Adult Responsiveness to Ultrasonic Signals from Gerbils of Varying Ages: Parity, Gender, and Housing Effects

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Groups of adult gerbils differing by gender, parity, and presence or absence of young in the nest were exposed to taped ultrasonic distress calls of male and female pups on postnatal Days 3, 8, and 13. Frequency and time spent performing several behavioral items as well as frequency and time spent in the compartment housing the recorded tapes were assessed in the adult groups. Adult groups differed in rates and durations of certain behavioral items only during playback, with females with young in the home nest exhibiting the highest frequency of nest building in the test apparatus. However, all groups spent more time in the arm from which calls of Day-8 pups were played. Calls of Day-3 and Day-13 pups did not produce significant differences in adult responsiveness. These results reveal significant characteristics of the ultrasounds for each group which parallel certain morphological and behavioral changes.

Among neonates of several species of rodents the rate of isolation-induced ultrasonic calling is high 4–7 days postnatal and declines almost to zero by 2–3 weeks of age. In attempts to assess the functional significance of neonatal ultrasonic vocalization in rodents several investigators have utilized the playback technique, which involves presentation of only the responses in the auditory modality of the distressed pup (Allin & Banks, 1972; Colvin, 1973; Sewell, 1970), that is, the pup exposed to isolation, cold stress, novel tactile stimulation, or a combination of these factors. Such studies have indicated that ultrasonic emissions alone—elicited by any one of the above stimuli or by stimuli in combination—are sufficient to induce adult orientation and

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localization of the signal by both female and male adults (Allin & Banks, 1972; Colvin, 1973). Few, if any, of the playback studies to date have examined adult responses to pups of differing ages and gender, nor have they systematically varied parental condition of the adults. The specific contributions of this study include the following:

1. *Use of a cross-sectional sample of pups.* Previous studies (Allin & Banks, 1972; Colvin, 1973; Sewell, 1970) have used a "constant" stimulus tape, i.e., recordings from only 1 age group. In light of the rather pronounced changes in signal parameters over time, adults may be differentially responsive to pups of varying age. Here, 3 different age groups will be examined.

2. *Separation of male and female pups at each age group.* Meier and Schutzman (1968) have indicated that female mice were more likely to retrieve a female pup first, and suggested that a difference in some characteristic of the vocalizations between female and male pups might account for preferential retrieving. Adults in the present study, therefore, were presented with taped calls of both male and female neonates in all 3 age groups.

3. *Classification of adult respondents by gender and by experiential factors.* Previous studies by Allin and Banks (1972) and Colvin (1973) have tested the responses of both female and male subjects to the recorded calls of distressed pups. In addition, Allin and Banks tested females with various reproductive histories (i.e., virgin females, primiparous females, and multiparous females). However, the parous females in that study all had pups in the nest at the time of testing. The changes in maternal responsiveness may be a function of internal physiological changes in the lactating mother, of changes in the physical/behavioral characteristics of the young, or of some combination of them (Rosenblatt, 1965). To tease out these interactive effects, we differentiated groups of parous females as to whether or not they were allowed to keep litters after delivery.

Experiment I: Recording of the Neonatal Stimulus Tapes

Method

Neonatal mongolian gerbils (*Meriones unguiculatus*), 3, 8, and 13 days of age, were used to produce ultrasonic vocalizations for the stimulus tapes. All pups were offspring of monogamous breeding pairs obtained from Tumblebrook Farms (Brant Lake, New York). Both male and female pups were recorded, 5 pups being selected for each gender from 2 litters per age. (Day of birth was designated as Day 1.) Animals were housed in clear plastic cages; strips of paper towelling were provided daily; and Purina lab chow and water were supplied ad lib. The colony room was maintained at approximately 23-24°C, with lights on from 0700 to 1900 hours. Pups were tested during the light period.

To induce ultrasound production, we isolated each pup for 5 min in an empty wooden container measuring 19 X 31 X 9 cm. The microphone was maintained manually approximately 2 cm from the pup's mouth. Vocalizations were recorded for 5 min, after which the pup was returned to the home cage.

