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Strain Differences in the Ultrasonic Behavior of Rats (*Rattus norvegicus*)

GILLIAN D. SALES

Department of Zoology, University of London King's College, London WC2R 2LS

SYNOPSIS. Wistar rat pups are generally more "vocal" than Lister pups; the ultrasonic calls that they produce are usually louder and emitted at a greater rate than those of Lister pups of the same age and studied under similar conditions of isolation or handling. These differences in ultrasonic behavior are apparently not related to differences in the development of homiothermy which follows a similar course in both strains. The calls of infants of the two strains do not show any marked differences in duration or frequency pattern that are consistent over age or treatment condition, but the Wistar pups appear to show greater changes with age than the Lister pups in certain parameters of the calls, while the latter strain show greater changes with treatment condition.

Similar strain differences in the rate and intensity of calling were seen during aggressive encounters between adult males, but these cannot be related to differences in aggressive behavior. The strains differ in the duration of both the long "22 kHz" calls (Lister greater than Wistar) and the shorter "50 kHz" calls (Wistar greater than Lister), as well as in the minimum frequency of these shorter calls (lower in Wistar than in Lister rats).

INTRODUCTION

It is now well established that both infants and adults of a wide variety of murid and cricetid rodents emit ultrasonic calls in various situations (*e.g.*, Colvin, 1973; Sales and Pye, 1974; Watts, 1975; Sales and Smith, 1978). The calls of the young are believed to be important in mother-infant interactions and there is evidence that they affect parental behavior such as searching and retrieving (Sewell, 1970; Allin and Banks, 1971; Colvin, 1973; Smotherman *et al.*, 1974; Smith, 1976), nest building (Noirot, 1974) and also play a role in the "early handling" phenomenon, the effect of infantile stimulation on subsequent development, which is possibly mediated by differential mothering towards stimulated as opposed to non-stimulated pups. Among adults the calls are believed to be important in mating behavior in many species (Sales, 1972*a*; Whitney *et al.*, 1973; Barfield and Geyer, 1975; Floody, 1977), in aggressive interactions in some species (Sales, 1972*b*) and in group interactions in woodmice, *Apodemus sylvaticus* and yellow-necked mice, *A. flavicollis* (Hoffmeyer and Sales, 1977). Indeed there is now good evidence that the mating calls of rats, *Rattus norvegicus*, and hamsters, *Mesocricetus auratus* serve to enhance subsequent mating behavior (Floody, 1977; McIntosh *et al.*, 1978) and circumstantial evidence that the "22 kHz" calls emitted by defeated rats affect the aggressive behavior of dominant animals (Sales, 1972*b*; Lore *et al.*, 1976).

It is still not exactly clear, however, what the precise roles of the various calls may be and more information is needed both on the causation of ultrasound emission, at a physiological as well as at an overt behavioral level, and on the effect these calls have on the behavior of other animals. Comparative studies can be important here; similarities in the ultrasonic behavior of different species and strains could indicate similar functions or motivating factors. For example, it has been shown that the calls of a wide variety of newborn murid rodents are similar in generally consisting of a single frequency component, which shows a gradual onset and cessation and relatively little frequency modulation over the duration of the call. Such calls probably

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function under similar circumstances, that is, in or near the nest, as they would not travel far and would not be easily localized by animals away from the nest (Sales and Smith, 1978). On the other hand, information on the motivation and function of the calls may also be gained by assessing any species or strain differences in acoustic behavior and then investigating whether such differences are associated with other differences in behavior, for example, in aggressive or parental behavior, or with differences in physiological mechanisms. Such an approach was successfully applied by Okon (1971, 1972, 1973) during a study of factors motivating ultrasound emission in rodent pups. Okon showed that in species such as mice, *Mus musculus,* in which homoiothermy was achieved within 21 days after birth, ultrasonic calling by isolated pups ceased by this age, but where the development of homoiothermy was not achieved until a later age, as in woodmice, ultrasonic calling by isolated pups was correspondingly prolonged. This was evidence that the calls could be associated with the development of thermoregulation.

A similar approach was suggested by Nitschke *et al.* (1975) as a means of assessing the role of neonatal ultrasound in mediating the early experience effects in rats and indirectly has provided evidence that strain differences in the ultrasonic behavior of infant mice may be related to differences in the parental behavior elicited by the pups. Thus Ressler (1962) found that BALB/c pups elicited more maternal behavior from mothers both of their own strain and of the C_{57}/BL/10 strains and this correlates well with the finding of Bell *et al.* (1972) that infant BALB/cJ mice call at a higher rate than infants of the C_{57}/BL/6J line.

The ultrasonic behavior of rats is of particular interest as rats are now becoming widely used in studies of ultrasonic behavior in both young and adults, yet strain differences do not appear to have received as much attention in rats as they have done in mice (*e.g.*, Bell *et al.*, 1972; Zachman *et al.*, 1972; Sales and Smith, 1978). In addition rats so far appear to be unique in being the only species which have been found to emit two distinct types of call during mating and aggression (see below). As argued above a comparative approach could help to elucidate the functions of both of these calls as well as those of the young, or such an approach could perhaps at least indicate useful lines of further study.

ULTRASONIC BEHAVIOR OF INFANT RATS

The first detailed report of the emission of ultrasound by rat pups was by Noirot (1968) who studied the rate of calling by pups of a mixed albino strain when isolated individually at room temperature for five minutes daily from birth to 27 days of age. Since then there have been a number of studies on single strains, generally Wistar (W) or Sprague-Dawley (S-D), investigating the effects on calling of variables such as ambient temperature (Okon, 1971 (W); Allin and Banks, 1971 (W); Sales and Smith, 1978 (W and W/Lister), tactile stimulation (Okon, 1972 (W); Oswalt and Meier, 1975 (S-D); Sales and Smith, 1978 (W and W/Lister)) and nutritional state (Hunt *et al.*, 1976 (S-D)). It appears that so far only one publication, by Nitschke *et al.* (1975), includes comparable studies on ultrasound emission by pups of more than one strain. This study also attempts to relate strain differences in calling to parental behavior.

