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Developmental changes in several parameters of ultrasonic calling by young Mongolian gerbils (*Meriones unguiculatus*)

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(With 6 figures in the text)

Mongolian gerbils were isolated for one minute on each of the first 14 days of life. The ultrasounds which they produced were displayed on a recorder which allowed measurement of the sound level, duration, shape and temporal patterning of the calls. These components of the pup's response changed with age in various ways. Most of the ultrasounds produced occurred in bouts of regularly spaced calls. The bout length was longest on day 4, and then decreased, but the rate of calling within bouts increased with age. The mean sound level of the calls reached a peak on day 6 and then declined, but one or more pups at each age could produce loud calls. The total rate of production of single calls changed little with age, but production of multiple calls reached a peak on days 4 and 5, and then declined. It seems that the ability of pups to call rapidly and to produce a series of loud, complex calls increases with age. As calling declined, scrabbling movements with the legs increased. Temperature regulation and motor ability are improving during this time. These changes in calling are related to changes in maternal behaviour, especially nest building, which peaked on day 6 in another study. Observations on control litters suggested that daily handling and testing increased the rate of calling on day 5.

Contents

Introduction

Ultrasounds are produced by a wide variety of young murid and cricetid rodents (Sewell, 1968, 1969) and can usually be elicited by cooling the animal or by tactile stimulation (Okon, 1972). Adult rodents have been found to have peaks of auditory sensitivity at frequencies similar to those of calls produced by the pups of that species (Ralls, 1967; Brown, 1973*a*, *b*) and to respond behaviourally to the calls. These responses include orientation towards the location of the sound-source and searching (Allin & Banks, 1972) and nest-building (Noirot, 1974).

The effectiveness of pups in eliciting maternal behaviour varies as a function of their age. Ultrasounds also vary with the age of the pup. For many species the rate of calling is low for the first few days, but peaks at about four to six days and then declines (Noirot, 1966, 1968; DeGhett, 1974). The rate of calling and the frequencies of the calls are the most easily measured aspects of ultrasounds and are often the only parameters which are measured (DeGhett, 1974). Noirot & Pye (1969) however, measured several parameters of sound frequency, as well as the length of the calls, and estimated the maximum sound level. Only the length, total band-width and sound level of the calls varied in a systematic manner. These, as well as the rate of calling, were considered to be the parameters which might influence maternal behaviour.

Maternal retrieving and nest-building by mice is temporally correlated with the production of ultrasounds by pups which are cooled, whilst those ultrasonic calls produced during rough handling are thought to reduce aggression (Noirot, 1966, 1972). Smith (1972) distinguishes two call types in *Peromyscus* and suggests that different calls may convey different "meanings". Bell (1974), however, thought that a continuum may exist both in the stimuli which elicit the calls and in their effects on the recipient adult. Noirot & Pye (1969) suggested that changes in the total sound energy of cold-elicited calls may produce changes in maternal behaviour but the evidence for this is not conclusive (Noirot, 1972). Thus, it has yet to be determined which characteristics of ultrasounds elicit maternal behaviour. In the present study a number of parameters of ultrasounds produced by Mongolian gerbils of different ages have been investigated. In particular, sound level has been measured precisely and the pattern of calling has been described. If such information is related to known changes in maternal behaviour, our understanding of the communicative functions of ultrasounds should be improved.

Methods

The subjects were Mongolian gerbil (*Meriones unguiculatus*) pups whose parents were maintained in the conditions described in Elwood (1975). Four experimental and two control litters of five or more pups were each reduced to five pups immediately after parturition. Since the dates of birth were spread over nine days, pups of various ages were tested on each day. In order to record vocalizations, each litter was removed from its home cage and placed in a small cotton-wool lined bowl at 35°C (temporary nest) on 14 successive days, at approximately 14.00 hrs, commencing a few hours after birth (day 0). Control pups were tested on day 5 only. The pups were tested in a partially sound-proofed room with no other animals in it. A round, glass bowl 7 cm deep and 13 cm in diameter was precisely positioned under a microphone which was held in a clamp so that it was always 5 cm above the bottom of the bowl. The temperature in the room varied little and that in the bowl was maintained at 20–22°C by minor adjustments of a bench lamp above the microphone.

