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Thermal versus Tactile Stimuli and Audible Vocalization in Rat Pups[1]

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Abstract — The influences of thermal and tactile stimuli on the audible vocalization in pup rats, *Rattus norvegicus* were investigated. Mother rats and their litters were exposed to ambient temperature (AT) depression by 5℃ for 2 hours every other day from 3 to 11 days postpartum. Nearly all audible calls emitted by pups were recorded during tactile contact with the mother. AT depression alone neither prolonged the total duration of pup-licking by mother nor intensified audible vocalization. It was found, however, that the frequency (No./h.) of nest exiting by the dam doubled during the cooling and that the sum of audible peeps emitted by cooled pups in 4 cooling days was higher than sum of peeps emitted by the control pups. This difference is apparently related to an increase in the mother's motor activity, especially nest-building activity, during cooling. Analysis of circumstances accompanying peep emission supports the hypothesis that the tactile stimuli delivered by mother play the predominant role in causing audible vocalization in pup rats.

There are two independent groups of stimuli inducing ultrasonic vocalization in young rodents (Sales and Pye 1974). First, ultrasounds appear as a consequence of tactile stimulation, *e.g.* during retrieving, grooming the pups by female or an experimental touching of the animals by the observer. The other group of the stimuli is related to the nest desertion by a pup (its separation from the siblings and mother), which is probably connected with cold stress. It corresponds to peep emission caused by experimental isolation and/or exposing of the pups to lowered ambient temperature.

Many authors (Hart & King 1966; Smith 1972; Sales & Pye 1974; Sales & Smith 1978; Haack *et al.* 1983; Ehret & Bernecker 1986) emphasize the existence of the two physically separated vocalization types (audible and ultrasonic) in rodents, and those related to pup age and to *kind of stimuli* which reach the pups.

Newborn Norway rats, *Rattus norvegicus* (Berkenhout, 1769) are able to emit sounds with various duration and intensity, both audible to humans and pure ultrasonics just after birth (Calhoun 1962; Okon 1971a, b; Peters & Kristal 1983). Even 21-day-old fetal rats removed surgically from their mother's body produced audible calls (Peters & Kristal 1983). So far attention has been concentrated mostly on ultrasonic communication in rats, whereas there are more data on audible sounds produced by pups of other rodents (Smith 1972; Sales & Pye 1974; Haack *et al.* 1983; Ehret & Bernecker 1986; Dempster & Perrin 1989). Only Noirot (1968) and Okon (1971a, b), who studied the ultrasounds emitted by pups in Norway rat, made some observations on their audible vocalization and suggested that these calls were probably elicited by tactile stimuli.

This work is an attempt to find more data to answer the still open question: which stimuli — thermal or tactile — influence the generation of the audible calls in rat pups.

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Subject and Methods

Sixteen mother rats with their litters, 4 pups per dam, were used in the experiment. Adult females were 12 outbred Wistar and 4 outbred hooded rats at the age of adout 150 days. All were bred to Wistar males. Before the experiment started, the rats had been kept at 21 ± 1℃ and adapted to 12/12 h light-dark cycle (light phase: from 8:15 a.m. to 8:15 p.m.). Four or five days before birth, pregnant females were separated and put singly in standard plastic cages provided with shavings and shredded sisal fibre. Each cage had one of its corners covered with a cardboard roof. Standard laboratory rodent food (Murigran) and water were available *ad libitum*.

Of 8-10 females bred at the same period, 2 dams, which delivered at the same day (± 18 hours), were chosen. On the 2nd day postpartum their litters were reduced to four pups (2 males and 2 females). Each time the observation was performed, one family was randomly assigned to experimental (Ex) group, the other control (C). They formed a pair to be observed simultaneously. The distance between the cages of observed pair was 30 cm.

After concluding tests on pair, another pair of families was chosen for next observation. Each of Ex and C groups comprised 8 families.