The studies on single strains of rats differ in the details of the methods used; for example, in the time of exposure to different ambient temperatures and, more importantly, in the means of measuring ultrasound emission. Differences in such methods can markedly affect the results, particularly for the rate of calling (Sales and Smith, 1978) and indeed the studies mentioned above do differ considerably in the maximum rates of calling (when all results are computed to rate per pup per minute) and also in the age at which the peak rate of calling occurs under any particular condition. But there is one feature that appears to be relatively consistent between different studies on the same strain and this is the differential response of the pups to isolation and to handling. Sales and Smith (1978) tape-recorded the calls emitted by individual Wistar pups when first isolated at room temperature (22°C) for 30 sec, then handled for 30 sec, by picking the pups up by the finger and thumb or rolling them on their backs. The pups were studied from day 3 to day 23, and up to day 10–11 the pups called at a greater rate during isolation then during handling. After this age the calls emitted during handling were produced at a greater rate than the "isolation" calls. Okon (1971) studied the ultrasonic behavior of Wistar pups isolated individually for 1 hr at three ambient temperatures, 2–3C°, 12°C or 22°C. He recorded both the sound pressure level of the calls (SPL; given here and throughout the text as dB re 2×10^{-5} N/m^2) measured after 10 min isolation, and the rate, measured from an oscilloscope screen between the 5th and 10th min of exposure. He also recorded the SPL of calls produced by further litters when handled by holding and lifting the pups loosely between two fingers. If Okon's results for the SPL of the calls of pups isolated at 22°C are superimposed on those of pups subjected to handling, then these too show that the isolated pups respond more vigorously, with louder calls, than handled pups up to day 10–11; but after this age the reverse is true: the calls emitted by the handled pups are louder then those of isolated pups. This similarity between the two studies could be coincidence, but a similar phenomenon can also be seen in the results of Nitschke *et al.* (1975) for Wistar/Furth rats and in work conducted in this laboratory on Wistar rats. Both of these last two studies have also involved two different strains.

Nitschke *et al.* (1975) studied the ultrasonic behavior of Sprague-Dawley and Wistar/Furth pups under similar, but not identical, conditions of cold stress. The pups were first exposed to 2–3°C either individually in a glass beaker in ice (Sprague-Dawley) or within litters in a refrigerator (Wistar/Furth). The ultrasonic calls of individual pups were then tape-recorded at room temperature (23–24°C) over three 10-sec intervals at 0–10, 60–70 and 300–310 sec. Additional Wistar/Furth litters were studied in a similar way but after handling, when each pup was dye marked before ultrasound emission was monitored as before. Litters were studied for seven consecutive days in either the first, second or third weeks after birth, and the tape recordings analyzed both for the rate of calling and using a sound spectrogram to determine the durations and frequency patterns of the calls.

The results of Nitschke *et al.* (1975) for the ultrasonic responses of Wistar/Furth pups to cold stress and to handling show that up to days 9–11 cold stressed pups called at a greater rate than handled pups, but in this study too, after this age, the handled pups called more (Nitschke *et al.*, 1975, Fig 2). The authors found that there was no significant difference in the mean peak frequency of the calls produced by cold stressed or by handled pups of this strain, nor was there any difference between the treatment conditions in the complexity of the frequency patterns (that is in the extent and number of frequency changes within each call. However, the cold stressed pups emitted calls that were significantly longer ($\overline{X}$ = 94 ms) than those of handled pups ($\overline{X}$ = 68 ms). Changes with age occurred in the mean peak frequency of the calls which was higher in the first week ($\overline{X}$ = 50.9 kHz) than in the second week ($\overline{X}$ = 43.3 kHz), but not in the duration or in the complexity of the calls.

The calls of Sprague-Dawley pups, like those of cold stressed Wistar/Furth pups showed no change in duration with age but again there was a significant decrease in the mean peak frequency between the first and second weeks after birth ($\overline{X}$ = 54.8 kHz for the first week, 47.8 kHz for the second week). In this strain there was a change in the complexity of the signals with age; pups of 8–14 days emitted significantly more complex calls than did pups of 1–7 days.

The authors point out that as there were differences in the procedure for chilling the pups as well as in the size of the litters, their ultrasonic behavior cannot be compared directly, but they point out "suggestive" differences between the acoustic behavior of the strains, for example that the Sprague-Dawley pups generally called at a higher rate than the Wistar/Furth pups particularly during the first week; the Wistar/Furth pups showed a peak in the rate of calling at an earlier age (5–10 days) than the Sprague-Dawley pups (about 12 days) and the Sprague-Dawley calls showed higher mean peak frequency, shorter durations and a greater proportion of simple frequency patterns than the calls of Wistar/Furth pups.

Nitschke and his colleagues also briefly studied retrieval behavior in the two strains on the first four days postpartum by placing all the pups in each litter at the far end of the cage away from the nest. They found that in both strains the average time for the mothers to retrieve all the pups tended to decrease with days postpartum and that the Sprague-Dawley mothers retrieved their pups far more rapidly than the Wistar/Furth mothers. This suggests that the retrieval behavior of the mothers is related to the rate of calling by the pups.

In this laboratory we have been investigating strain differences in the ultrasonic behavior of Wistar (albino) and Lister (hooded) rats, both infants and adults, as part of a wider study on the role of ultrasound in mother-infant interactions and also in aggressive encounters between adults (see below). Two studies have been made on infant rats. In the first, G. Donovan observed individual pups of both strains for 15 secs during isolation followed by 15 sec of handling, both after an initial 10 sec period beneath the microphones to overcome any effects of placing the pups there. The SPL of the calls was measured directly from an oscilloscope screen and simultaneously tape-recordings were made for later determination of rate and for sonagraphic analysis (see Sales and Smith, 1978 for methods). The pups were studied daily from days 1–3 to days 17–19 after birth

There was some variation in the rate of calling both within and between litters, a phenomenon also noticed by Noirot (1968). Figure 1 shows mean values averaged across adjacent pairs of days (1 and 2, 3 and 4, etc.) and it can be seen that, in general, the Wistar pups tended to call at a greater rate than the Lister pups during both isolation and handling, particularly over the first 10 days. After this age the difference in the strains was less marked but the rate of calling by Wistar pups in each condition was always greater than that of Lister pups. In the Wistar pups the rate of calling during isolation was greater than that during han-

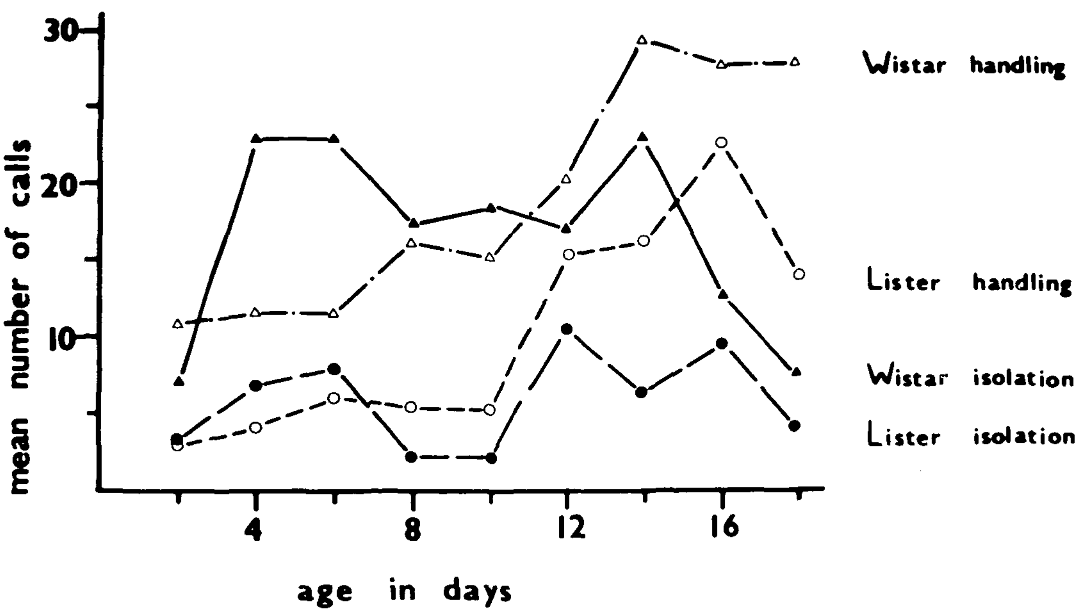

FIG. 1. Changes with age in the mean number of calls per pup emitted over a 15-sec period during isolation and during handling in Wistar and Lister rats. ▲ —▲ Wistar-isolation; △ —·— △ Wistar-handling ● — — ● Lister-isolation; ○ ---- ○ Lister-handling.