Presence of ultrasonic signalling was first monitored with a Holgate ultrasonic receiver. When ultrasonic signals were detected, the vocalizations were then recorded

on ½-in. (1.27-cm) Scotch instrumentation tape using an Ampex SP 300 instrumentation recorder. This recorder, using a direct mode recording procedure, gave a reasonably flat response up to 50 kHz at a tape speed of 15 in./sec (37.5 cm/sec). A modified videotape transducer, showing an upper sensitivity of 55 kHz, was used as a microphone. A Tektronix type 545-B oscilloscope with type 2A63 differential amplifier served as preamplifier as well as signal monitor. To check for possible distortion during taping, we compared stored oscillographic tracings of pup vocalizations with tracings of the stimulus tapes. No differences between the 2 modes of presentation were detectable. All recordings were conducted in a sound-shielded chamber (Industrial Acoustics, Inc., model 404-B) with the recording equipment placed outside of the chamber. Room temperature was maintained at about 22-23°C.

From the 5-min records of pups in each age X gender category, the 60-sec segment containing maximal rate of calling was selected. Sound spectrograms of the recorded calls were made with a Sonagraph (Kay Electric Co., Model 6061-A). All spectrograms were made at the wideband setting, with a bandwidth of 300 Hz. Because the frequency of the sonagraph was from 85 to 8000 Hz, the tape recorder speed was reduced by a factor of 8, rendering the sounds audible and appropriate for spectrographic analysis. A bandpass filter (Krohn-Hite, Model 310-C) set to 4 and 200 kHz was used to reduce low frequency noise.

Rate of calling was measured for each 60-sec segment on all tapes. Bandwidth, duration, and initial, terminal, maximal, and minimal frequencies were measured for each call on the selected stimulus tapes.

Results

Day-8 animals produced the highest rates of vocalization followed by Day-13 and Day-3 pups, in that order ($F = 24.95$, $df = 2/24$, $p < .001$). (See Table 1 for all vocalization measures.) Their calls were also the longest in duration, with Day-3 pups producing calls of medium duration ($F = 6.26$, $df = 2/24$, $p < .01$). Bandwidths of calls from Day-3 and Day-8 pups were similar, whereas those of Day-13 animals were narrower ($F = 24.22$, $df = 2/24$, $p < .001$). Bandwidths of male calls were narrower than those of female calls ($F = 5.19$, $df = 1/24$, $p < .05$). Because no significant differences were evident on any frequency measure, only means and standard errors

TABLE 1. Rate, Duration, and Bandwidth Characteristics of Neonatal Ultrasonic Vocalizations.

	Rate (calls/min)		Duration (msec/call)		Bandwidth (kHz)	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Day 3 ♂	17.00	5.11	266.25	51.35	6.50	1.04
Day 3 ♀	23.00	9.71	225.00	21.75	8.00	.32
Day 8 ♂	55.00	.84	228.67	29.21	6.20	.67
Day 8 ♀	68.00	7.64	428.90	62.39	7.12	.79
Day 13 ♂	38.00	.89	62.44	14.75	1.94	.56
Day 13 ♀	37.00	5.05	167.38	20.63	3.41	.34

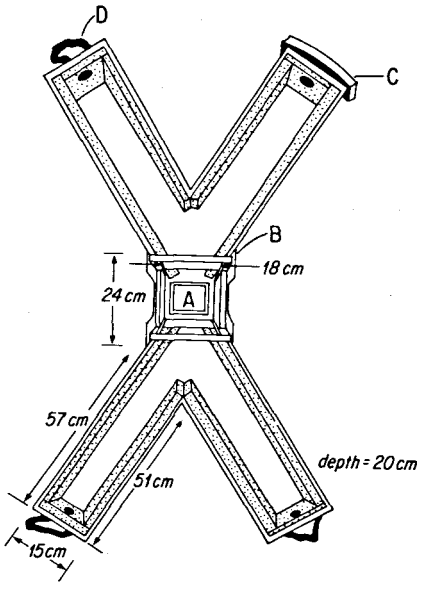

Fig. 1. Apparatus for behavioral testing of adults.

will be presented. Calls began at a mean frequency of 36.58 kHz (±.41) and terminated at a mean frequency of 40.95 kHz (±.54). Minimal and maximal frequencies were 34.17 kHz (±.60) and 44.33 kHz (±1.4), respectively.

