ISSN 1517-6770

Exploratory behavior of female rats born to differently raised mothers

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ABSTRACT: Fifty-four rats (*Rattus norvegicus Berkenhout, 1832*) were raised under four different conditions: individually or in groups and with or without frequent handling. At 90 and 120 days of age were tested in the exploration of a complex environment. Differences in performance between raising types were detected. The animals were mated and after pregnancy and delivery mothers and their litters were weekly submitted to the same exploration tests. After weaning, female pups from each litter were randomly selected and raised under standard conditions. These females were then tested at the same time intervals, i.e. at 90 days and them again at 120 days using the same device for the evaluation of exploratory performance. The results showed no systematic influence of the conditions under which the corresponding mothers had been raised on the 4 groups of daughters. However, there was a significant difference ($p<0,05$) in latency to leave the den, time outside the den covered distance between mothers and daughters. Explanatory possibilities are discussed, with emphasis on the complexity and subtlety of the structuring of the behavioral regulation processes.

Key words: Exploratory Behavior, Maternal Influence, Mother-pup Interaction.

INTRODUCTION

In the development of behavioral regulation of rodents, environment influences are of paramount importance. Fossorial altricial rodents such as the rat receive their first environmental stimuli from their social (mother and siblings) and physical (burrow and nest) environment. These stimuli are of three main types: thermotactile from the mother and siblings (nursing, licking, occasional retrieving and general body contacts), olfactory (urine, feces, nesting material, body odors of the mother and siblings), and gustatory, mainly milk and the substances secreted into it (for a review, see PORTER, 1983).

There is vast experimental evidence of the importance of the importance of such stimuli. Raising male rats in groups or

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Pesquisa apoiada por: CNPq (n° 142395/93-5)

depriving them of social contact affects not only their subsequent social interactions (HOLE, 1991) but also their performance in other nonsocial behaviors such as the level of activity in an open field test (SYME, 1973; REBOUÇAS & SCHMIDEK, 1997) or the exploration of a complex environment (REBOUÇAS & SCHMIDEK, 1997). Females react in a similar manner when tested in an open field but inversely in the exploration of a complex environment (GENARO & SCHMIDEK, 2002) which is explored less by animals reared in isolation.

Experimental tactile fur stimulation provided by regular handling also affects subsequent behavior. Postnatal handling of rats has been reported to reduced neophobia (BODNOFF *et al*., 1989), to increase the subsequent resistance to stress (HILAKIVI-CLARKE, *et al*., 1991), and to have positive effects on emotional behavior depending on the intensity of the benzodiazepine receptor system (ESCORIHUELA *et al*., 1991). The activity levels of adults in open field tests are increased and the subsequent corticosterone release is reduced (HESS *et al*., 1969). Male rats handled daily during development have a substantially reduced latency to enter a new and complex environment and an increased exploration of this environment (REBOUÇAS & SCHMIDEK, 1997). In female rats, handling has a marked influence in reduced their emotionality and consequently increasing both the activity in open field tests (NÚÑEZ *et al*., 1995) and the initial exploration of a complex environment (GENARO & SCHMIDEK, 2002). It is important to take into consideration the conditions of the exploratory test since the properties required for the performance of exploratory behavior may be involuntarily forgotten when the animals, rodent in particular, are submitted to laboratory conditions. Thus, it is fundamental importance to provide the animals with appropriate conditions for a full performance of this behavior (GENARO *et al.*, 2004; POUCET & HERMANN, 2001).

Stimuli from the mother are considered to be an important source of social stimuli received by rat pups (PORTER, 1983; RIUL *et al*., 1999; YA AMSTISLAVSKY *et al*., 2001). Absence of olfactory stimuli in situations in which the pups are isolated and placed on clean bedding material induces ultrasonic vocalizations (OSWALT & MEIER, 1975). Lactating females have been described to produced a pheromone that is especially attractive from the 12th day on but whose attractiveness decreases after the third week of life their pups (PORTER, 1983). This is the time interval between the first attempts to leave the nest and weaning (leaving the burrow). The attractiveness of this smell is at least in part learned and influences the later social behavior of the animals as shown by cross-fostering experiments (STARK & HAZLETT, 1972).

