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Postnatal Handling Reduces Anxiety as Measured by Emotionality Rating and Hyponeophagia Tests in Female Rats

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FERRÉ, P., J. F. NÚÑEZ, E. GARCÍA, A. TOBEÑA, R. M. ESCORIHUELA AND A. FERNÁNDEZ-TERUEL. *Postnatal handling reduces anxiety as measured by emotionality rating and hyponeophagia tests in female rats.* PHARMACOL BIOCHEM BEHAV 51(2/3) 199-203, 1995.—The present studies evaluated the short- and long-lasting effects of postnatal handling (administered during the first 21 days of life) on the emotional behavior of female Sprague-Dawley rats. The performance of postnatally handled (H) and control nonhandled (NH) animals was compared in two different situations: an emotionality rating (ER) test (when they were 40 days or 4 months old), and a hyponeophagia (neophobia) test of anxiety, at the age of 4 months. The results showed that postnatal handling induced both short-term and long-term reductions of spontaneous emotional reactivity in the ER test, although the effects on some measures disappeared in 4-month-old rats. Postnatal handling also induced enduring decreases of anxiety as measured by the hyponeophagia test. None of the observed effects were attributable to changes in basal locomotor activity. ER measures were significantly related to hyponeophagia, because animals showing the highest emotionality scores in the ER test (preferentially NH animals) were those that showed the highest eating latencies and spent less time eating in the neophobic situation (i.e., hyponeophagia test).

Postnatal handling   Emotionality ratings   Hyponeophagia   Anxiety   Developmental course   Rats

POSTNATAL handling (H) of rats (during the first 21 days of life) has been shown to reduce emotional reactivity or anxiety on a long-term basis, as measured either by endocrine reactivity to stress or by defecation, exploration, and avoidance behavior in different situations involving novelty and/or conflict (7-12,15-17,19,24,25,28,29,32,33). However, some important inconsistencies have appeared among results from different laboratories using similar testing situations.

Thus, a reduction in anxiety as a consequence of postnatal handling has been reported by Fernández-Teruel et al. (12) using the elevated plus-maze test, whereas Wakshlak and Weinstock (31) did not find significant effects of that treatment in the same test. In a different model of anxiety, the hyponeophagia test, Bodnoff et al. (4) reported an anxiolytic-like action of postnatal handling, whereas Hilakivi-Clarke et al. (21) failed to find it. Finally, Eells (8) as well as Ader (1) found a reduction of emotionality after postnatal handling by using an "emotionality rating" test, but such an effect was not replicated by Maier and Crowne (27).

It is worth considering, in that regard, that (as it is widely accepted) emotionality is a "complex of factors" (20), and that trying to analyze emotional reactivity or anxiety on the basis of single measures within them can be one of the reasons to explain those inconsistencies. Two other major sources to account for the frequent unreplicability could be constitutional differences among the animals [i.e., sex and strain or stock differences (13,18)] as well as differences in postnatal handling procedures.

The present study was aimed to test postnatal handling effects on emotionality rating (ER; see description below) measures at two different developmental stages, because previous studies have not evaluated the persistence of H effects on ER test measures along development (8,27,33). A further purpose was to study H effects on hyponeophagia in adult

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rats, as well as to evaluate the possible relationships among both emotionality measures.

Emotionality rating (ER) tests are based on scoring such spontaneous emotional behaviors as vocalization, defecation, resistance to capture, and/or to handling and preference for novel environments [e.g., (8,22,23,26,33)]. Since the earliest studies of postnatal handling from the 1960s, different types of ER tests have been used to evaluate emotional differences among H and nonhandled (NH) rats [e.g. (1-3,8,11,16,22, 23,27,33)] and more recently among animals habituated to adult handling and their nonhandled controls [(5,6,14); and unpublished results]. The evidence overall gives support to the validity of ER test measures as reflecting emotionality in rats [e.g. (14,26)]. On the other hand, hyponeophagia (unconditioned inhibition of feeding due to novelty) has been used to assess emotional reactivity or anxiety (20), and it iss considered to be a valid animal model of anxiety because it is sensitive to anxiolytic (30) and anxiogenic drugs (our unpublished results). Studies on hyponeophagia have also shown that, to ascertain whether treatments affect anxiety or/and other behavioral aspects (e.g., activity) in that test, measuring several parameters is better than recording only one measure.

Accordingly, in the present study several parameters were scored in both the ER and hyponephagia tests, to maximize both the appearance of H effects and their possible interpretation.