The testing procedure involved removing the pup from the temporary nest and placing it into the pre-warmed glass bowl (20–22°C), placing a cover over the bowl, carrying the bowl to the test room 15 m away, placing the bowl in position under the microphone and removing the cover. The door of the room was then quietly closed and, 5 sec after the bowl was in position, the recorder was switched on for 1 minute. At the end of the minute the pup in the covered bowl was returned to the home cage and another pup was fetched from the temporary nest. The pups were always carried and set down as gently as possible.

The microphone used was that supplied with a Holgate Ultrasonic Receiver Mark V. It was connected, via an amplifier constructed by A.J.P., to a Bruel & Kjaer 2305 Sound Pressure Level

Recorder which produced a plot of sound level against time. The recording system was tested using standard sound inputs and was found to give an accurate representation of sound level over the whole range of sound frequencies which gerbil pups produce. The system was also calibrated by this means so that the base line sound level on the record could be measured. This base line was 34 dB (re. 0·0002 dynes/cm^2) and sounds of up to 84 dB could be recorded. The background noise level in the room and the noise of small movements made by the pup were always lower than 37 dB, so this level was chosen as the threshold when calls were counted.

The rise-time of the recorder was adjusted so that the sound pressure level of a call lasting 50 msec would be accurately recorded without the production of onset or offset transients and a good indication would be given for calls as short as 25 msec. Sewell (1969) found that almost all gerbil calls lasted much longer than 50 msec. Calls appeared on the record as spikes, most of which were very much above the threshold sound level. These spikes could be single or multiple and on each 60 sec long record the number of single and multiple calls in each of eight sound level ranges was counted. The delay before calling started, loudest call, length of longest bout of calling and number of calls in that bout were also recorded. A bout of calling was defined as a regular series of more than three calls. Bouts were considered to have terminated when no call was made for at least twice the previous inter-call interval. Most bouts included many more than three calls but measurements of bout-length were inevitably more accurate for longer bouts. The scrabbling movements made by older pups made sounds which were loud enough to be clearly visible on the record as small spikes superimposed upon a continuous elevation of sound level to more than 36 dB. When such a bout of scrabbling lasted for more than 0·5 sec its duration was measured.

In order to check the recording method, some 5-day-old pups were put on a flat bench at a similar temperature and their ultrasonic calls were recorded on an oscillograph in which the paper was travelling at 5 cm per sec and in which the rise time was 16 msec.

Results

In pilot experiments by J. L. and S. J. W., gerbil pups were watched while in the test situation and their calls were monitored using both an ultrasonic receiver (bat detector) and the sound pressure level recorder. These observations facilitated the interpretation of the record obtained in the main experiment and indicated that there were changes with age in ultrasonic calling and in the locomotor movements which are here referred to as scrabbling. It was possible to distinguish calls from scrabbling movements until day 9, but the quietest calls could be confused with scrabbling after this age, so the graphs for call frequency and mean sound level refer to days 0–9, but those concerning loudest calls, bouts of calling, and scrabbling refer to days 0–13. During the pilot experiments and during the 5 sec before recording commenced for each animal, the experimenters listened to the pups. No calls were ever heard, even when the recorder showed a high calling rate, so it was concluded that all calls recorded during the experiments were ultrasonic. Audible calls were produced in other situations.

The mean number of calls given during the 60 sec test (Fig. 1) increased markedly during the first day and then continued to increase until day 4 and day 5. After this age, calling declined until day 13 when very few calls were given. Mean values are shown because they provide a good indication of the sound output from a litter. The median delay before calling started was less than 1 sec until day 8 but increased rapidly to 28 sec on day 13. The type of calls produced changed during this period. Most were single calls on days 0, 1 and 2, but the increase on days 3 to 6 was largely due to multiple calls. Multiple calls had two or more peaks of sound level. There was considerable