dling up until days 10–12, after which, as in the studies mentioned earlier, the handling calls were emitted at a greater rate. In the Lister pups the "crossover point," the age at which handling calls were emitted at a greater rate than isolation calls, occurred earlier—between days 6 and 8.

The strains also differed in the SPL of the calls. Those of Lister rats were generally below the noise level of the equipment (approx. 54 dB SPL). Changes with age in the SPL of the calls of Wistar rats during isolation and handling are shown in Figure 2. Again there is generally a greater acoustic response to isolation than to handling in younger pups, at least from day 6, but here the "crossover point," when handling elicits a greater response than isolation, occurs at 14 days, somewhat later than for the rate of calling.

I later analyzed the tape-recordings made by Donovan on days 6 and 14 during both isolation and handling using a sound spectrogram to determine any differences between the strains in the durations and frequency patterns of the calls and any changes of these with age and with treatment. From the sonographs, the duration of the calls, the maximum and minimum frequencies and also the bandwidth (maximum minus minimum frequency) were measured and the frequency pattern of the call was noted as consisting of slow drifts in frequency or more marked changes such as rapid frequency changes, instantaneous frequency steps, rapid sweeps or frequency fluctuations (see Sales and Smith, 1978).

The calls of both strains were similar in basic structure, generally consisting of a single frequency component, although both strains produced a few calls that showed a second and sometimes a third component appearing for a few milliseconds at the beginning or end of the call, and not related harmonically to the main component. There were some differences between the strains in certain parameters of the calls (see Table 1). During isolation the calls of Wistar pups tended to be longer in duration and lower in frequency than those of similarly aged Lister pups (a similar situation to that indicated by Nitschke *et al.* (1975) for Wistar/Furth and Sprague-Dawley rats). However the differences in the present study were significant only for duration on day 14 and for maximum and minimum frequencies on day 6 (Table 1). There were no significant differences between the strains in any parameters of the calls produced during handling on either day 6 or day 14; neither were there any strain differences in the frequency patterns produced by each

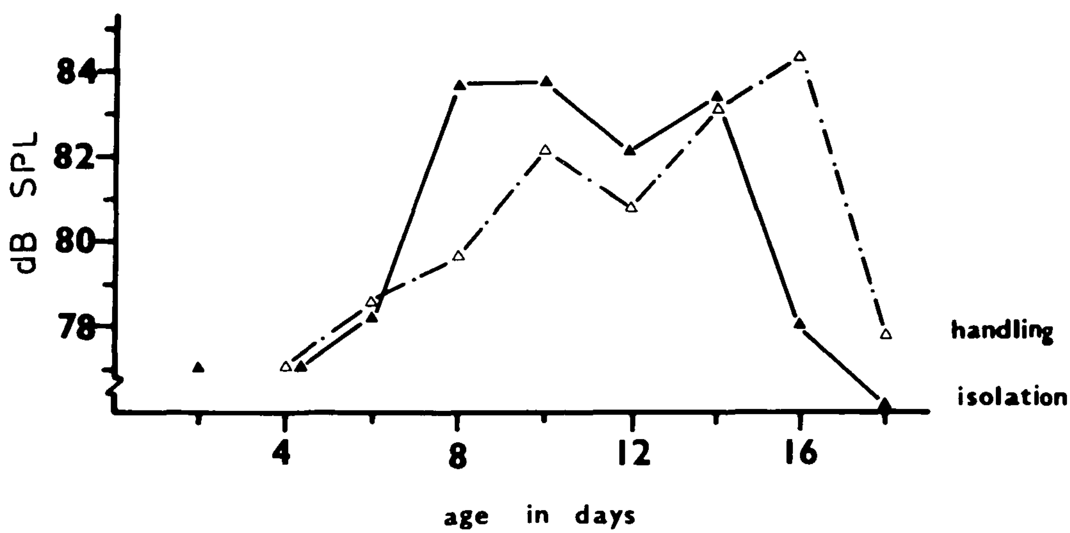

FIG. 2. Changes with age in the sound pressure level (SPL) of the calls of infant Wistar rats. ▲——▲ during isolation; △—·—△ during handling.

TABLE 1. *Comparison of the ultrasonic calls emitted by day 6 and day 14 Lister and Wistar rats during isolation and during handling.*

Age, treatment and parameters		Lister Range mean (SD)	Wistar Range mean (SD)	P^a
Day 6	Isolation (N_L = 26; N_W = 30)[b]			
	Duration (ms)	3–80 41.3 (22.0)	5–120 61.2 (38.2)	NS
	Max. freq. (kHz)	40–68 51.2 (6.8)	40–78 46.1 (8.2)	0.025
	Min. freq. (kHz)	38–62 48.3 (6.2)	38–75 42.2 (7.2)	0.001
	Bandwidth (kHz)	(−)[c]–8 3.0 (2.4)	1–12 4.1 (3.3)	NS
	Handling (N_L = 21; N_W = 26)			
	Duration (ms)	3–120 29.3 (30.2)	2–110 38.5 (27.8)	NS
	Max. freq. (kHz)	45–64 53.6 (5.3)	38–94 52.8 (11.0)	NS
	Min. freq. (kHz)	43–59 50.5 (6.5)	36–84 49.3 (10.4)	NS
	Bandwidth (kHz)	1–9 3.6 (2.7)	1–8 3.3 (2.3)	NS
Day 14	Isolation (N_L = 23; N_W = 21)			
	Duration (ms)	5–135 21.6 (22.5)	2–110 62.5 (42.3)	0.001
	Max. freq. (kHz)	40–78 61.6 (12.6)	38–74 49.1 (11.2)	NS
	Min. freq. (kHz)	18–70 52.0 (16.8)	30–72 43.4 (10.1)	NS
	Bandwidth (kHz)	2–38 9.3 (9.4)	2–28[d] 6.4 (5.4)	NS
Day 14	Handling (N_L = 24; N_W = 32)			
	Duration (ms)	3–105 34.6 (32.2)	4–167 42.7 (41.8)	NS
	Max. freq. (kHz)	40–102 61.4 (16.1)	40–96 59.6 (10.4)	NS
	Min. freq. (kHz)	34–90 51.7 (16.1)	36–67 54.0 (8.3)	NS
	Bandwidth (kHz)	(−)[c]–40 10.8 (11.0)	(−)[c]–44 6.2 (8.4)	NS

[a] Ranges, mean values and standard deviations (SD) are given for raw data. An analysis of variance was used to compare the various parameters of the calls in the two strains, but because of the apparent skewness of the data, all scores were transformed by the formula $(x + 0.5)^{\frac{1}{2}}$ prior to analysis.