METHOD

Animals

Twenty-four pregnant Sprague-Dawley (IFFA-CREDO, France) rats were used. They were individually housed and maintained with food and water freely available, with a 12L : 12D cycle (light on 0800 h) and controlled temperature (22 ± 2°C). Rats were randomly distributed across the two experimental groups to which their offspring would be assigned. Experimental groups were "handled" and control "nonhandled" (H and NH, respectively; see below) and consisted of animals from 9-11 different litters in each group.

Handling Procedure

Postnatal handling treatment (administered between 1 and 22 postnatal days) consisted of first removing the mother and then the pups (10 per litter, half of each sex) from the nest (between 1000 and 1200 h daily). Pups were then individually placed in plastic cages lined with paper towel while gently caressing them for 2 s, and 15 min later they and their mothers were returned to their home cages (this step also involved individually caressing the pups for 2 s). Nonhandled (NH) pups were left undisturbed until weaning, except for routine sawdust change that was done only once during the first 22 postnatal days.

After weaning (23rd postnatal day) all pups were housed (two per cage) in standard laboratory conditions.

Behavioral Testing

Experiment 1. Forty female offspring (40 days old; weight 184.5 ± 3.5 g), 20 from each experimental group, were used.

Rats were submitted to an emotionality rating (ER) test, adapted from several previously published procedures (1-3,5,6,8,11,14,22,23,26,33), to assess their emotional reactivity. The experimental situation consisted of two adjacent cages (50 × 27 × 15 cm), one of them being the rats' home cage and the other being an identical (but new) cage containing clean sawdust. Because the walls of both cages were in contact, animals could easily climb from one cage to the other when the home cage cover was removed. Thus, testing started by removing the home cage cover and then an experimenter, who was unaware of the animal's treatment, recorded the following parameters during 1 min: 1) number of ambulations (one ambulation was recorded each time the animal moved horizontally in the cage) performed in the home cage (AMB1) and in the new one (AMB2), and 2) number of rearings (standing on its hindlegs) performed in the home cage and in the new one (REAR1 and REAR2, respectively). Thus, the total activity performed in the home cage (ACT1 = AMB1 + REAR1) was distinguished from that exhibited in an unknown (new cage) environment (ACT2 = AMB2 + REAR2).

Immediately after that 1-min test, the experimenter picked up and weighed the animal while scoring its "reaction-to-handling" (i.e., whether the animal exhibited resistance to being captured and/or to being handled while the experimenter picked it up for and after weighing; scoring "0" as absence, and "1" as presence). Defecation and vocalization were not included because no rats defecated or vocalized. Testing was performed between 1000 and 1300 h.

Experiment 2. Forty female offspring (4 months old, weight 326.3 ± 6.8 g; NH = 18 and H = 22) were tested in the ER test as described in Experiment 1. One month later, they performed a "hyponeophagia" test. All the animals were food deprived according to a progressive 15-day schedule resulting in a final 10-day period during which animals ate for 1 h (1700-1800) daily.

Testing was performed in a brown wooden box (57 × 28 × 32 cm) with eight holes in the bottom (diameter 5 cm), each containing a plastic recipient with a piece of chocolate. Testing started by placing the animal in the apparatus facing a wall corner. The following parameters were recorded during 10 min: approach latency – time (s) elapsed until the animal performed the first exploration of a hole; eating latency – time (s) elapsed until the rat started to eat; time spent eating – time (s) spent eating chocolate; amount of food eaten (in g) during the 10-min test.

The hyponeophagia test was performed between 1600 and 1900 h.

TABLE 1

MEAN ± SEM OF "ER" TEST VARIABLES

	n	ACT1	ACT2	Reaction to Handling (% of Animals)
Experiment 1				
Nonhandled	20	5.5 ± 0.9	3.1 ± 0.8	85.0
Handled	20	6.3 ± 0.9	5.7 ± 0.8*	5.0†
Experiment 2				
Nonhandled	18	10.1 ± 0.9	3.3 ± 0.9	72.2
Handled	22	10.7 ± 1.1	3.0 ± 0.9	18.1†

ACT1, activity in the home cage; ACT2, activity in the new cage; "% of animals," percentage of animals showing reaction to handling (reaction to capture and/or to being handled) in each group.

*$P < 0.05$ vs. nonhandled group (Student's t-test after significant ANOVA).

†$P < 0.001$ vs. nonhandled group (chi-square test).

TABLE 2

MEAN ± SEM OF HYPONEOPHAGIA VARIABLES

	Approach Latency (s)	Eating Latency (s)	Amount Eaten (g)	Time Spent Eating (s)
Nonhandled (N = 18)	51.8 ± 9.9	525.5 ± 34.8	0.15 ± 0.1	22.7 ± 9.7
Handled (N = 22)	49.4 ± 13.1	334.3 ± 43.6*	0.61 ± 0.2†	75.6 ± 15.2*

*$p < .01$ vs. nonhandled group (Student's t-test).
†$p < .05$ vs. nonhandled group (Student's t-test).