Procedure

Interactions were observed from the 3rd to 11th day of the pups' lives. On cooling days (3rd, 5th, 7th, 9th and 11th days of age), the temperature inside the Ex group cages was lowered by 5℃ (from 21 ± 1℃ to 16 ± 1℃) for 2 hours. Temperature was decreased by placing the cage in cool water (5 cm deep). During the whole experiment the control group cages remained at 21 ± 1℃ . The temperature was measured with a mercury-in-glass thermometer placed inside the cage, in the corner diagonal to the corner with the nest.

On the 3rd day the cooling of the Ex group rats was only aimed at making them sensitive to thermal stimuli. On the remaining days (from the 4th to the 11th one) both families of a given pair (*i.e.* the Ex group family and the C group family) were tested simultaneously for 2 hours per day: between 6:00 and 8:00 p.m. Recorded were: (1) total number of audible calls, (2) total duration of puplicking by mother rats, (3) total number of each female's leaving/entering the nest, (4) circumstances accompanying emission of pup calls.

Vocalization bouts jointly emitted by offspring were scored as one. Bouts were separated by at least 1 second. Uninterrupted "choral vocalization" lasting several seconds and a short single peep of one pup were both recorded as a single vocalization bout.

Durations of all pup-licking episodes by mother rats lasting at least 5 seconds were recorded.

For statistical analysis the following pairs of noncooling/cooling days were formed: the 4th & 5th, 6th & 7th, 8th & 9th and 10th & 11th. In turn, the analysis of differences between the Ex group and the C group became available due to the comparison of results obtained for the groups on each day postpartum (the pups' age in both groups was the same). Mann-Whitney U-test and sum of ranks test were used (Sokal & Rohlf 1981; Gren 1982).

Results

The results of 1st and 2nd hours of each observational session were jointy represented in Figures 1 and 4. There were no statistically significant differences (sum of ranks test) between the data collected during the 1st and 2nd observation hours.

The number of audible calls recorded during the absence of the females from their nests was frequently close to zero for control and experimental rats (Fig.1). Conversely the levels of pups' vocalization during periods of mother nest residence vary from about 35 calls/hour on day 5 to about 10 calls hour on day 11. These levels thus are always much higher than those during periods of no mother-pup tactile contact ($\chi^2 = 11.29$, $P < 0.001$, sum of ranks test). On the other hand, in the Ex group the differences between cooling and noncooling days — comparison within pairs of days — shows no statistical significance (Mann-Whitney U-test).

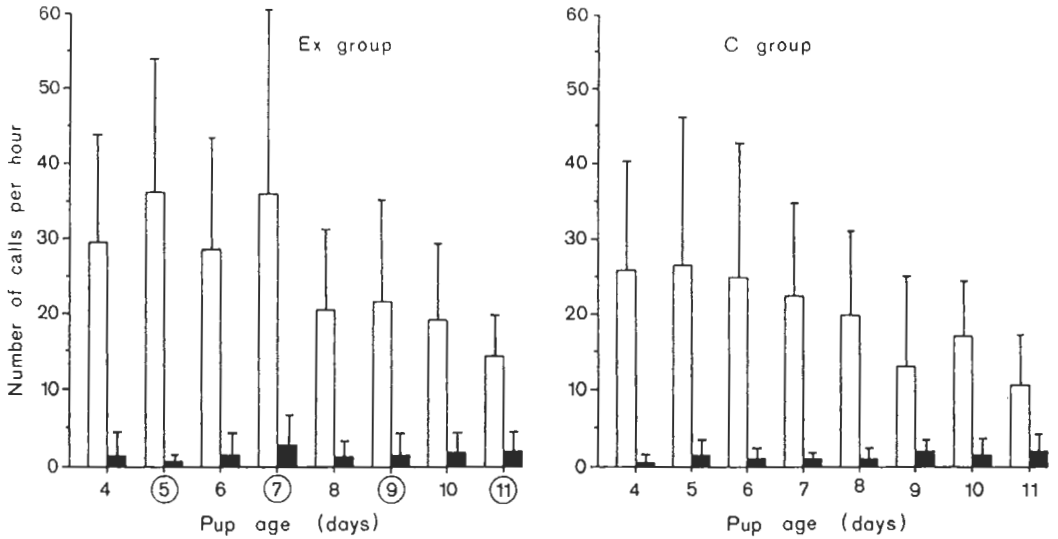

Fig. 1. Mean daily number of audible vocalization bouts per hour emitted by pups during two hours of observation on the subsequent days of the pups' lives. Bars represent the number of pup calls emitted during presence (open) or absence (closed) of the mother in the nest. In each group, the data were obtained on eight litters. Numbers in circles stand for days when the Ex group was subject to cooling by 5℃ for 2 hours. The SD are showed.