Other pheromone-like substances are secreted by rats in stressful situations such as forced swimming (ABEL, 1991_a) and have a long-lasting effect on the behavior of other rats (ABEL, 1991_b; 1992). A stress-inducing effect of these substances could be detected already in 24 day old rats (ABEL, 1993). Hypothetically such substances might be secreted in smaller but still detectable amounts also in less fear-inducing situations. Animals differing in emotionality might thus differ in secretions levels of such substances, producing different “emotional environments” for their social group (e.g. their offspring).

In the present experiment we tested the hypothesis that rearing conditions (isolation x non isolation; handling x non handling) of female rats might not only affect their own individual behavior but also change the stimulation pattern they will present to their pups, leading to alterations in the behavioral regulations of the latter.

MATERIAL AND METHOD

We used 54 females rats (*Rattus norvegicus* Berkenhout*, 1769*) of the Long-Evans black hooded strain born in our laboratory and raised under four different conditions: with (H) or without (N) systematic handling (1 min of daily gentle fur rubbing from the 28th to 120th day of life) and in groups of four (S) or in isolation (I).

Four experimental groups (HS, HI, NS and NI) respectively of 16, 13, 12, and 13 animals were formed, for a total of 54 dams. The animals were housed in plastic boxes (31 x 46 x 17 cm), water and food were available *ad libitum* and the light cycle was 12:12 h with lights on 06:00 h.

These females were evaluated for performance in exploration of a complex environment (GENARO & SCHMIDEK, 1999; 2002) at 90 and 120 days of life. The animals were mated and after pregnancy and delivery were submitted weekly to the same exploratory test together with their young which were from litters of 8 to 10 pups from primiparous mothers, with mating and pregnancy occurring simultaneously in each female.

A weaning female was randomly chosen from each litter for a total of 54 daughters. These daughters were all raised under intermediate conditions, i.e. they were kept in pairs (daughters of mothers submitted to the same treatment) and handled only once a week when the bedding of the cages was changed. They had already been tested together with their mothers (when these daughters were 2, 8, 14, 21 and 28 days old; however, due to the late state of locomotion of these animals, they were able to move to the testing environment only they reached 21 and 28 days) and were again tested in exploration of the complex environment, now individually at 90 and 120 days of age.

Schematic presentation of the experiment. Experimental Procedure (Exploratory Test):

Test at (days): ———90———120——————————————90———120: [**Figure 1**].
(Mothers) (Daughters)
———21———28—————90——120: [**Table 1**].
(Daughters)

For the exploratory procedure the animals (mother and daughter) were adapted to a den cage (with an opening that could be kept open or closed and thus be connected to the behavioral test environment) similar to the maintenance cage for 30 min before the test. The test, lasting 15 min, was performed between 11:00 and 16:00 h and started by the opening of a tunnel, a rigid plastic tube 7.6 cm in diameter and 10 cm long, that connected the den cage with an "environment" consisting of a series of five communicating 20 x 60 x 25 cm compartments with cover and front wall of transparent glass. Each compartment consisted of two 20 x20 wooden platforms separated by a 20 x 20 x 10 cm water tank. Access to the rear platforms was provided by cross walks of different widths. After each test the floor and walls of the system were cleaned with a moist cloth and then carefully dried.

Every test was recorded on video tape and subsequently transcribed to a computer for analysis. In the first series of exploratory tests we measured the latency for the first exit of a young from the den cage on the 21st and 28th days (Tab1, only daughters). In the subsequent individual exploratory tests at 90 and 120 days (Fig 1: mothers and daughters, and Tab. 1, only daughters) we measured the latency for first exit from the den, total time outside the den, and traveled distance.

RESULTS

The mothers reduced intensely their exploratory performance during the first two exploratory tests together with the two and eight day old young. In the third test the adult females began to increase their time outside the den, reaching the usual values for this variable, and increased the traveled distance in the fourth and fifth tests when the young were already 21 and 28 days old and followed the mother in her exploration. In these last two tests there were significant differences in the time outside the den and the traveled distance among the adult females according to their raising conditions (GENARO & SCHMIDEK, 2002). Thus, different litters exploring this new and complex environment together with their mothers had contact with the patterns of their mothers. No significant differences in latency for the first young to reach the “environment” (see Tab. 1) could be detected between groups. Nevertheless, the latency decreased by two thirds from the test at 21 days to the test at 28 days of age.