Statistics

Activity measures from the ER test were analyzed by two-factor ANOVAs (factors: handling and activity location; with repeated measures on the latter) followed by Student's t-test. Reaction-to-handling results (ER test) were analyzed by a chi-square test. Hyponeopahgia variables were analyzed by Student's t-test. Chi-square contingency tests were also applied to assess the relationship or independence between performance in the ER and hyponeophagia tests after distributing eating latency, time spent eating, and amount eaten variables into six equal intervals.

RESULTS

In Experiment 1 (Table 1), postnatal handling significantly increased activity in the new cage (ACT2; $t = 2.25, p = 0.03$ vs. NH group) without affecting behavior in the home cage (ACT1) [ANOVA with repeated measures, $F(1, 38) = 4.82, p = 0.034$]. Moreover, H animals showed less emotional reactivity than the NH group, as reflected by their absence of reaction to being picked up and handled ($\chi^2 = 25.86, p < 0.001$, Table 1).

In Experiment 2 (Table 1), ER results from 4-month-old rats showed again that H animals exhibited less reaction-to-handling than NH ones ($\chi^2 = 11.83, p < 0.001$). Nevertheless, unlike results from Experiment 1, there were no between-group differences in ACT1 nor in ACT2 [ANOVA with repeated measures, $F(1, 38) = 0.05$, NS].

In the hyponeophagia test (Table 2), H treatment significantly decreased eating latency ($t = 3.32, p = 0.002$) and increased both the time spent eating ($t = 2.78, p = 0.008$) and the amount of food eaten ($t = 2.16, p = 0.037$), whereas no effects were observed on approach latency.

Chi-square contingency tests also showed that the majority of animals (88.2%) that exhibited reaction to handling displayed the longest eating latencies in the hyponeophagia test [interval 500–600 in Fig. 1(A)], whereas rats not exhibiting resistance to handling showed shorter eating latencies [contingency coefficient = 0.56, $p = 0.003$, Fig. 1(A)]. A similar