[b] N_L, N_W are the number of calls analyzed for Lister and Wistar rats respectively.

[c] Bandwidth was unmeasurable, the frequency appeared to be absolutely constant for the whole duration of the pulse.

[d] Only one out of 21 calls had a bandwidth greater than 10 kHz and this was 28 kHz. If this point is omitted, the mean and standard deviation become 5.3 (2.3), but the difference between the two strains is still insignificant.

age during isolation or handling.

The strains differed more in the changes in their own calls with treatment or with age. In general the Wistar pups showed more marked changes on handling while the Lister pups showed greater changes with age. Thus on day 6 the calls of Wistar pups when handled were shorter in duration and higher in both maximum and minimum frequency than the calls produced by this strain during isolation (F = 5.94, P = 0.01; F = 6.4, P = 0.025; F = 10.4, P = 0.005 respectively). The same trends were seen on day 14 but only the increase in maximum frequency on handling was significant (F = 14.9, P = 0.001).

These results are comparable to those of Nitschke *et al.* (see above). The Lister pups showed little difference in frequency between calls emitted during isolation and during handling. The latter tended to be shorter in duration but the difference was not significant.

Both strains showed changes in frequency with age, the calls of day 14 pups being higher in frequency and having a greater bandwidth than those of day 6 pups. These differences were significant for the maximum frequency of both strains during handling (for maximum frequency $F = 4.16$, $P = 0.05$ in Lister pups; $F = 5.9$, $P = 0.025$ in Wistar pups. For bandwidth $F = 8.2$, $P = 0.005$ in Lister pups; $F = 5.07$, $P = 0.005$ in Wistar pups). In addition the calls of older Lister pups during isolation also showed a greater bandwidth and were shorter in duration than the isolation calls of younger pups ($F = 11.8$, $P < 0.005$ for bandwidth; $F = 13.6$, $P < 0.001$ for duration). This is in marked contrast to the study of Nitschke *et al.*, who showed that the mean peak frequency of both Wistar/Furth and Sprague-Dawley strains decreased with age. The day 14 Lister pups when handled produced significantly more calls with marked changes in frequency than did handled younger pups ($X^2 = 4.6$, $P < 0.05$). There was no such significant difference in the frequency patterns of the calls at different ages in Wistar pups on handling or in pups of either strain during isolation. In this respect these results are very similar to those of Nitschke *et al.* (see above).

It appears then that while Wistar pups show more marked changes in the structure of calls between the isolation and handling treatments, the Lister pups show greater changes in acoustic parameters with age. However, this could be a result of the very restricted ages sampled and an analysis spread over greater age ranges would be needed to confirm this.

In the first study the pups were observed for only a short time, 15 sec in each treatment condition. It was possible that Lister pups responded acoustically to various conditions with a greater latency than Wistar pups and so did not reach maximum rates of response within the brief observation period. This possibility was taken into account in a pilot study by R. Roberts on the development of homoiothermy and ultrasound emission in the two strains. In this study three litters each of Wistar and Lister pups were subjected to one hour of exposure to temperatures of 2–3°C, 12°C and 22°C on 2 to 6 different occasions up to day 16 after birth. Body temperatures were measured using copper/constantan thermocouples and the pups were put together in an artificial nest, a glass bowl lined with a tissue. They were placed in groups of two or three in a constant temperature cabinet and the number of ultrasonic calls emitted during each five-minute period was noted throughout the hour-long exposure, together with the body temperature of each pup at the end of each five-minute period. As the litters were small and were observed irregularly, the results must be considered tentative.

As in the previous study, the Wistar pups generally showed greater acoustic responsiveness than the Lister pups (Fig. 3) and this was particularly marked at 3°C. However, there were no significant differences in the final body temperatures of the two strains at any of the three ambient temperatures. There was also little difference between the strains in the rate of fall of body temperature during exposure; the only significant differences occurred between the 40th and 50th minutes of exposure of day 10 pups at 3°C. An examination of the rate of calling throughout the hour-long exposure period at different ages and ambient temperatures showed that there was no consistent difference between the strains in the temporal pattern of calling; neither was there any evidence that the Lister pups either began calling after a longer latency or ceased calling earlier than the Wistar pups. In some cases, particularly in younger pups at 3°C, both strains ceased calling after 40–45 min, with the Lister pups showing a higher rate of calling initially. In most cases, however, the Lister pups called at a fairly constant rate throughout the exposure period at a low level while the Wistar pups either showed a marked peak of calling after a certain period or else showed more constant rates, but generally higher than those of Lister pups.

It appears that Lister pups generally show lower acoustic responses than Wistar pups to stimuli such as cold stress and handling and this is reflected in the intensity and probably also in the rate of calling. It is, of course, possible that the lower recorded rates merely reflect the lower intensity of the calls in Lister pups; more of these calls being below the noise level of the detecting equipment than those of Wistar rats. This possibility cannot be ruled out. However the QMC Instruments Bat Detector used in this study has a sensitivity down to 10–20 dB SPL and although rats are certainly more sensitive than this at some frequencies (*e.g.*, Kelly and Masterton, 1977) it seems unlikely that such calls would be of use at anything but extremely close range.

ULTRASONIC BEHAVIOR OF ADULT RATS DURING AGGRESSION

As mentioned above, rats so far appear to be the only species which have been found to emit two distinct types of call during aggressive behavior. These are a short call of 3–65 ms duration, and at a frequency of 40–70 kHz that shows varying degrees of frequency modulation (short 50 kHz calls) and a much longer call up to 3400 ms commonly at a frequency of between 25 and 30 kHz which shows little or no frequency modulations (Sales, 1972*b*). This latter call is commonly named the "22 kHz call" after the discovery by Barfield and Geyer (1972) of an almost identical call at 22 kHz after ejaculation in rats.

