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BRIEF COMMUNICATION

Effect of Neonatal Handling on Learned Helplessness Model of Depression

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COSTELA, C., P. TEJEDOR-REAL , J. A. MICO AND J. GIBERT-RAHOLA. *Effect of neonatal handling on learned helplessness model of depression.* PHYSIOL BEHAV 57(2) 407–410, 1995.—The present study was undertaken to investigate the effects of neonatal handling on learned helplessness (LH) model of depression in the rat. We also investigated the effect of neonatal handling on behavior in an open field test of emotionality. The handling procedure reduced helplessness behavior, with a decrease in the number of escape failures, an increase in the number of avoidance responses, and a decrease in the escape latency in the shuttle-box after induction of LH. In addition, handling during infancy decreased the number of boli in an open field test, which suggests that the level of emotivity in adulthood was reduced. It is suggested that handling in infancy improves behavioral adaptation to the environment, including enhanced adaptive response to stress.

Handling   Learned helplessness   Rat   Emotivity   Depression

THE term handling has been used to describe a variety of neonatal environmental alterations that modify adult behavior. For example, it has been shown that handled (H) rats exhibit attenuated fearfulness in novel environments (9), show a higher exploratory behavior, and a low emotional reactivity (4,7). Likewise, H animals have been found to have shorter escape latencies than nonhandled (NH) rats in a shuttle-box (11), an effect which suggests that early handling increases the reactivity to foot shock. On the other hand, handling reduces cognitive, learning, and memory impairments in old rats (10).

Physiological effects in adulthood of infantile handling are also well documented. Thus, it has been reported that handling in infancy induces long-lasting modifications in the hypothalamic–pituitary–adrenal (HPA) axis (9). These differences in HPA axis activity observed during stress cannot be accounted for by differences in prestress, basal levels of corticoids. Likewise, levels of both CRF and plasma ACTH are higher during and after stress in NH animals (9). It has been reported that H rats secret less corticosterone in adulthood than do NH rats, and their stress-induced secretion is also more short-lived (2,9,10). It has also been shown that H rats have a permanent increase in glucocorticoid receptors in the hippocampus (10).

Moreover, other studies have shown that postnatal handling increases exploratory behavior and decreases emotional reactivity, as expressed by the reduction in defecations (2,4,7) and immobility in the swimming test (5). Deficits in escape from shock observed in animals previously exposed to inescapable, uncontrollable, and unsignaled electric shock have been postulated as a model of depression, referred to as Learned Helplessness (13). This experimental procedure has been extensively employed as an animal model of depression (17). In a previous study in which we selected NH animals according to their emotivity, we found that the high-emotive rats showed a higher escape failure than the low-emotive group in the shuttle-box after induction of learned helplessness (14). The present experiments were designed to determine whether rats handled during early postnatal period are less susceptible to helplessness in adulthood.

METHOD

Animals

Pregnant Wistar rats, obtained from the Central Animal Service of the University of Cadiz, were maintained on a 12:12 light:dark schedule with free access to food and water. Animals were housed in a room with controlled temperature and humidity with noise kept to a minimum. On the day of birth (day 0), the three litters were cross-fostered and culled to six–eight male pups.

Handling

Litters were randomly assigned to one of two infantile treatment conditions: nonhandled control (NH) (n = 12) or handled

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TABLE 1

NUMBER OF BOLUSES EXCRETED IN AN OPEN FIELD

	NH Rats	H Rats
First session	4.00 ± 0.51	4.75 ± 0.86
Second session	4.42 ± 0.54	2.50 ± 0.50*

Values given are mean ± SEM.
* Versus NH animals, $p < 0.05$.

(H) (n = 8) on days 1–21. Mothers of the H litters were removed daily from their cages and then the pups were removed and placed in a plastic container with a paper towel for about 15 min, at room temperature. After handling, the pups, followed by their mother, were returned to their cage. This procedure was repeated daily from day 1 to day 21 at 1200 h. The NH litters were left undisturbed. Offspring were weaned at 21 days of age. Subjects were then left undisturbed until testing on days 47–50.