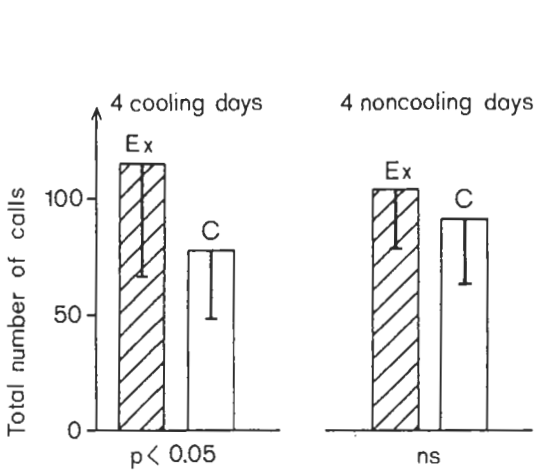

Fig. 2. Mean (n = 8) number of all audible calls emitted by offspring of females from the Ex and the C group within 4 cooling days and 4 noncooling days. The SD are showed.

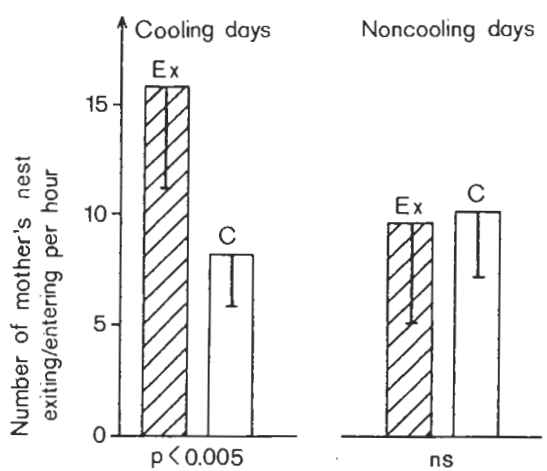

Fig. 3. Mean (n = 8) number of mother's nest exiting / entering per hour for the Ex and the C group on cooling days and noncooling days. The SD are showed.

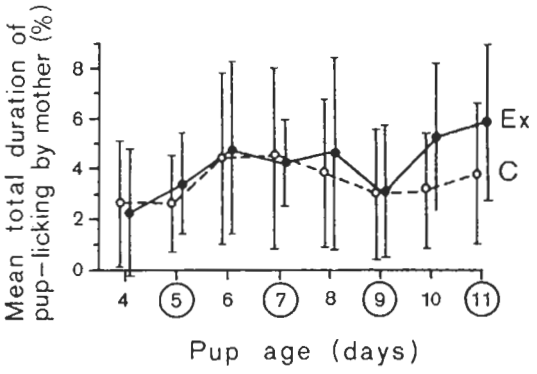

Fig. 4. Mean (n = 8) daily duration of pup-licking by mothers from the Ex and the C group during two hours of observation. The data are presented in percents where 100% = 60 min. The SD are showed. Abscissa denotations as in Fig. 1.

The sum of peeps emitted during the presence and absence of mother in the nest on each of the succeeding days (especially the cooling days) is somewhat higher (but not significantly so) in the Ex group than in the C group. Only on the 11th day postpartum did the difference between groups attain statistical significance ($U = 49$, $P = 0.05$, Mann-Whitney U-test).

Nevertheless, the total number of calls emitted during all four cooling days is significantly higher in the Ex group than in the C group at the same days ($\chi^2 = 3.98$, $P < 0.05$, sum of ranks test; Fig.2). The analogous comparison of calls recorded within four days without cooling does not show any significant difference.