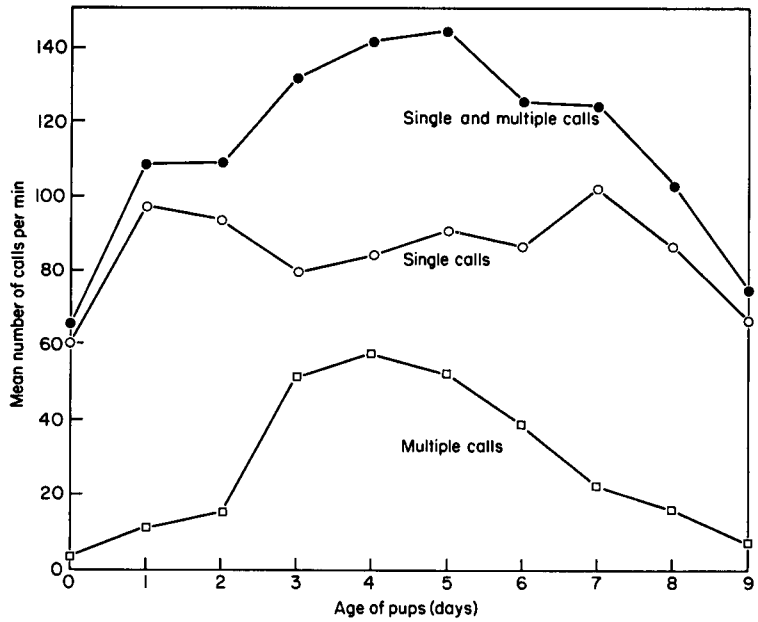

FIG. 1. The mean number of calls given during a one minute observation period is shown for gerbil pups of ten ages. Each point is the mean of 20 animals. Single calls ○ appeared as single peaks and multiple calls □ as two or more peaks of sound level on the record.

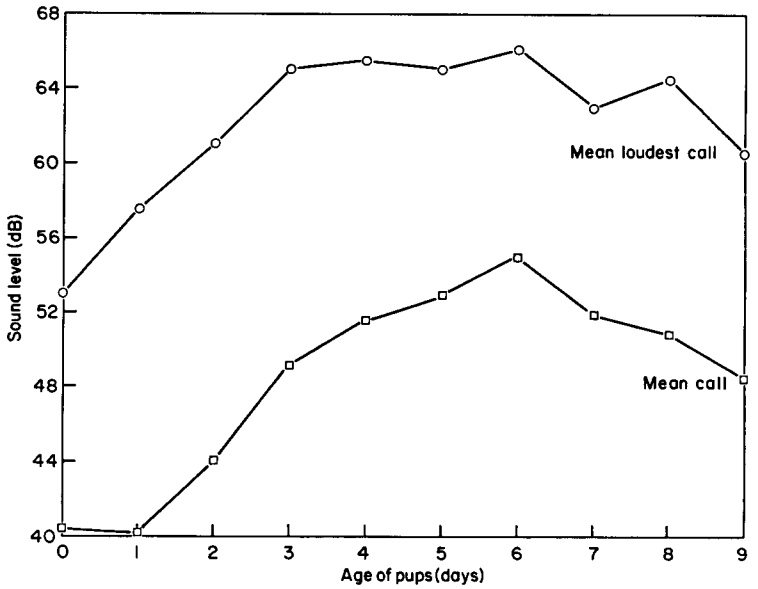

FIG. 2. The sound level in dB (re. 0·0002 dynes/cm²) of the mean call (□) produced by the 20 animals at each age and the median of the loudest calls (○) by these animals is shown for gerbil pups of ten ages.