Open Field Test

Emotivity was tested in a circular open field (1 m diam., 50 cm high walls). The open field was illuminated by a 100-W bulb suspended 60 cm above it, and the apparatus was situated in a dark and soundproof room. A white noise provided a deep and uniform sonorous background. The test was carried out individually in two daily sessions of 5 min during 2 consecutive days. The number of boluses excreted, used as a measure of emotivity (1), was recorded during each period.

Induction of Learned Helplessness

Seventy-two hours after the last open field session, all the animals received an inescapable shock session to induce helplessness. Electric foot shocks were delivered in 20 × 20 × 10 cm chambers with Plexiglas walls and cover. The floor was made of stainless steel grids (1.5 cm apart). A constant current shocker was used to deliver 60 scrambled, randomized, and inescapable shocks (duration 15 s, intensity 1 mA, intershock interval 10–90 s) to the grid floor. The inescapable shock session was performed in the morning, starting at 0900 h.

Conditioned Avoidance Training

To evaluate escape and avoidance performance, avoidance training was initiated 48 h after the inescapable shock session in an automated two-way shuttle-box, divided into two equal-sized chambers by a plastic partition with a gate that provided access to the adjacent compartment through a 7 × 7 cm space. The animals were placed singly in the shuttle-box and subjected to 30 avoidance trials.

During the first 3 s of each trial a light signal was presented (CS). The animals were allowed to avoid shock during this period. If an avoidance response did not occur, a 1-mA shock lasting 3 s was delivered. If no escape response occurred within this last period, shock and light were terminated. The response required from the rat during each trial was to pass only once through the gate into the other compartment of the shuttle-box (FR1). Althought escape failure is defined as failure to escape within a 30–60-s period in most procedures used for helplessness assessement, the very first seconds following shock onset seem to be critical for detecting interference effects in animals preexposed to inescapable shocks, especially under a simple FR1 schedule (8,16). The intertrial interval was 30 s. Avoidance sessions were performed for 3 consecutive days in the morning. The number of escape failures and the escape latency were recorded during shock delivery. Escape failure refers to the failure of the rat to change compartment during the electric foot shock. The number of avoidance responses during CS period and the intertrial interval (ITI) activity were also recorded.

Statistical Analysis

Values of emotivity were expressed as the average number of boluses excreted in the open field during each period. Values for learned helplessness were expressed as: a) the average number of escape failures ± SEM, b) the mean time of escape latency ± SEM, and c) average number of avoidance responses ± SEM recorded over 30 trials during each shuttle-box session.

Mann–Whitney U-test and Spearman's rank correlation were employed to compare the results, which were considered significant if $p < 0.05$.

RESULTS

Animals, previously subjected to handling during early postnatal life and tested in adulthood, excreted less boluses during the second session [$U = 20$, $p < 0.05$ (n = 8–12)] of the open field test than did the NH rats (Table 1).

The escape deficit was also reduced in H rats compared with NH rats. The control animals preexposed to inescapable shocks exhibited significantly more escape failures than did H animals. This effect was observed during the second and third shuttle-box sessions (second session: $U = 0$, $p < 0.01$ (n = 7–11); third session: $U = 8$, $p < 0.01$ (n = 7–12)] (Fig. 1).

Infantile stimulation (handling) reduced the mean escape latency after induction of helplessness during the three daily shuttle-box sessions. This difference was statistically significant during the second and third shuttle-box sessions [second session: $U = 16$, $p < 0.05$ (n = 8–12); third session: $U = 3$,$p < 0.01$ (n = 8–12)] (Fig. 2).