Experiment II: Playback of Neonatal Tapes to the Adult Groups

Method

Selection of Adult Subjects

In order to assess the relative contributions of the presence of young, we established 2 conditions: (1) Females with litters. Three primiparous and 5 multiparous females reared their own litters to weaning, and young were present in the home cage at the time of testing. (2) Females without litters. Litters were removed within 24 hr after parturition from 4 primiparous and 5 multiparous females.

Primiparous and multiparous females were tested 5 days postpartum. In addition, 5 older females that were no longer bearing young, the "multiparous nonbreeding" females, were observed in order to separate the experiential effect from the postpartum physiological changes experienced by the other females that had recently delivered. Five nulliparous females were also tested.

Finally, 5 males were housed with nulliparous females and termed "nulliparous" males and 5 were housed with multiparous females and termed "multiparous" males. The latter group had previously been in contact with litters, and were tested to assess the effects of exposure to neonates. None of the females housed with the "multiparous" males had young in the nest at the time of testing.

Testing Apparatus for Playback

The behavior of adult gerbils during playback of recorded sounds was observed in a 4-armed plywood structure with a central nest compartment. (See Fig. 1.) The

nest chamber was partitioned off from the arms of the apparatus by the use of aluminum doors which could be raised and lowered. An aluminum nest box (18.5 × 16 × 5 cm) was situated in the center of the nest chamber and contained standard bedding and strips of paper. Plexiglas doors covered the top of the apparatus. The floor was varnished, and the arms were completely covered with acoustic tiling of 1.5-cm thickness to eliminate echoing of the sounds during playback.

In the end of each arm was a circular opening of 3-cm diameter through which sounds could be directed. In 1 arm the stimulus tapes of 3-, 8-, and 13-day-old neonates were played through the ultrasonic transducer. Random noise in the audible range (designated the control tape) was produced by a frequency generator (Hewlett-Packard Model 200CD/CDC). This was transmitted via a Fedtro PC-200 TDC speaker in the arm diagonal to the stimulus tape arm. To hinder echoing of sounds which might obscure the sound source, all speaker openings were backed with foam rubber lining 2 cm thick. Given stimulus and control tapes were played simultaneously during each trial.

A single sheet of plastic (Saran Wrap) covered the walls of the apparatus. The plastic was changed for each animal. In addition, the floors and ceiling of the apparatus were vacuumed and then washed with a detergent (Alconix) after each animal was tested.

Pre/Poststimulus Measures

To minimize handling effects we transported the adult animal from the home cage to the nest chamber of the apparatus via a clear plastic tubular module from the Habitrail system. The animal was allowed to move freely about the entire apparatus for 10 min prior to presentation of the tapes.

During the last 5 min of this period (the Prestimulus period) and during subsequent testing periods, behavioral items were recorded on an event recorder (Esterline-Angus Model AW). Items during the Prestimulus period included: frequency of entry and time spent in each arm of the testing chamber; frequency of entry and time spent in the nest box; and frequency and time spent in the activities of grooming, nest building, sleeping, vocalizing, and sitting alert. The behavioral categories adopted are those developed by Kaplan and Hyland (1972).

Following presentation of each trial, a 5-min intertrial interval elapsed. During this time, behavioral measures and frequency and time spent in the arms and in the nest box were recorded. These were the same as those recorded during the Prestimulus period.

Presentation of the Tapes

Following the Prestimulus period the Habitrail tube was again used to transport the animal to the nest box. The aluminum doors of the nest chamber were then lowered and remained closed until the onset of the stimulus period.

The animals received 6 trials lasting 60 sec each (Day-3 female vs control tape, Day-3 male vs control tape, Day-8 female vs control tape, etc.). Position and order of presentation of the stimulus tapes were randomized.

During each trial, data concerning frequency of entry and time spent in each arm of the chamber, frequency of entry and time spent in the nest box, and frequency and time spent in the behavioral measures were collected.

Results

Behavior in Nest Chamber

Because inspection of Pre- and Poststimulus measures did not reveal any significant differences, only the behavior during the Stimulus periods will be described.