TABLE I

Statistical comparisons for each measure

Measure	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13
Total calls	1 2 3* 4* 5* 6* 7* 8*	0 4 5 9	0 4 9	0* 8 9	0* 1 2 8 9*	0* 1 8 9*	0* 9	0* 8 9	0 3 4 5 7	1 2 3 4* 5* 6 7	No data	No data	No data	No data
Multiple calls	1 2 3* 4* 5* 6* 7* 8	0 3* 4* 5* 6* 7	0 3* 4* 5* 6*	0* 1* 2* 7 8* 9*	0* 1* 2* 7* 8* 9*	0* 1* 2* 7 8* 9*	0* 1* 2* 7 8* 9*	0* 1 3 4* 5* 6 9	0 3* 4* 5* 6* 9	3* 4* 5* 6* 7 8	No data	No data	No data	No data
Sound level	2 3* 4* 5* 6* 7* 8* 9*	2 3* 4* 5* 6* 7* 8* 9*	0 1 4 5* 6* 7* 8* 9	0* 1* 5 6	0* 1* 2 6 9	0* 1* 2* 3 8 9*	0* 1* 2* 3 4 7 8 9*	0* 1* 2* 6 9	0* 1* 2* 5 6	0* 1* 2* 4* 5* 6* 7	No data	No data	No data	No data
Median loudest call	3 4 5 6 7 8	3 4 5 6* 7 8	6	0 1 9	0 1 9	0 1	0 1* 2 7 9	0 1	0 1 9	3 4 6 8	No data	No data	No data	No data
Median longest bout of calling	9 10 11* 12* 13*	8 9 10* 11* 12* 13*	8 9 10* 11* 12* 13*	7 8 9* 10* 11* 12* 13*	7 8* 9* 10* 11* 12* 13*	7 8* 9* 10* 11* 12* 13*	7 8* 9* 10* 11* 12* 13*	3 4 5 6 9 10* 11* 12* 13*	1 2 3 4* 5* 6* 10 11* 12* 13*	0 1 2 3* 4* 5* 6* 7 11 12* 13*	0 1* 2* 3* 4* 5* 6* 7* 8* 11* 12* 13*	0* 1* 2* 3* 4* 5* 6* 7* 8* 12* 13*	0* 1* 2* 3* 4* 5* 6* 7* 8* 9* 10* 11* 13	0* 1* 2* 3* 4* 5* 6* 7* 8* 9* 10* 11* 12
Median rate of calling in longest bout	1 2 3 4 5* 6 7* 8* 9* 10* 11* 12	0 10 11	0 8 10 11	0 7 8 9 10* 11 12	0 8 10 11	0* 6	0 5 7 8 9 10 11 12	0* 3 6	0* 2 3 4	0* 3 6	0* 1 2 3* 4 6	0* 1 2 3 4 6	0 3 6	No data
Duration of scrabbling	6 7 8 9* 10* 11* 12* 13*	6 7 8 9* 10* 11* 12* 13*	6 7 8 9* 10* 11* 12* 13*	6 7 8 9* 10* 11* 12* 13*	6 7 8 9* 10* 11* 12* 13*	8 9* 10* 11* 12* 13*	0 1 2 3 4 9* 10* 11* 12* 13*	0 1 2 3 4 9* 10* 11* 12* 13*	0* 1* 2* 4 5 9 10* 11* 12* 13*	0* 1* 2* 3* 4* 6* 7* 9 10 11* 12* 13*	0* 1* 2* 3* 4* 5* 6* 7* 8* 9 12 13*	0* 1* 2* 3* 4* 5* 6* 7* 8* 9* 12 13*	0* 1* 2* 3* 4* 5* 6* 7* 8* 9* 10 11	0* 1* 2* 3* 4* 5* 6* 7* 8* 9* 10* 11*

The results of two-tailed Mann–Whitney *U* tests comparing each day with each other day.
The number of each day which is different from the day at the top of the column is listed if $P<0{\cdot}05$ and an asterisk * is added if $P<0{\cdot}001$.

variation amongst pups in the number of calls which they produced and the distribution was not normal, so the calling at each age has been compared using Mann–Whitney *U* tests. Each age was compared with each other age and the results are shown in Table I. From this it can be seen that for total calls, day 0 differs from days 3, 4, 5, 6 and 7, and day 9 differs from days 4 and 5 at the $P = <0{\cdot}001$ level (two-tailed).

The mean sound level of calls produced by the gerbil pups was much greater on day 6 than on days 0 and 1 or day 9 (Fig. 2, Table I). Even the youngest pups were capable of producing calls of over 52 dB, as is shown by the "median loudest calls" in Fig. 2. The loudest call produced by any pup was between 75 dB and 82 dB on each of days 0 to 8. Thus the difference between the mean sound levels of 40 dB on days 0 and 1 and 55 dB on day 6 is due to the production of a larger proportion of loud calls by the older pups (Fig. 3). It is apparent from this Figure, which shows the distribution of calls produced by

