalization, can increase her reproductive fitness by retrieving the pup to the nest. In the case of the adult, however, the answer is maybe; there is, as yet, no evidence that conspecifics attend and respond to the 22 kHz vocalization. Furthermore, it is not clear what the potential value of the 22 kHz vocalization is nor how a conspecific could use the message inherent in the vocalization to improve its reproductive success. Perhaps this accounts for the continued difficulty in determining its communicatory significance?

Even though the communicatory significance of the 40 kHz vocalization is well established, we cannot consider this vocalization as an adaptation: to do so is to confuse current utility with historical origin (26). By this argument, the larynx is adapted for the control of respiration (40), while the 40 kHz vocalization is a nonadapted consequence of laryngeal braking, as may be the 22 kHz vocalization. Gould and Vrba suggest that such nonadapted but useful characters be called exaptations to distinguish them from those characters that have been directly shaped by natural selection, i.e., adaptations. Thus, the 40 kHz vocalization can be viewed as an exaptation for maternal retrieval. The 22 kHz vocalization, however, is a nonadapted consequence of laryngeal braking for which we cannot assign a use. It is, perhaps, an exaptation in waiting—whether it is waiting to acquire a value or waiting for us to discover its value may be the next analytic challenge.

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