The H rats also made more avoidance responses after inescapable shock session than did the NH rats. These changes were

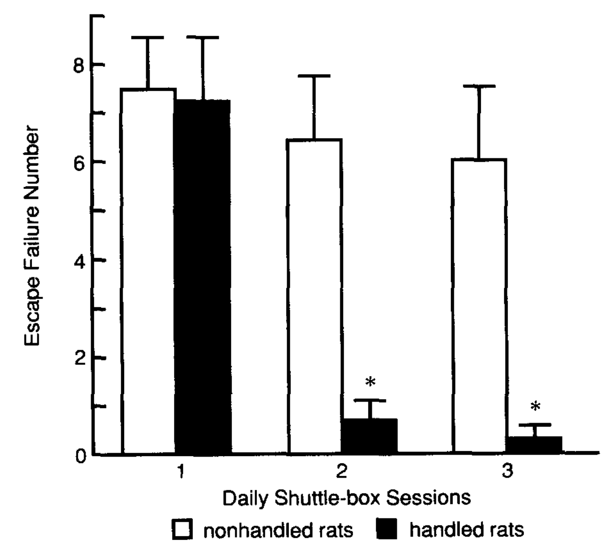

FIG. 1. Mean ± SEM number of escape failures during the 30 trials of the three daily shuttle-box sessions in nonhandled (NH) rats and handled (H) rats (n = 8–12 rats per group) in infancy. Escape failures refers to failure of the rat to change compartment during the electric foot shock (1 mA, 3-s duration). *$p < 0.01$ vs. NH group (Mann–Whitney U-test).

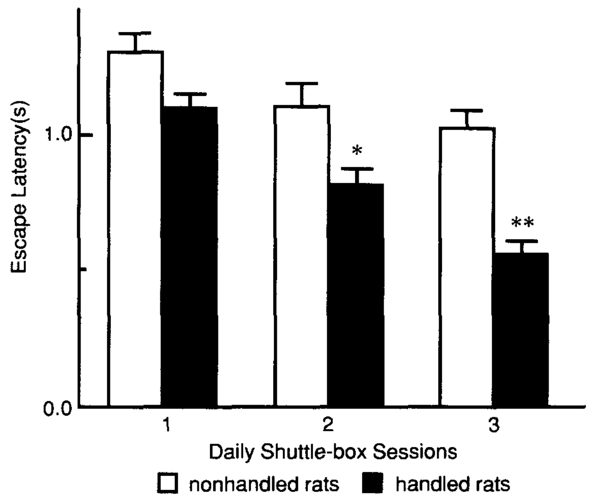

FIG. 2. Mean ± SEM of escape latency during the three shuttle-box sessions, after inescapable shock pretreatment, in nonhandled (NH) rats and handled (H) rats (n = 8–12 rats per group). Escape latency refers to the time from the start of the shock to the escape response. *$p < 0.05$; **$p < 0.01$ vs. NH rats (Mann–Whitney U-test).

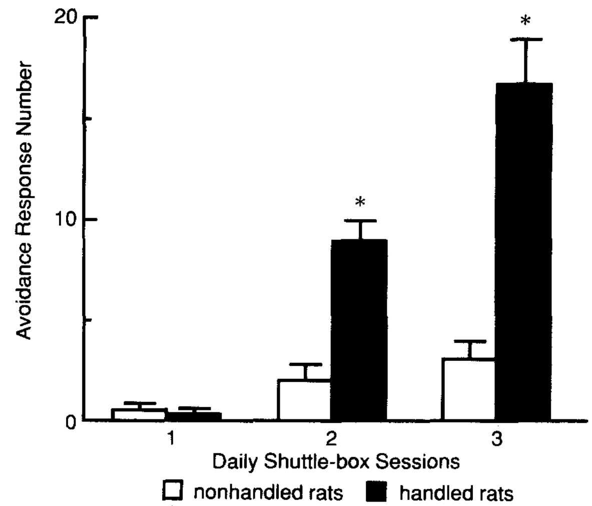

FIG. 3. Mean ± SEM number of avoidance responses during the three shuttle-box sessions in nonhandled (NH) rats and handled (H) rats, after inescapable shock pretreatment (n = 8–12 rats per group). Avoidance response refers to the crossing of the rat into the other compartment during the conditioned stimulus (light). *$p < 0.01$ vs. NH rats (Mann–Whitney U-test).

statistically significant during the second and third shuttle-box sessions [second session: $U = 2.5$, $p < 0.01$ (n = 8–12); third session: $U = 0$, $p < 0.01$ (n = 8–11)] (Fig. 3).