The first detailed study of the emission of ultrasound during aggression in rats was by Sales (Sales, 1972*b*). This used mixed breed rats of hooded and albino strains that were observed during a series of "round robin" encounters each lasting an hour. During this time the occurrence of different behavior patterns such as attack, wrestle, box, submission were recorded together with any simultaneous emission of either short 50 kHz calls or long 22 kHz calls. It was shown that the shorter calls were particularly associated with aggressive behavior patterns such as attack and boxing but not with submissive postures. The long 22 kHz calls were associated with submissive pos-

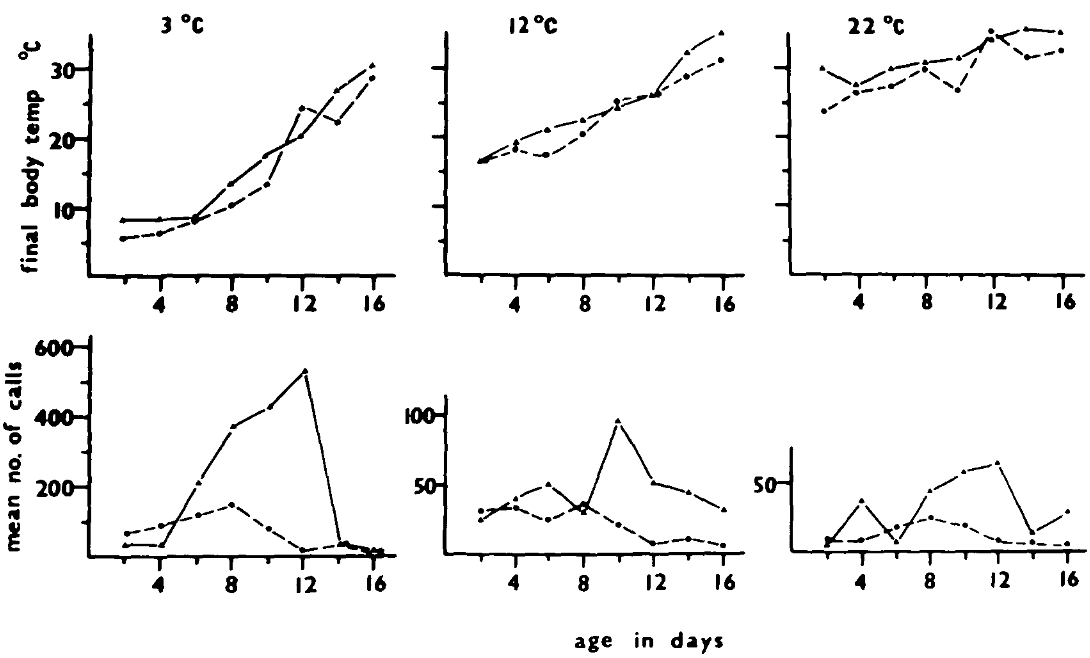

FIG. 3. Mean final body temperature and mean number of ultrasonic calls per pup in Lister and Wistar rats isolated for one hour at three different ambient temperatures. ●—● Lister rats; ▲—▲ Wistar rats

tures and the occurrence of attacking decreased after the first 10–20 min in those encounters where such long calls were produced. It was therefore suggested that these calls might serve to inhibit aggression in the dominant animal.

Evidence for such suppression of aggression was given by the work of Lore *et al.* (1976) on aggressive encounters in Sprague-Dawley rats. The authors studied the behavior of rats (intruders) that were each placed into the home cages of two other rats (residents) for a period of 24 hr. The two encounters were separated by 7–8 days and the animals were observed during the first 30 min of each encounter. During these aggression tests ultrasounds of 20–25 kHz were detected using an ultrasonic detector.

Lore and his colleagues found that while six out of nine naive intruders elicited aggression from residents during the first observation period, none of the nine intruders were attacked on their sound encounters, and that the latency to the first aggressive act was significantly increased in the second encounter. This change in aggression could not be related to any obvious change in the social behavior of the intruders, but during the second aggression test the intruder emitted 22 kHz calls much sooner than in the first test (mean latency 1,459 sec in the first test, 687 sec in the second). In addition, the number of ultrasonic sequencies detected as well as the duration of ultrasound emission was much greater during the second test (mean number of sequencies: 4.2 in the first test, 14.1 in the second; mean duration of ultrasound: 46.1 sec in the first test, 431.0 in the second). These results certainly do suggest that the emission of long 22 kHz calls is accompanied by a decrease in fighting and appears to be associated with the failure of resident animals to initiate an attack.

Lehman and Adams (1977) also found that the emission of long calls at 25 kHz tended to inhibit approach and attack. They used hybrids of DA agouti, Fischer albino and Wag-Rij albino rats and observed encounters between naive animals for 20 min. Ultrasounds were detected with a receiver sensitive to 20.5–24.5 kHz. The authors found that the intruder frequently emitted long ultrasonic calls after being attacked and particularly during submissive postures. When ultrasounds were emitted during such submissive behavior patterns, they were seldom followed by any kick or bite attacks. The authors suggested that if the ultrasonic calls do inhibit bite and kick attacks, these would continue at a high level if the transmission or reception of these calls was interfered with. To investigate this, two intruders were muted and two residents deafened. In encounters involving these operated animals there were the same number of bite and kick attacks in the post-operative test sessions as there were during the pre-operative sessions. The authors therefore suggest that their results do not confirm their original hypothesis.

In this laboratory I have been carrying out an investigation into strain differences in the ultrasonic behavior of male Lister and Wistar rats during aggression (Sales, unpublished) Animals were isolated in individual cages and a naive intruder introduced into the home cage of a naive resident for 30 min (first encounters). In some further encounters a previous intruder acted as resident to a previous resident (second encounters) and in Lister rats two encounters involved a previous intruder acting as resident to a naive intruder. During each encounter the occurrence of the aggressive acts attack, wrestle, box, aggressive upright, aggressive sideways and aggressive/submissive postures was recorded during each 15-sec period, together with the emission of long 22 kHz and short 50 kHz calls (see Sales, 1972*b* for description of behavior patterns). The ultrasonic calls were detected using two QMC Instruments bat detectors, one tune to 25 kHz the other used in the broadband mode. The calls emitted during some encounters were tape-recorded for later sonographic analysis and where possible the SPL of the calls was measured by a calibrated microphone held 30 cms above the floor of the cage.

Statistical comparisons were made between the strains in the latency to first aggressive act, to the first emission of short 50 kHz calls and of long 22 kHz calls, as well as in the total numbers of aggressive acts, of short and of long calls. No significant differences were found for any of the latency scores, except for the latency to first emission of short 50 kHz calls during second encounters (Table 2), when that for Lister rats was shorter than that for Wistar rats. The most marked difference between the strains was in the much higher rate of emission of short 50 kHz calls by the Wistar rats than by the Lister rats. A similar difference was found by S. Hall (personal communication) during 5-min encounters between naive males of these strains.

There were no clear strain differences in the temporal pattern of aggression and ultrasound emission throughout each encounter and these varied with individual encounters (Figs. 4 and 5). As in infants, the main difference between the strains was in the generally higher occurrence of ultrasound, here the short 50 kHz calls, throughout the observation period in Wistar rats. In one encounter between Lister males a burst of aggression occurred with no detectable emission of ultrasound (Fig. 5, bottom line); this never occurred in Wistar rats.

From Table 2 it can be seen that there are suggestive trends in the changes in aggression and ultrasound emission between the first and second encounters and differences between the two strains in some of these trends. However, there was not enough data on second encounters to test these trends statistically and given the variation that was seen in individual encounters, they may well not stand up to statistical analysis. Such trends include an apparent reduction in the latency to aggression between first and second encounters in Lister rats, but an increase in latency in Wistar rats and a decrease in latency to emit short 50 kHz calls in Lister rats but not in the Wistar strain.