Table 1. Exploratory performance of daughter animals grouped according to the raising condition of their mothers. Mean values and corresponding ANOVA values of the variables: Latency for the first young of the litter to leave the den in the exploratory test at 21 and at 28 days of life (litter and mother tested together); Latency to leave the den; Time in the environment and Distance traveled by the young adult daughter animals tested individually. CV: coefficient of variation; MSD: minimal significant difference; DF: degrees of freedom; ns: not significant

	21 days Latency	28 days Latency	Latency	90 days Time	Distance	Latency	120 days Time	Distance
	✧	✧	✧	◆	⌘	✧	◆	⌘
Social Factor								
Non Isolated	45.40	15.97	18.41	707.36	2147.81	9.03	720.60	2076.24
Isolated	48.86	14.42	13.30	741.16	2153.14	9.71	713.84	2142.50
F Test	0.06^{ns}	0.07^{ns}	0.65^{ns}	1.69^{ns}	0.001^{ns}	0.04^{ns}	0.04^{ns}	0.20^{ns}
Handling Factor								
Non-Handled	52.53	13.34	16.84	726.27	2292.26	10.55	724.94	2173.17
Handled	71.73	17.04	14.88	722.25	2008.69	8.19	709.50	2045.56
F Test	0.59^{ns}	0.39^{ns}	0.10^{ns}	0.02^{ns}	3.59^{ns}	0.44^{ns}	0.21^{ns}	0.76^{ns}
Interactions	0.11^{ns}	1.00^{ns}	1.09^{ns}	1.61^{ns}	5.16*	0.26^{ns}	1.95^{ns}	1.71^{ns}
CV	107.89	133.28	121.64	10.98	20.94	114.64	14.44	21.10
MSD	28.33	11.96	12.90	52.80	304.19	7.22	69.06	297.72
DF	48	48	35	35	35	35	35	35

✧ - Latency (in s) for the first exit from the den compartments.
◆ - Total Time (in s) outside the den compartment during the 15 minutes of the test.
⌘ - Total Traveled Distance (in cm) during the test.
* - $p<0.05$

From 28 to 90 days of life the randomly selected young females from 4 mother groups were kept under equal conditions in 45 x 30 x 12 cm plastic cages with only one female companion (with the same original mother treatment) with brief handling once a week.

At 90 and at 120 days of age these animals were put again in contact with the exploration set. Now they were tested individually and their results are also presented in Table 1. When their individual results were grouped according to the raising conditions of their mother no significant effect or systematic tendencies of these conditions could be detected. Thus, we conclude that raising conditions of the mothers had no detectable effect on the exploratory performance of their young adult daughters in this situation.