On cooling days Ex group mothers (see Fig.3) left and returned the nest about twice as often as the C group mother ($\chi^2 = 8.65$, $P < 0.005$, sum of ranks test). On the days without cooling the two groups did not differ in this regard.

In the Ex group (see Fig.4) the mean total duration of pup-licking by a mother on the cooling days does not differ statistically when compared to the days without cooling. In the C group the analogous comparison does not show statistically significant differences either. The differences between values obtained in the Ex group on the succeeding days and the ones from the C group are statistically insignificant except days 10 and 11 (on these two days $U = 52$ and $U = 50.5$ respectively, $P < 0.05$, Mann-Whitney U-test). Thus, the experimental reduction of temperature did not cause any perceptible changes in pup-licking behaviour.

Audible vocalization was observed in the following situations:
- pup-licking by mothers (vocalization almost always occurred and lasted dozen of seconds),
- mother's self-grooming,
- before and during position changes of mother in the nest,
- mechanical traumas *e.g.* when the female pressed a pup down, trod on it or mouthed it (vocalization was sometimes very loud and usually shorter than during pup-licking),
- other presumable discomforts *e.g.,* when a pup could not reach the teat or squeeze under the mother, when it fell out of the nest or when the mother pulled it out of the nest while it was still attached to her teat,
- when the mother rebuilt the nest being inside or outside it,
- at the moment of the mother's return to the nest (often choral calls),
- during the mother's absence from the nest: when pups displayed huddling behaviour (single peeps).

Any prolonged and/or loud squealing of the pups almost always made the mother react and change her position.

Discussion

It appears that pups, regardless of treatment group, emitted almost all of their audible calls during tactile contact with their mothers. Only a few peeps were recorded during the mother's absence from the nest. It happened when the female was rebuilding the nest being outside it and during pups' huddling behaviour. These facts suggest that audible peeps are emitted chiefly as a consequence of tactile stimulation most often delivered by their mother.

Rat pups are devoid fo efficient thermoregulatory mechanisms until 24th day of their life (Melanie *et al.* 1986). Thus, if the ambient temperature is below optimum, pups begin to suffer from a cold-stress soon after the interrup-

tion of the tactile contact with their mother (Leon *et al.* 1978; Chaber & Korda 1984). This regularity applies to the present work where the ambient temperature was lowered by 5℃ (the Ex group pups). Lack of audible vocalization by pups remaining in the nest without their mother was observed even in a lowered temperature. Therefore, it can postulated that audible peeps of rat pups are not caused by thermal stimuli, and that their function is neither to inform the mother about the cold-stress nor to call her back and arouse the retrieving reaction, as has been suggested of pup ultrasonic vocalization (Bell *et al.* 1974; Jans & Leon 1983; Ehret & Haack 1984; Ehret & Koch 1989).

It was ascertained that the number of peeps emitted by cooled pups from the Ex group was slightly higher than that of C group. This was not the result of an intensification of mother's pup-licking because the total duration of licking was not increased during cooling. However, the observed difference between the groups was apparently caused by the increased motor activity of the mothers in Ex group during nest building activity (Korda & Komorowska 1987; Rychlik & Korda 1989). Steming from it more numerous mother nest exiting / entering as well as more frequent position changes in the nest could deliver more frequent tactile stimuli to pups.

There are at least three functions which can be attributed to pups' audible vocalization in rodents. The first is to inform the mother of the discomfort felt by her offspring. The second is to inhibit mother's or other adults' aggression towards intraspecific pups (Noirot 1966; Allin & Banks 1972; Smith 1972; Haack *et al.* 1983; Peters & Kristal 1983). And the third, perhaps audible vocalization plays a very important role also in the establishment and maintenance of the care-giving maternal responsiveness after parturition which largely depends on pup-derived stimuli (Terkel *et al.* 1979; Rosenblatt & Siegel 1980; Peters & Kristal 1983). The methods used in this work were insufficient to verify above possibilities and further studies should be carried out.

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