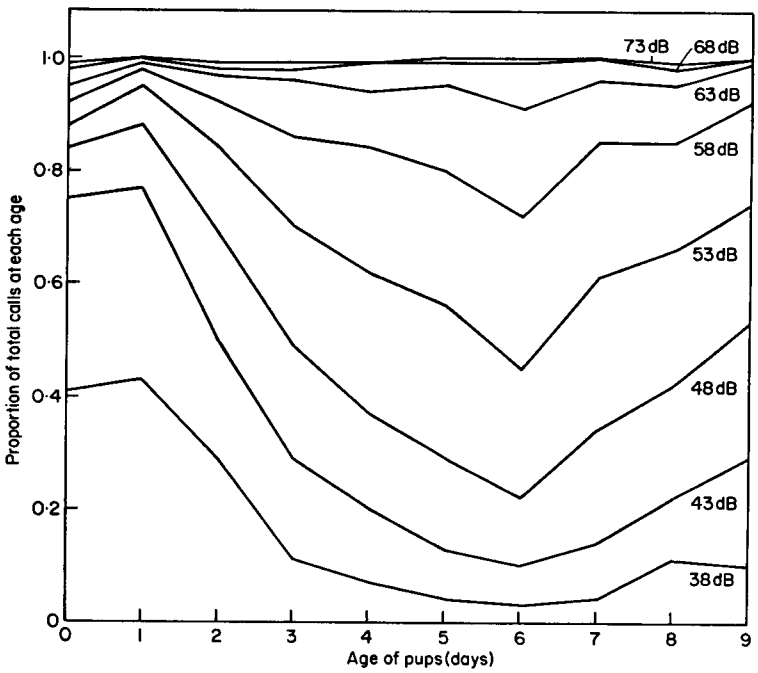

FIG. 3. The proportions of calls below each of eight sound pressure levels are plotted for ten ages of gerbil pups ($n=20$). The majority of calls given by the youngest pups are below 43 dB but on day 6 only a tenth of calls are below 43 dB and more than half are above 53 dB.

pups of each age, that there is a steady decline in the proportion of quiet calls (e.g. less than 43 dB) and a steady increase in the proportion of louder calls from day 1 to day 6.

Most pups did not call continuously, so a greater total number of calls in the 60 sec could have been due to more bouts or longer bouts of calling, or to a greater rate of calling during a bout. The duration of the longest bout of calling and the rate of calling during that bout were ascertained for each pup and the median values for each age are shown in Figs 4 and 5. The longest bout was chosen for analysis because it could be defined precisely, whereas attempts to define the mean bout are subject to error when all

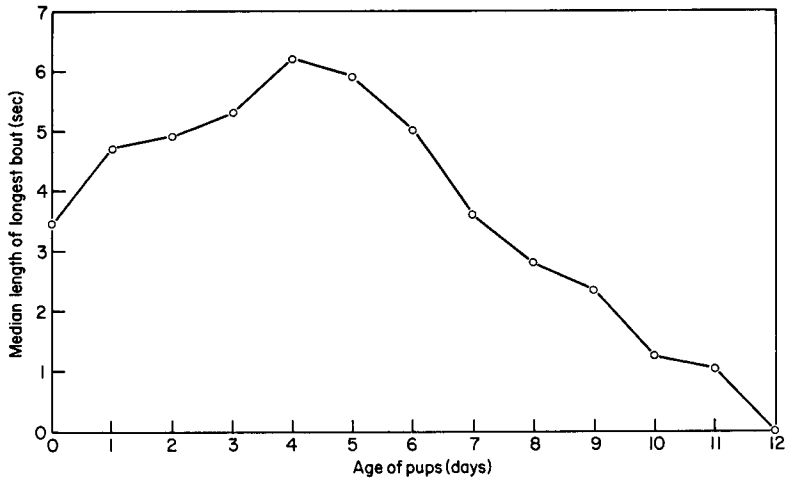

FIG. 4. The median of the longest bouts of calling by 20 gerbil pups is plotted for 13 different ages.

short bouts have to be measured (see Methods). From these figures it is apparent that the increase from day 0 to day 1 in the total number of calls in the test was due in part to an increase in the rate of calling within a bout but that later changes were due to differences in the duration or number of bouts of calling. The rate of calling within a bout continued