TABLE 2. *Comparison of aggressive behavior and ultrasound emission in Lister and Wistar rats during encounters between males.*

	Lister	Wistar	
Behavioral measure and encounter[b]	Mean (SD)	Mean (SD)	P^a
Latency to aggression[c]			
1st encounter	157.0 (122.0)	176.4 (87.5)	NS
2nd encounter	115.0 (15.8)	339.6 (363.7)	NS
All encounters	137.5 (98.3)	161.3 (85.9)	NS
Latency to short "50 kHz" calls			
1st encounter	52.5 (76.3)	41.4 (27.9)	NS
2nd encounter	12.0 (22.0)	45.0 (22.9)	0.057
All encounters	47.6 (61.0)	42.4 (25.6)	NS
Latency to long "22 kHz" calls			
1st encounter	369.7 (258.9)	515.5 (280.2)	NS
2nd encounter	223.7 (122.9)	339.7 (363.7)	NS
All encounters	304.9 (180)	467.5 (269.0)	NS
Total aggressive acts			
1st encounter	16.8 (7.8)	42.6 (30.6)	NS
2nd encounter	39.3 (41.5)	38.0 (26.6)	NS
All encounters	28.1 (27.2)	39.1 (26.2)	NS
Total short "50 kHz" calls			
1st encounter	136.3 (124.7)	545.9 (279.6)	0.028
2nd encounter	116.8 (55.5)	558.0 (312.6)	0.056
All encounters	114.1 (85.6)	557.5 (273.2)	0.002
Total long "22 kHz" calls			
1st encounter	166.8 (235.7)	150.5 (185.0)	NS
2nd encounter	334.5 (216.0)	247.3 (62.3)	NS
All encounters	229.2 (205.0)	162.0 (171.0)	NS

[a] The differences between the two strains were assessed by a Mann-Whitney U test, two tailed.
[b] For 1st encounters, $N = 8$ for Wistar, $N = 4$ for Lister rats; for second encounters, $N_W = 3$, $N_L = 4$; for All encounters $N_W = 11$, $N_L = 10$.
[c] Latencies in sec.

There was a diffe ence in the SPL of the calls of the two strains. Those of Wistar rats were between 56 and 63.5 dB, both short and long calls with the latter occasionally reaching levels of 86 dB. In Lister rats the short calls were generally below the noise level of the equipment (54 dB), while some of the long calls were just above this level and a few reached levels of 86 dB.

The durations and frequency patterns of the calls emitted during aggressive encounters differed somewhat between the two strains (Table 3). The long 22 kHz calls of Lister rats were significantly longer than those of Wistar rats and generally showed little change in frequency over their length, although some showed a downward drift at the beginning and an upward drift in frequency at the end of the calls. The long calls of Wistar rats were far more variable. Some showed one or more instantaneous steps in frequency within the main part of

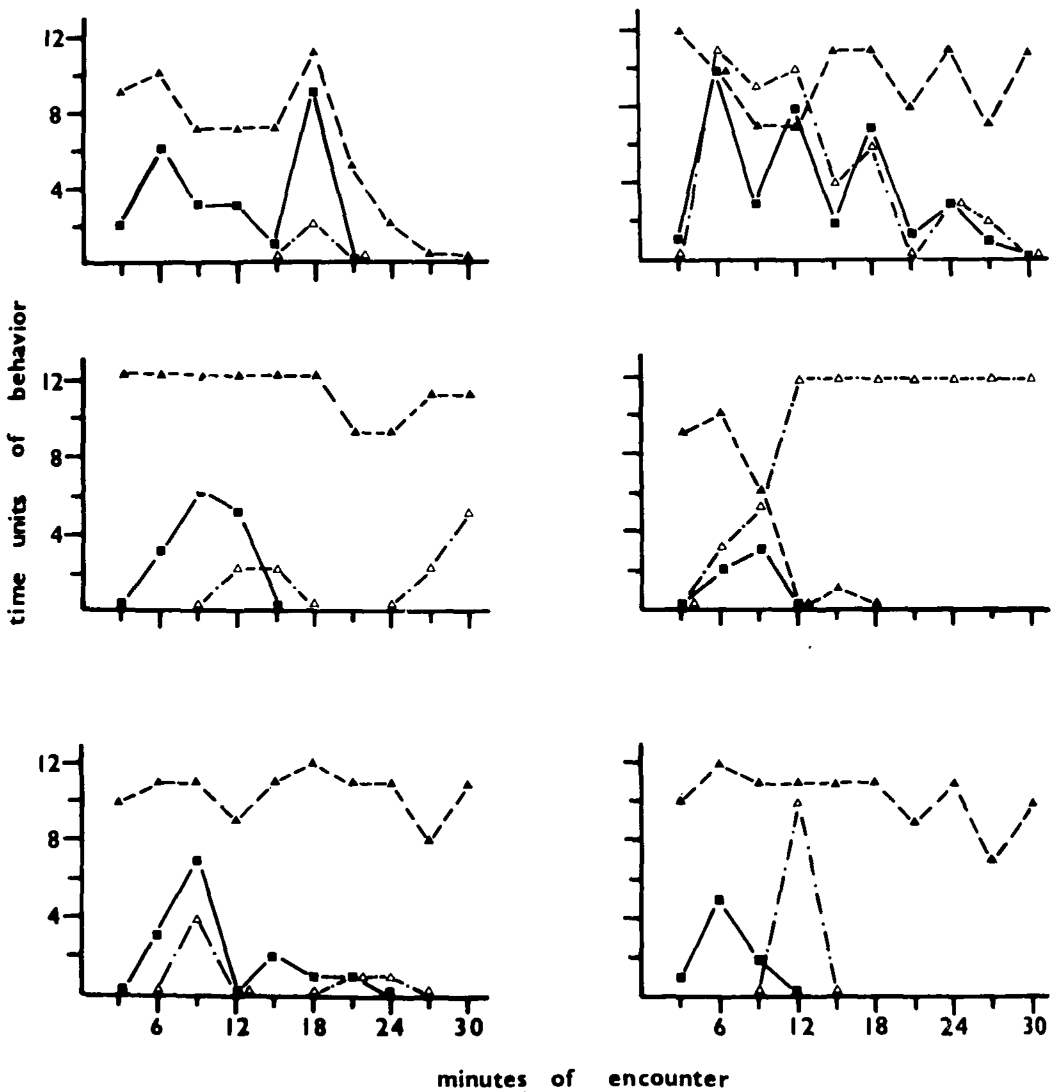

FIG. 4. Temporal patterns of aggressive behavior and ultrasound emission in individual "first" encounters between Wistar rats. The number of 15-sec periods (time units) in which the behavior was recorded is summed over successive 3-min intervals. ▲— — —▲ short 50 kHz calls; △ —·— △ long 22 kHz calls; ■——■ aggression.

