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NEONATAL HANDLING REDUCES EMOTIONAL REACTIVITY AND SUSCEPTIBILITY TO LEARNED HELPLESSNESS. INVOLVEMENT OF CATECHOLAMINERGIC SYSTEMS

PURIFICACION TEJEDOR-REAL, CARLOS COSTELA, JUAN GIBERT-RAHOLA

Department of Neurosciences, School of Medicine, University of Cádiz, Plaza Fragela s/n. 11003 CADIZ, SPAIN

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Summary

Environmental circumstances during the neonatal period are critical for the establishment of adult responses to stressful environmental situations. As these responses are underpinned by adaptations in the functioning of brain neurotransmitter systems, the present study was designed to assess the mediation of noradrenergic and dopaminergic systems in the long-lasting effects of neonatal handling on both emotionality and learned helplessness behaviour. Animals received either prazosin, propranolol, haloperidol or saline before infantile handling. When the animals were 2 months old, they were subjected first to an open field test and then to the learned helplessness paradigm. Non-treated handled animals exhibited lower emotional reactivity and reduced susceptibility to helplessness compared to non-treated non-handled rats. The results suggest that noradrenergic, but not D2-dopamine receptor systems mediate the influence of neonatal handling on the acquisition of learned helplessness in the adult. Only beta-adrenoceptors appear to play a role in emotional responsiveness.

Key Words: neonatal handling, learned helplessness, open field test, prazosin, propranolol, haloperidol, emotional reactivity, noradrenergic system, dopaminergic system

There is now considerable experimental evidence for the existence of a critical period for brain development in the rat soon after birth. During this neonatal period the brain is particularly sensitive to both environmental conditions and pharmacological treatments. The influence of neonatal handling on adult behaviour has been the subject of numerous studies. For example, it has been shown that handled rats are less fearful in novel environments, and exhibit low emotional reactivity (1,2). Furthermore, handling has been shown to reduce the impairments in cognitive, learning and mnesic functions in old rats (3). In previous studies, we found

Corresponding author: P. Tejedor-Real, Department of Neurosciences. School of Medicine, University of Cádiz. Plaza Fragela s/n. 11003 CADIZ, SPAIN
Tel: 34-56-228717; Fax: 34-56-223139; E-mail:tejedor.real@uca.es

that animals handled during childhood are less susceptible as adults to helplessness after being subjected to inescapable footshocks (1).

The physiological repercussions of infantile handling in adulthood are also well documented. Thus, the development of the adrenocortical stress response in the rat is modified by daily handling during the early postnatal period (4), and handled (H) animals show faster adrenocortical recovery from stress than do non-handled (NH) animals (5). This adult response to early environment is thought to be mediated by glucocorticoid receptors in the hippocampus, as H animals have been found to have increased levels of these receptors in this brain region (6). Various neurotransmitter systems have also been shown to be modified after handling during childhood such as cyclic AMP (7) and the GABA/benzodiazepine (8, 9) and serotoninergic systems (10).

There is also evidence that neonatal treatment with drugs which affect cerebral aminergic systems leads to disturbances in the maturation of brain and behaviour in mammalian offspring (11). Interactions with specific mechanisms of neurotransmission during early life have been shown to alter the course of neuronal development (12), which in turn has behavioral consequences. Neurotransmitter receptors are expressed to various extents during gestation, but development and coupling to second messengers take place also during the neonatal period.

The mechanisms mediating the effects of neonatal handling on behaviour are not well understood, and the exact neuronal consequences of handling during the developmental period are unknown. The present pharmacological study was designed to throw more light on this question. The noradrenergic system, and to a lesser extent the dopaminergic system, have been reported to be modified in depression (13). We examined the involvement of both noradrenergic and dopaminergic systems in the behavioral changes induced by neonatal handling on the acquisition of learned helplessness in adult rats.

Learned helplessness is a behavioral depression caused by inescapable stress, and is a well validated and widely used model of depression (14). In this paradigm, exposure of rats to inescapable shocks induces a high rate of escape failures on subsequent testing in a shuttle-box. It has been suggested that exposure to uncontrollable stress teaches animals to be helpless (15). Escape deficits in this paradigm have been found to be reversed by tricyclic antidepressants (16,17,18). It has also been reported that the learned helplessness responses are the result of transient neurochemical changes induced by the uncontrollable stress (19,20,21). These behavioral and neurochemical changes, so-called learned helplessness effects, are akin to the symptoms found in depressed patients (22).