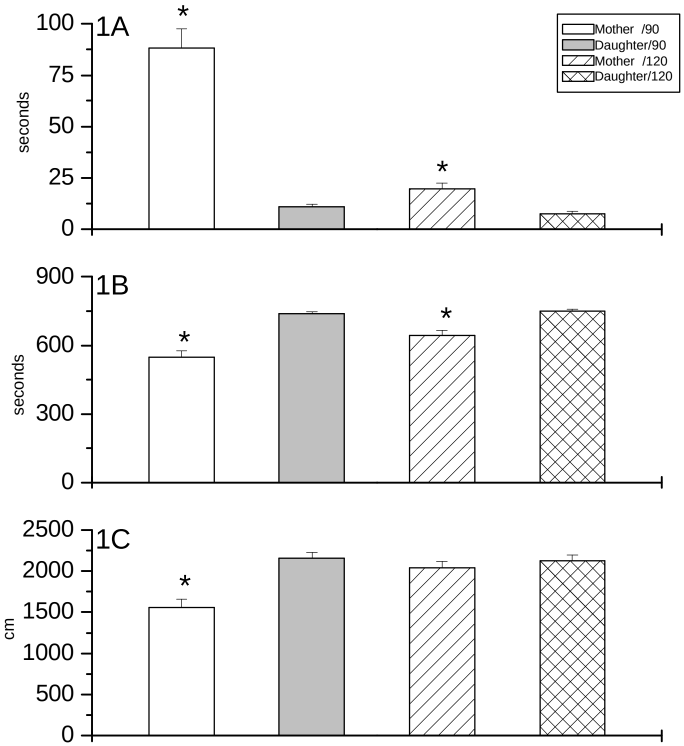

Figure 1. Exploratory performance of mother and daughter animals at 90 and 120 days of life. Mean ± SEM: (1A) latency to leave the den (in s); (1B) time outside the den (in s); (1C) covered distance (in cm). The number of animals used (mothers and daughters) was 54 for each condition. * $p<0.05$.

DISCUSSION

Several possibilities can be considered in the discussion of these results. First, we may assume that the testing conditions were too nonspecific to elicit measurable effects. However, these same equipment and procedure have been used in previous studies yielding evidence both of individual and group differences between male and female rats and mice (NEGRÃO & SCHMIEDK, 1987; PINTO & SCHMIDEK, 1994; REBOUÇAS & SCHMIDEK, 1997; GENARO & SCHMIDEK, 1999, 2000, 2002, 2004).

A second possibility is that the stimuli provided by the experimental situation were too feeble to have a long lasting effect on the young. However, when we compared globally the performances of the daughter rats with the overall performances of their mothers at the same ages (Figure 1) an important picture appeared. Daughter rats first tested as young animals (at 21 and at 28 days of age) showed a markedly different performance compared to their mothers at the corresponding ages (at 90 and at 120 days of age, both for mothers and daughters). Daughter animals left the den compartment within the first 10 seconds, a latency that was substantially and significantly lower than their mother's. They also remained a longer time in the environment and covered a greater distance. At 120 days of age this difference between groups decreased but remained significant for the latency to leave the den and the time spent in the environment.

This strongly suggests that even a brief exposure to the testing conditions at a young age imposed a definitive mark on the subsequent behavioral performance of the daughter animals. Actually this is not surprising in view of the evident excitation that was shown by the young animals during their first excursions out of the "den". An additional possibility is that the signals emitted by the different mothers were too weak to compete with the other stimuli occurring to introduce a differential mark in the behavioral regulation process of the young. Differences in emotionality due to the studied raising conditions and leading to differences in exploratory performance have been clearly detected in the set used by our group (REBOUÇAS & SCHMIDEK, 1997; GENARO & SCHMIDEK, 2002). However, it is possible or even probable that such differences are not expressed in the monotonous and safe breeding cage. Even the exploratory set with which the mothers had been acquainted prior to breeding (test at 90 and 120 days) may have been an insufficiently stressful situation to trigger evident signs of fear. In fact, the described alarm pheromone (ABEL, $1991_{a;b}$) is released by rats in the forced swimming test, a quite radical situation. Contributing also to this stimulus blurring are the other young of the litter that probably emit a variety of different and even contradictory signals both in the maintenance cage and in the exploratory set. They showed remarkable ambivalence at the opening of the tunnel where they exhibited repeated to and fro movements.

A final possibility is the age to which the social contact with the mother and the first contact with the testing situation were restricted. In fact, ABEL (1993) showed that the reaction to the alarm pheromone was only discernible when the young animals reached 24 days of age. PORTER (1983), when discussing communication between adult and infant rodents, pointed out that in altricial and fossorial species such as the rat the young do not leave the nest in the first two weeks of life and do not exit from the burrow until weaning. In such species social and especially alarm signals are of small biological relevance during this first period of life. Thus, a possibility that remains to be tested is the effect of a longer mother-daughter contact extending well beyond weaning and including exposure to more stressful situations.

ACKNOWLEDGEMENTS

The author thanks Humberto Giusti for technical assistance and Prof. Dr. José Carlos Barbosa for statistical analysis.

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Recebido: 22/06/04
Aceito:31/03/05