Analysis of ITI activity (Table 2) showed that the H rats were more active than undisturbed (NH) rats during the three shuttle-box sessions [first session: $U = 0$, $p < 0.01$ (n = 8–11); second session: $U = 2$, $p < 0.01$ (n = 7–11); third session: $U = 5$, $p < 0.01$ (n = 8–11)]. Spearman's rank correlation was employed to determine whether the higher ITI activity was related to an improved performance (a decrease in the number of escape failures). The results showed a negative relationship for the NH animals during the three shuttle-box sessions, being statistically significant during the second session (first session: $r = -0.107$, $df = 12$, NS; second session: $r = -0.626$, $df = 12$, $p < 0.03$; third session: $r = -0.514$, $df = 12$, NS). There was also a negative relationship between ITI activity and escape failures during the three shuttle-box sessions for the H animals (first session: $r = -0.157$, $df = 8$, NS; second session: $r = -0.365$, $df = 8$, NS; third session: $r = -0.327$, $df = 8$, NS), although this failed to reach statistical significance.

DISCUSSION

The present results show that handling reduces the level of emotivity, which is in agreement with previous findings (2,4,7). In addition, infantile stimulation (handling) reduced the susceptibility to learned helplessness. Thus, handled rats made less escape failures, had a shorter escape latency, and made more avoidance responses than did the nonhandled rats. However, these differences were only observed in the second and third session. This may indicate the involvement of an enhanced adaptivity to stress.

It is possible that the results were due to changes in locomotor activity, induced by infantile handling. However, we failed to find any statistically significant negative relationship between intertrial activity and the number of escape failures in experimental animals. This would tend to rule out the possibility that the increased number of escape failure in the H rats was due to higher activity.

It has been shown that handled animals show different behavioral, endocrinological, and neurochemical adaptive responses to those of nonhandled animals (see the Introduction). A variety of neuroanatomical and neurochemical pathways could be involved in this handling effect. For example, early postnatal handling has been found to lead to permanent increase in size of the adult corpus callosum (3).

Recent studies in our laboratory have shown that administration of two inhibitors (RB 38A and RB 38B) of enkephalin-degrading enzymes, which increase the level of endogenous opioids (12), induced an overall reduction in the number of escape failures in the shuttle-box in animals subjected to inescapable shocks (15). These results could suggest that endogenous opioid peptides may be a physiological substrate of this model. In this view, the resistance of handled animals to depression in the LH paradigm may result from raised levels of endogenous opioid-induced during infancy. This may improve adaptive responses, which in turn attenuate the consequences of stressful stimuli in neonatally handled animals. In this sense, Iny et al. (6) found that handling resulted in an increase in plasma β-endorphin in breast-fed rats. Nevertheless, other neurotransmitters (norepinephrine, dopamine, GABA, serotonine, etc.) could be involved.

It could be suggested that one of the major consequences of handling in infancy is to improve matching of behavioral and neuroendocrinological responses to the demands of the environment. The handled animals thus make more appropriate re-

TABLE 2

INTERTRIAL INTERVAL ACTIVITY

	NH Rats	H Rats
First session	5.90 ± 0.86	15.00 ± 2.03*
Second session	5.90 ± 1.51	23.86 ± 2.03*
Third session	8.10 ± 1.74	47.50 ± 8.43*

Values given are mean ± SEM.
* Versus NH animals, $p < 0.01$.

sponses to stress, and hence exhibit less cognitive, motivational, and emotional impairments.

In conclusion, the results obtained in this study show that a handling procedure reduces susceptibility to learned helplessness, an animal model of depression. Given the wide range of biochemical changes observed after neonatal handling, further experiments are in progress in our laboratory to elucidate the biobehavioral mechanisms involved.