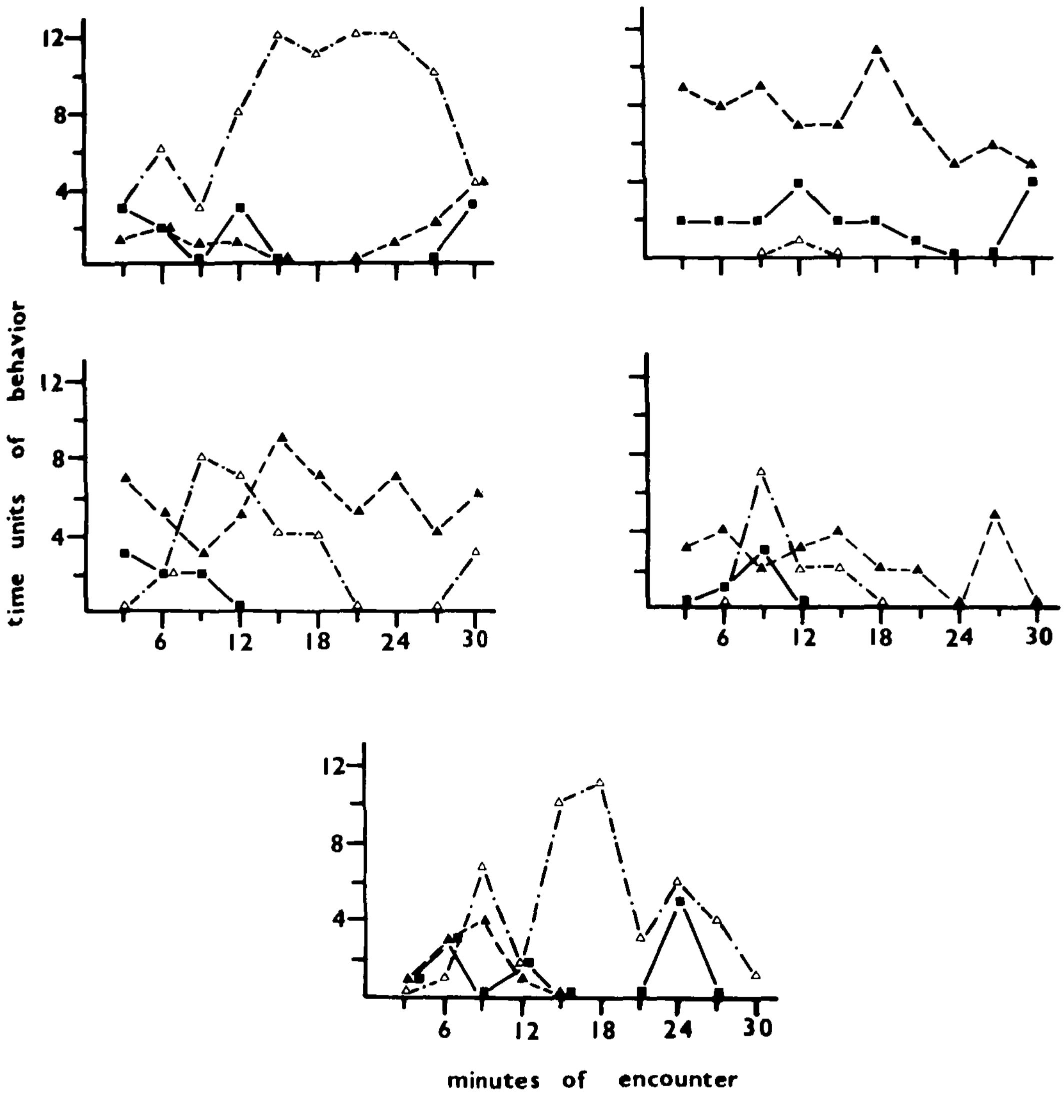

FIG. 5. Temporal patterns of aggressive behavior and ultrasound emission in individual encounters between Lister rats. Top and middle rows—"first" encounters; bottom—"second" encounter. ▲ — — ▲ short 50 kHz calls; △ —·— △ long 22 kHz calls; ■——■ aggression.

the call and often one or more upward steps at the end of the call to very brief components, a few milliseconds long. Some of these higher frequency components showed both amplitude and frequency modulation and were similar to the short calls described below, but as they occurred instantaneously after the end of the longer more constant frequency component, they were taken to be part of the same pulse. Statistical analyses have been carried out including these high frequency components (H.F.) and on the more constant frequency part of the calls only (C.F. only.) (Table 3.) In these calls then the distinction between the long 22 kHz calls and the shorter 50 kHz calls breaks down. If the higher frequency components are included in the analyses, then there is a significant difference between the strains in the maximum frequency of the calls and in the bandwidth, but if these components are excluded, there is no significant difference between the calls of the different strains.

The short 50 kHz calls emitted by Lister rats were significantly shorter than those of Wistar rats and also tended to be higher in frequency and have a lower bandwidth. In both strains these short calls showed varying degrees of frequency modulation and the more marked modulations, up to 30 kHz within 5 ms in Lister rats and up to 80 kHz in Wistar rats, occurred within trains of pulses, each 2–12 ms long and separated by 3–12 ms. Often such trains appeared to be part of a continuous frequency-modulated signal and for the statistical analyses they were treated as single calls rather than a series of single pulses. They were similar to the calls regularly recorded from rats during mating (Sales, 1972*a*).

It is difficult to compare the results of this study directly with those of Lore *et al.* (1976) and of Lehman and Adams (1977) discussed above. These detected only the emission of long 22 kHz calls and from their results it appears that the Sprague-Dawley strain shows a longer latency to the emission of long calls than was recorded for any encounter in the present study, while the hybrid strain observed by Lehman and Adams appear to emit some long calls within the first minute of the encounters.

DISCUSSION

It is clear that there are some differences in the ultrasonic behavior of Lister and Wistar rats in both infants and adults. It is less clear whether or not these differences are related to differences in behavior. Further, more detailed study is needed of overt behavior, but from the various studies discussed here it seems possible that differences between strains may be more apparent in changes in the responses of each strain to changing conditions rather than in a direct comparison of their behavior in any particular condition. Thus while a detailed examination of mother-infant interactions

TABLE 3. *Comparison of the ultrasonic calls emitted by Lister and Wistar rats during aggressive encounters.*

Call type and parameters	Lister range mean (SD)	Wistar range mean (SD)	P[a]
Long "22 kHz" calls (N_L = 16; N_W = 23)[b]			
Duration (ms)	300–3000	300–920	
	120 (707)	500.5 (208.1)	0.001
Max. freq. (kHz)	26–36	22–78	
(+ H.F.)[c]	31.3 (5.0)	46.8 (13.4)	0.001
Max. freq. (kHz)	as above	22–36	
(C.F. only)[c]		28.8 (3.3)	NS
Min. freq. (kHz)	24–28	21–32	
	26.4 (3.5)	26.5 (3.2)	NS
Bandwidth (kHz)	1–12	1–50	
(+ H.F.)[c]	5.1 (5.2)	18.7 (11.5)	0.001
Bandwidth (kHz)	as above	1–6	
(C.F. only)[c]		2.6 (1.5)	NS
Short "50 kHz" calls (N_L = 32; N_W = 35)			
Duration[d] (ms)	1–160	7–380	
	31.1 (30.7)	53.7 (62.0)	0.05
Max. freq. (kHz)	35–108	35–116	
	72.9 (16.9)	62.3 (20.1)	NS
Min. freq. (kHz)	34–68	20–78	
	56.3 (7.7)	46.1 (11.3)	0.001
Bandwidth (kHz)	2–68	2–82	
	14.8 (16.9)	21.5 (22.5)	NS

[a, b] See footnotes to Table 1.
[c] For explanation see text.
[d] Where trains of frequency modulated pulses occurred, the durations of the whole train was measured rather than those of individual parts within the train.

in different strains would be informative and cross-fostering experiments especially so, it might well prove useful to compare changes in parental behavior with time or with different treatment of the pups.