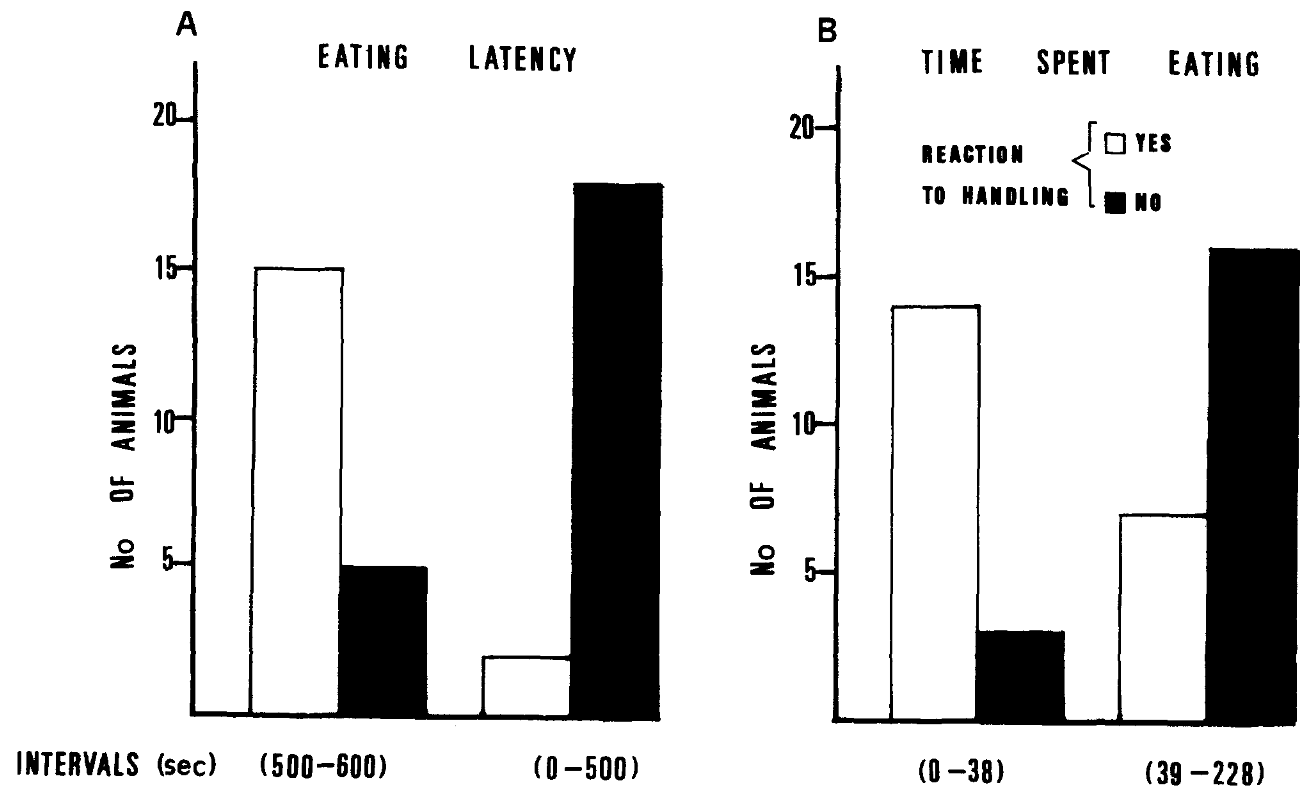

FIG. 1. The significant associations (see the Results section) between eating latency and time spent eating (A and B, respectively; hyponeophagia test) with reaction-to-handling (ER test) measures are represented. To that purpose each of both hyponephagia measures were divided into six equal intervals (in seconds). For the eating latency variable each interval had an amplitude of 100 s. Thus, in (A) the number of animals included in the sixth interval (with scores comprised from 500–600 s) and the number of animals that scores ranged from 0 to 500 s (first to fifth interval) are represented. In (B) the number of animals included in the first interval (with scores in time spent eating ranging from 0 to 38 s) and the number of subjects comprised from the second to the sixth intervals (scores ranging from 39 to 228 s; interval amplitude = 38 s) are represented.

association pattern was found with respect to the time spent eating; that is, the majority of animals that showed reaction to handling (82.3%) were those subjects that spent less time eating chocolate [contingency coefficient = 0.49, p = 0.02, Fig. 1(B)], whereas no significant association appeared among reaction-to-handling and "amount eaten" parameters.

DISCUSSION

The ER test used in the present experiments was adapted from previously reported emotionality rating procedures (which included similar measures) (1,2,8,22,23,26,27,33) and from our previous studies (5,6,11,14). Results have shown that, in young female rats, H treatment selectively increases exploration of a novel environment (i.e., ACT2) without affecting exploratory behavior of the familiar (home cage) one (i.e., ACT1). They also indicate that reactivity to handling (in the ER test) is decreased by H in young as well as in adult animals, whereas handling effects on the ACT2 measure disappeared in the latter. In this regard, the present report is, to our knowledge, the first study evaluating the stability of postnatal handling effects on ER scores through two different developmental stages. Thus, although postnatal handling seems to be a powerful treatment in short-and long-lastingly reducing fearfulness or emotionality (7–12,15–17,19,24,25, 28,29,32,33), the present ER results suggest that maybe all its effects would not be equally potent, because some of them (i.e., those more related to exploratory behavior) do not appear in adulthood. These findings also suggest that pooling information from different ER measures to form a single emotionality score could have masked the appearance of H effects in previous studies [e.g., (27)].

Hyponeophagia has been validated as an anxiety test in rodents (see the Introduction), but little attention has been paid to the effects of nonpharmacological treatments on that task (4,21). The present report is the first detailed study of postnatal handling effects on hyponeophagia, using female rats as well as a sufficient number of subjects (from a considerable number of different litters) and focusing on several behavioral parameters (4,21). Our results indicated a strong positive association among reactivity to handling (ER test) and hyponeophagia; that is, animals reactive to handling showed the highest eating latencies and spent less time eating, whereas no significant relationships appeared among activity in the ER test (ACT1 and ACT2) and any hyponeophagia parameter (data not shown). These are outstanding findings because: 1) associations among measures from different tests in handled animals have not always appeared (21,33), and 2) because the absence of between-group differences in ACT1 and ACT2 and approach latency (Experiment 2) appear to exclude the possibility that H effects on hyponeophagia could be due to changes in general activity.

In summary, the present results give support to the contention that several ways of measuring emotionality may be assessing different aspects of the same construct (33). They are also in agreement with our previous findings (mainly in males) of H treatment effects in different tests involving anxiety or conflict (9–12). Finally, the relatively inconsistent effects observed in measures of exploration in novel situations [i.e., the ER and elevated plus-maze tests (12,31), and present results] compared with positive H effects in tasks involving a stronger conflict component [i.e., the hyponeophagia and shuttle-box acquisition tests (9–11), and present results] appear to raise the interesting possibility that postnatal handling consequences could be more marked in situations involving strong conflict than on novelty/exploration measures (in which conflict is apparently less intense).

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