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REFERENCES

1. Broadhurst, P. L. Applications of biometrical genetics to the inheritance of behaviour. In: Eysenck, H. J., ed. Experiments in personality. vol. 1. New York: Praeger; 1960:2.
2. Denenberg, V. H.; Brumaghim, J. T.; Haltmeyer, G. C.; Zarrow, M. X. Increased adrenocortical activity in the neonatal rat following handling. Endocrinology 81:1047–1052; 1967.
3. Denenberg, V. H.; Fitch, R. H.; Schrott, L. M.; Cowell, P. E.; Waters, N. S. Corpus callosum: Interactive effects of infantile handling and testosterone in the rat. Behav. Neurosci. 105:562–566; 1991.
4. Fernandez–Teruel, A.; Escorihuela, R. M.; Driscoll, P.; Tobeña, A.; Bättig, K. Infantile (handling) stimulation and behaviour in young roman high- and low-avoidance rats. Physiol. Behav. 50:563–565; 1991.
5. Hilakivi–Clarke, L. A.; Turkka, J.; Lister, R. G.; Linnoila, M. Effects of early postnatal handling on brain β-adrenoceptors and behavior in tests related to stress. Brain Res. 542:286–292; 1991.
6. Iny, L. J.; Gianoulakis, C.; Palmour, R. M.; Meaney, M. J. The b-endorphin response to stress during postnatal development inthe rat. Dev. Brain Res. 31:177–181; 1987.
7. Levine, S.; Mullins, R. F. Hormonal influences on brain organizationin infant rats. Science 152:1585–1592; 1966.
8. Martin, P.; Soubrié, P.; Simon, P. Shuttle-box deficits induced by inescapable shocks in rats: Reversal by the beta-adrenoreceptor stimulants clenbuterol and salbutamol. Pharmacol. Biochem. Behav. 24:177–181; 1986.
9. Meaney, M. J.; Viau, V.; Bhatnagar, S.; et al. Cellular mechanisms underlying the development and expression of individual differences in the hypothalamic–pituitary–adrenal stress response. J. Steroid Biochem. Mol. Biol. 39:265–274; 1991.
10. Meaney, M. J.; Aitken, D. H.; van Berkel, C.; Bhatnagar, S.; Sapolsky, R. M. Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 239:766–768; 1988.
11. Rocha, J. B. T.; Vendite, D. Effects of undernutrition and handling during suckling on shuttle avoidance and footshock escape behavior and on plasma glucose levels of young rats. Dev. Psychobiol. 23:157–168; 1990.
12. Roques B. P.; Fournié–Zaluski, M. C. Enkephalin degrading enzyme inhibitors: A physiological way to new analgesic and psychoactive agents. In: Opioid peptides: Molecular pharmacolgy, biosynthesis and analysis. Maryland: NIDA Research Monograp Series; 1986:128–154.
13. Seligman, M. E. P.; Rosellini, R. A.; Kozak, M. Learned helplessness in the rat: Reversibility, time course and immunization. J. Comp. Physiol. Psychol. 88:542–547; 1975.
14. Tejedor–Real, P.; Gibert–Rahola, J.; Leonsegui, I.; Mico;, J. A. Relationship between emotivity level and susceptibility to the learned helplessness model of depression in the rat. In: Olivier, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhäuser Verlag; 1991:217–224.
15. Tejedor–Real, P.; Mico, J. A.; Maldonado, R.; Roques, B. P.; Gibert–Rahola, J. Effect of mixed (RB 38A) and selective (RB 38B) inhibitors of enkephalin degrading enzymes on a model of depression in the rat. Biol. Psychiatry 34:100–107; 1993.
16. Telner, J. E.; Singhal, R. L. Effect of nortriptyline treatment on learned helplessness in the rat. Pharmacol. Biochem. Behav. 14:823–826; 1981.
17. Willner, P. The validity of animal models of depression. Psychopharmacology (Berlin) 83:1–16;1984.