The behavioral items, *sleep* and *vocalize,* were eliminated from analysis due to negligible levels of occurrence. Differences among groups on the behavioral measures, *in nest, nest build, groom,* and *sit alert,* were evident.

Primiparous Females Without Young entered the nest chamber more frequently than did the other groups ($F = 4.12$, $df = 7/174$, $p < .01$). However, the Primiparous Females With Young spent more time in the nest chamber ($F = 7.99$, $df = 7/174$, $p < .001$) and engaged in nest building most frequently ($F = 4.6$, $df = 7/174$, $p < .01$). Multiparous Females With Young showed the 2nd highest frequency of nest building. Duration of nest building did not differ from group to group.

The Nulliparous Females, Primiparous Females Without Young, and Multiparous Females With Young engaged in the most frequent bouts of grooming ($F = 2.41$, $df = 7/174$, $p < .03$). The latter 2 groups also spent the most time engaged in grooming activity ($F = 2.68$, $df = 7/174$, $p < .02$). Finally, Nulliparous Females and Males, and Multiparous Nonbreeders showed both the highest rates and durations for the item *sit alert* ($F = 2.58$, $df = 7/174$, $p < .03$ for duration; $F = 5.71$, $df = 7/174$, $p < .01$ for rate). (See Fig. 2.)

When the rate and duration of the behavioral items were examined for the 6 stimulus tapes, no significant differences were revealed.

Orientation to the Stimulus Tapes

Localization of the stimulus tapes was measured by noting whether or not the animal entered the arm from which the neonatal call was being amplified. Both rate of entry and time spent in each arm were recorded.

For all groups more time was spent in the stimulus arm than in the control arm ($F = 4.3$, $df = 1/174$, $p < .05$). No significant differences among the groups in frequency of entry or time spent within the stimulus arm were revealed, nor did the groups differ in response to male versus female pups. However, tests of orthogonal contrasts revealed that all groups spent more time localizing the calls of Day-8 young ($F = 6.4$, $df = 2/28$, $p < .01$).

Discussion

We have demonstrated that there are maturational and gender-related differences in several parameters of ultrasonic vocalizations of gerbil neonates. Spectographic analysis indicated narrower bandwidths and shorter durations of calls on Day 13, compared with those on Days 3 and 8. Day-8 calls of both males and females elicited the greatest amount of adult localization. The relative lack of difference between characteristics of Day-3 and Day-8 calls is problematic. Although Day-8 females did evidence much longer durations than Day-3 animals, the relationship did not hold for Day-8 males.

However, both Day-8 females and Day-8 males showed the highest rates of calling among the 6 groups. This is consistent with previous data derived from comparisons of cross-sectional and longitudinal litters of pups tested within the 1st 21 days of life (Kleese, 1976). Both of these groups also evidenced peak rates of calling on Day 8. These data are somewhat inconsistent with De Ghett's (1974) report of a peak on Day 4 and of higher frequencies in calls of 1- and 3-day-old gerbils. We do not know whether differences in equipment, experimental procedure, or subjects account for this discrepancy. Bandwidth was the only parameter on which gender differences occurred; this factor did not differentiate adult localization of male and female calls.

Presence of young in the female's home nest resulted in increased nest-related activity in the experimental chamber during presentation of the tapes. Thus, the hormonal and experiential concomitants of recent parturition were sufficient to increase time in the nest or frequency of nest building. Recent parturitional state was sufficient to increase number of entries into the experimental nest chamber by primparous females without young; however, these entries were brief and did not result