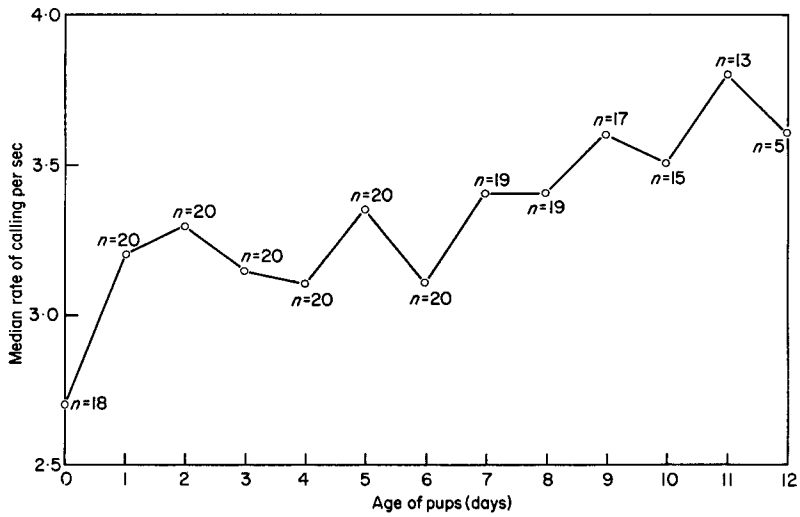

FIG. 5. The median rate of calling during the longest bout of calling by gerbil pups is plotted for 13 different ages Since some animals did not produce bouts of calling, the number from which the median is calculated is given for each age.

to rise slowly from day 4 onwards at the same time that the bout length and the total calls given in the test were declining (see also Table I). Bouts of calling had ceased to occur by day 13. The change in the behaviour of the pups over the 14 day period is emphasized in Fig. 6 which shows the decline in the percentage of pups which gave a bout of calling and

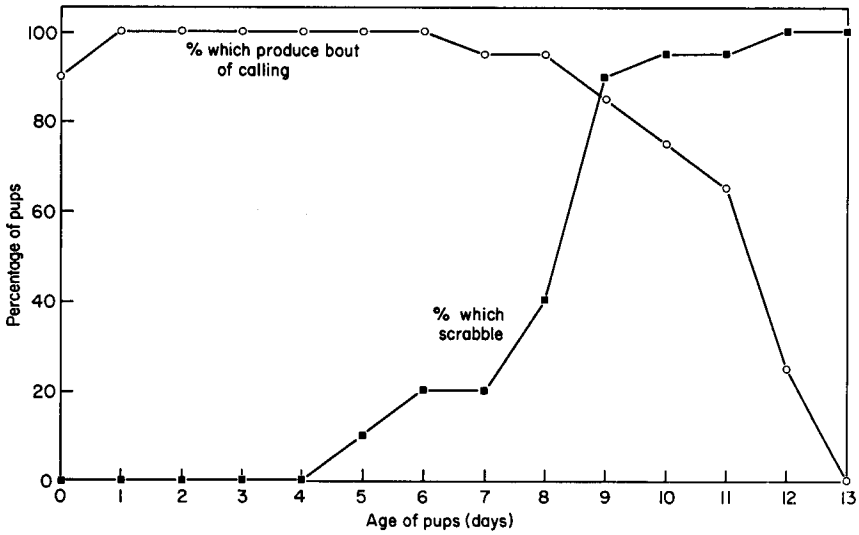

FIG. 6. The percentage of 20 gerbil pups which scrabble (■) and the percentage which produce a bout of calling (○) is shown for 14 different ages.

the increase from day 5 onwards in the percentage of pups which scrabbled audibly. The median duration of scrabbling was nil until day 8, but pups spent 50% of their time scrabbling on day 13 (see also Table I).

Since the same animals were tested on each day, it was considered necessary to test a naive control group on one day in order to look for any habituation or sensitization to the testing procedure. Two control litters of five pups were tested on day 5 and the results are

TABLE II

Control and experimental groups on day 5

		Day 5 (n=20)	Day 5 controls (n=10)	Mann–Whitney *U* test
Mean no. of calls		144	111	$P<0{\cdot}05$
Mean no. of multiple calls		53	29	$P<0{\cdot}05$
Mean sound level	dB	53	53	
Mdn loudest call	dB	65	66	
Mdn longest bout	sec	5·9	3·1	$P<0{\cdot}02$
Mdn rate of calling during longest bout	calls/sec	3·3	3·1	
% which scrabble		10	10	

compared to the experimental group on day 5 in Table II. There was no difference in the sound level of the mean or loudest call or in the rate of calling in the longest bout. It seemed, however, that repeated testing may have increased the lengths of bouts of calling and hence the total number of calls, especially multiple calls, given in 1 minute.