Similarly more information about the communication value of adult calls during aggressive encounters may come from a comparison of the behavior of the strains to changing circumstances, such as that used by Lore *et al.* (1976), that is, during consecutive encounters for either resident and/or intruder. Rats are colonial in the wild and so would probably experience far fewer "first" encounters than "second" or subsequent encounters. The aggressive behavior of laboratory strains has apparently been modified by domestication and the extent of this modification probaly varies with the different strains. If so then this might show more clearly not so much during initial encounters between animals but during subsequent encounters when adaptive changes in behavior must occur if the animals are not to treat each other as strangers at each encounter and so lose one of the benefits originally gained from colonial life; a reduction in intraspecific aggression.

REFERENCES

Allin, J. T. and E. M. Banks. 1971. Effects of temperature on ultrasound production by infant albino rats. Dev. Psychobiol. 4:149-156.

Allin, J. T. and E. M. Banks. 1972. Functional aspects of ultrasound production by infant albino rats (*Rattus norvegicus*). Anim. Behav. 20:175-185.

Barfield, R. J. and L. A. Geyer. 1972. Sexual behavior: Ultrasonic post-ejaculatory song of the male rat. Science 176:1349-1350.

Barfield, R. J. and L. A. Geyer. 1975. The ultrasonic post-ejaculatory vocalization and the post-ejaculatory refractory period of the male rat. J. Comp. Physiol. Psychol. 88:723-734.

Bell, R. W., W. Nitschke, T. H. Gorry, and T. A. Zachman. 1971. Infantile stimulation and ultrasonic signalling: A possible mediator of early handling phenomena. Dev. Psychobiol. 4:181-192.

Bell, R. W., W. Nitschke, and T. Zachman. 1972. Ultrasounds in three inbred strains of young mice. Behav. Biol. 7:805-814.

Colvin, M. A. 1973. Analysis of acoustic structure and function in ultrasounds of neonatal *Microtus*. Behaviour 44:234-263.

Floody, O. R. and D. W. Pfaff. 1977. Communication among hamsters by high-frequency acoustic signals: III. Response evoked by natural and synthetic ultrasounds. J. Comp. Physiol. Psychol. 91:820-829.

Hoffmeyer, I. and G. D. Sales. 1977. Ultrasonic behaviour of *Apodemus sylvaticus* and *A. flavicollis*. Oikos 29:67-77.

Hunt, L. E., W. P. Smotherman, S. G. Wiener, and S. Levine. 1976. Nutritional variables and their effect on the development of ultrasonic vocalizations in rat pups. Physiol. and Behav. 17:1037-1039.

Kelly, J. B. and B. Masterton. 1977. Auditory sensitivity of the albino rat. J. Comp. Physiol. Psychol. 91:930-936.

Lehman, M. N. and D. B. Adams. 1977. A statistical and motivational analysis of the social behavior of the male laboratory rat. Behavior 61:238-275.

Lore, R., K. Flannelly, and P. Farina. 1976. Ultrasounds produced by rats accompany decreases in intra-specific fighting. Aggressive Behav. 2:175-181.

McIntosh, T. K., R. J. Barfield, and L. A. Geyer. 1978. Ultrasonic vocalizations facilitate sexual behavior in female rats. Nature 272:163-164.

Nitschke, W., R. W. Bell, M. J. Bell, and T. Zachman. 1975. The ontogeny of ultrasounds in two strains of *Rattus norvegicus*. Exptal. Aging Res. 1:229-242.

Noirot, E. 1974. Nest building by the virgin female mouse exposed to ultrasound from inaccessible pups. Anim. Behav. 22:410-420.

Noirot, E. 1968. Ultrasounds in young rodents. II. Changes with age in albino rats. Anim. Behav. 16:129-134.

Okon, E. E. 1972. Factors affecting ultrasound production in infant rodents. J. Zool. Lond. 168:139-148.

Okon, E. E. 1971. The temperature relations of vocalizations in infant Golden hamsters and Wistar rats. J. Zool. Lond. 164:227-237.

Okon, E. E. 1970. The effect of environmental temperature on the production of ultrasounds by isolated non-handled albino mouse pups. J. Zool. Lond. 162:71-83.

Oswalt, G. L. and G. W. Meier. 1975. Olfactory, thermal and tactual influences on infantile ultrasonic vocalizations in rats. Dev. Psychobiol 8:129-135.

Ressler, R. H. 1962. Parental handling in two strains of mice reared by foster parents. Science 137:129-130.

Sales, G. D. 1972*a*. Ultrasound and mating behaviour in rodents with some observations on other behavioural situations. J. Zool. Lond. 168:149-164

Sales, G. D. 1972*b*. Ultrasound and aggressive behaviour in rats and other small mammals. Anim. Behav. 20:88-100.

Sales, G. D. and J. D. Pye. 1974. Ultrasonic communication by animals. Chapman and Hall, London.

Sales, G. D. and J. C. Smith. 1978. Comparative studies of the ultrasonic calls of infant murid rodents. Dev. Psychobiol. 11:595-619.

Sewell, G. D. 1970. Ultrasonic communication in rodents. Nature 217:682-683.

Smith, J. C. 1976. Responses of adult mice to models of infant calls. J. Comp. Physiol. Psychol. 90:1105-1115.

Smotherman, W. P., R. E. Bell, J. Starzec, J. Elias, and T. A. Zachman. 1974. Maternal responses to infant vocalization and olfactory cues in rats and mice. Behav. Biol. 12:55-66.

Watts, C. H. S. 1975. Vocalizations of Australian hopping mice (Rodentia *Notomys*). J. Zool. Lond. 177:247-263.

Whitney, G., J. R. Coble, M. D. Stockton, and E. F. Tilson. 1973. Ultrasonic emissions: Do they facilitate courtship of mice? J. Comp. Physiol. Psychol. 84:445-452.

Zachman, T. A., R. W. Bell, W. Nitschke, and B. Irvin. 1972. Ultrasonic signalling in three inbred strains of mice. J. Aud. Res. 12:137-145.