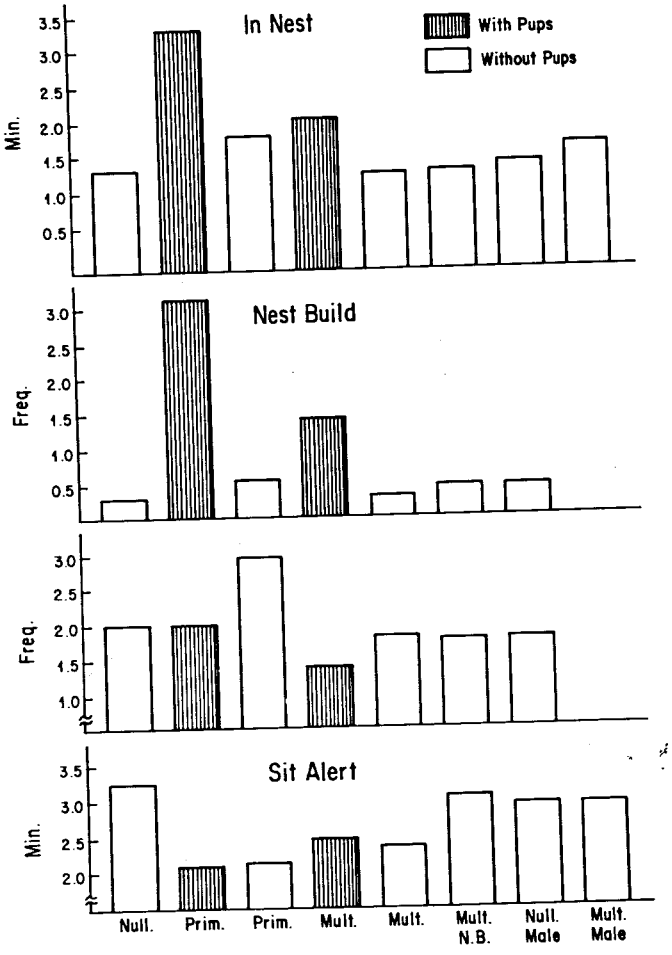

Fig. 2. Adult behavior during playback (6 tapes, 6 min).

in organized nest-building. Note that primiparous females engaged in greater amounts of nest-related behavior in the experimental chamber than did multiparous females. This is in contrast to the lack of differences between primiparous and multiparous females on a number of measures of maternal behavior in the home nest reported by Moltz and Robbins (1965). Nulliparous and multiparous nonbreeding females and males in this experiment all exhibited similar low rates of nesting activity and similar high rates of sitting alert, a behavior usually indicative of attention to a novel stimulus. Thus, neither gender nor previous reproductive experience differentiated the behavior of these currently nonreproductive animals.

In contrast to the effects on localization, all stimulus tapes were similarly effective in eliciting adult behavior in the nest chamber. Furthermore, the responses reported here do not simply reflect general tendencies for the animals to engage in the reported activities. Significant differences in behavior occurred only during the presentation of the stimulus tapes.

We view the role of ultrasonic signalling within a broader system of redundant stimuli that facilitate parent-young contact, in which the primary mover assuring successful contact shifts as a function of time. For the 1st few days postnatal, the pups are unable to leave the nest because of inadequate sensory-motor development. They are poikilothermic and require the mother's heat as well as her milk and grooming functions. Leon (1977) suggests that the temperature needs of the mother may determine length of nest bouts during the 1st 2 weeks postpartum. Initially, the mother spends a great deal of time crouching over and nursing the pups, which in turn produce few ultrasonic vocalizations except in response to rough handling. By Day 8 pups are able to leave the nest but are only beginning to be able to regulate their own temperature. The mother still spends considerable time with the pups and retrieves them very readily. At this time ultrasonic vocalizations reach their peak. By Day 13 thermoregulation and sensory-motor development have advanced but are not complete. The pups generate too much heat for the mother to spend as much time with them as before (Leon, 1977), and they are able to leave and return to the nest. In rats, by Day 14 the maternal phenomone begins to serve as an attractant (Leon & Moltz, 1973). By Day 18 or 19 thermoregulation is well developed and the mother spends relatively little time on the nest. The pups' eyes are open and ambulation is rapid and well directed. Ultrasonic vocalization ceases except in the event of rough handling. However, the maternal phenomone continues to attract the rat pups back to the nest until about 27 days postnatal. Thus, the increased rate of calling on Day 8 and the corresponding increase in adult responsiveness to those calls occur at the time of greatest need for active maternal retrieval. Furthermore, these vocalizations are able to elicit nest-building activity, in addition to localization, only in those females which have recently given birth and which continue to have young in their home nests.

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