The production of multiple calls by 5-day-old gerbil pups was verified using an oscillograph. Calls which included two, three or four peaks were clearly seen. These multiple

calls were no longer or louder than single calls recorded a few seconds earlier. The durations of calls were as great as 180 msec. This figure agrees well with the estimate of 150–200 msec obtained by using the Bruel and Kjaer recorder to measure the length of the longest calls of 5-day-old pups.

Discussion

Several parameters of the ultrasonic calling of gerbil pups vary with the age of the litter. The number of calls produced, the sound level, the length of bouts and the rate of calling within those bouts all change in a systematic manner. The mean sound level of calls showed a clear increase to a peak on day 6 and then declined. This does not necessarily reflect the sound producing ability of the pups, for the median loudest call reached a plateau on day 3 and some pups at each age were able to make very loud calls. Noirot & Pye's (1969) estimates of the maximum sound level of mouse-pup calls showed no change over the first five days but then dropped. They did not measure the sound level of all calls and thus could not assess the mean sound levels. The lengths of bouts of calling showed no significant change during the first four days but became markedly shorter after the maximum length on day 4. The rate of calling during those bouts, however, increased markedly during the first day and continued to increase throughout the period of ultrasound production. It appears that, whilst some young pups can call loudly, only older pups can call rapidly.

The total number of calls per minute increased from day 0 to day 4 and declined after day 5. These data are very similar to those obtained by DeGhett (1974) for the same species. It seems that at least two distinct types of call are produced and that the peak in the rate of calling per minute is largely due to the more complex multiple calls. The oscillograph study showed that a range of complexity exists amongst multiple calls. Noirot (1974) found that different maternal responses were elicited by the calls of mouse-pups in different situations and Smith (1972) showed that *Peromyscus* pups could produce calls at two different frequencies. It seems unlikely that these two call types are comparable with those found in the present study. The multiple calls would probably be more obvious to the mother, but their precise function has yet to be determined.

Noirot & Pye (1969) thought that the "total sound energy" produced by cooled pups, rather than just rate of calling, determine the effectiveness of pup calling in eliciting maternal behaviour. DeGhett (1974) measured only rate of calling by gerbil pups, but did find a close correlation between the number of calls produced and McManus's (1971) data on the thermoregulatory ability of the pups. The present study shows that the peak in calling at four to six days is a peak in sound level, total rate of calling per minute, duration and complexity of individual calls (more multiple calls) and length of bout of calling. These would all contribute to "total sound energy" produced over a number of seconds. Data for the nest-building of female gerbils with litters shows a peak of activity on day 6 (Elwood, 1975) when the pups have passed their peak rate of calling per minute, but when the sound level is at its peak. As the calling response wanes, scrabbling movements increase. Eventually, pups could return to a nest unaided if they were displaced a short distance from it. Thus it seems that as the pups' homoiothermy and motor ability develop, their calling response declines. It should be emphasized, however, that there is considerable individual variation in the response and the various components of the response do not change with age in the same way. The ability of the mother to hear the sounds produced has been clearly demonstrated by Brown (1973*a*, *b*) but the precise effects

of the various aspects of the ultrasonic calls on maternal behaviour have not yet been clarified.

The control litters did not differ from experimentals on day 5 in the maximum or mean sound level of calls or in the rate of calling within a bout. The daily testing of the experimental animals does, however, appear to have increased the total number of calls per minute, especially multiple calls, and the length of the longest bout. It is possible that pups have more control over these aspects of calling and that increased maternal behaviour when pups were returned to the nest on days 0 to 4 led to increased calling on day 5. The fact that maternal behaviour is modified after handling, with consequent effects on the behaviour of the young, has been emphasized by Russell (1971) and others. The difference between the control and experimental animals also leads to speculation about the extent to which the increase in calling during the first four to six days is a response to the behaviour of the mother.

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