In an attempt to assess the involvement of catecholaminergic systems in the resistance of H animals to helplessness, we modified pharmacologically the activity of noradrenergic and dopaminergic systems prior to neonatal handling. Neonatal exposure to antagonist drugs was designed to block partially one of these systems and alter their development in an attempt to modify the influence of neonatal handling. Animals were treated with either prazosin,

propranolol or haloperidol. Although few studies have examined the effects of drug administration in pups and functional receptor ontogeny, the antagonists were selected on the basis of their relevance to depression and helpless behaviour (21,23,24,25,26,27). Although alfa2-adrenoceptors have been implicated in depression (28), we did not administer an alfa2-adrenoceptor antagonist to avoid interference with presynaptic mechanisms.

We also examined the involvement of catecholaminergic systems in the observed reduction in emotionality of adult rats following neonatal handling (1). In a previous study, we found that emotive rats were more susceptible than non-emotive ones to become helpless (29). Our second objective was to find out whether changes in both learned helplessness and emotivity induced by handling during the neonatal period were mediated by the same neurotransmitter systems.

Methods

Animals
Subjects were the offspring of 6 male and 18 female Wistar albino rats. Pregnant 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 (commercial diet for rodents A03: PANLAB/U.A.R., Barcelona, Spain) and water. Animals were housed in a temperature and humidity-controlled room with noise kept to a minimum. On the day of birth (Day 0), all the 18 litters were cross-fostered and culled to 10 male pups with 8 mothers. Females were rejected to avoid hormonal effects during adulthood, the period of behavioral testing. Neonates remained with the dams at all times except during drug administration and neonatal handling, and were weaned at 21 days of age. The behavioral testing was performed in adulthood at 60 days of age.

The experiments were conducted according to the guide-lines laid down in the "Guide for Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources National Research Council. The experimental procedure was also approved by the Local Ethical Committee for Animal Experimentation of the School of Medicine of the University of Cadiz (License nº 079604).

Handling
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 mothers were returned to their cage. This procedure was repeated daily from day 1 to day 21 at 12:00h. Fifteen minutes before this procedure both H and NH animals received a drug or saline injection. The only manipulation the NH animals received, apart from injection at the same time as that H animals, was cleaning the cages once a week. Offspring were weaned at 21 days of age. After weaning, the pups were then housed in groups of five belonging to the same initial treatment group and left undisturbed until testing on day 60.

Treatment groups and drug administration
Litters were randomly assigned to one of two infantile treatment conditions: non-handled control (n=40) or handled (n=40) on days 1-21. Offspring, H and NH, were treated with either DL-propranolol

hydrochloride (PRP) (4 mg/Kg), prazosin hydrochloride (PRZ) (2 mg/Kg), haloperidol (HLP)(1 mg/Kg) (Sigma Chemical Co.) or saline (SS). Each of the 8 groups contained 10 animals.

Drugs or saline were administered subcutaneously once daily from birth to the 21st day (when they were weaned) at a dose volume of 10 ml/Kg, 15 minutes before neonatal handling. There were no noticeable differences in behaviour between the treated and untreated pups during the handling period.

Only one dose of each drug was administered. The doses were selected on the basis of their reported receptor blocking activity (26,30,31).

Open-field test

Emotivity was tested in a white circular open field (1 m diam. 50 cm high walls) with a floor divided into 25 sections of similar area by two concentric circles and a series of radii. The open field was illuminated by a 100 W bulb suspended 60 cm above it, and the apparatus was situated in a dark and sound-proof room. A white noise provided a deep and uniform sonorous background. The test was carried out individually in a session of 5 min. From the behavioral point of view the first day of the open field testing is considered to be the most relevant because of the concomitant neophobic effect from its being the first time that the animal was exposed to the test. The duration of the session was based on our previous experimental designs (1,29). Total ambulation (number of squares adjacent to the wall and in the central area) and the number of boluses excreted, used as a measure of emotionality, were recorded during the period. The rate of defecation rate in a new environment is a widely used index of emotivity (2,32,33).

Induction of Learned Helplessness

Induction of learned helplessness was carried out as described elsewhere (34). Seventy two hours after the open field session, all the animals received a session of inescapable shocks in order to induce helplessness. Electric footshocks were delivered in 20x20x10 cm chambers with Plexiglass 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 0.8 mA, intershock interval 10-90 s) to the grid floor. The inescapable shock session lasted 60 minutes.

Conditioned avoidance training

In order 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 7x7 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 (conditioned stimulus). The animals were allowed to avoid shock during this period. If an avoidance response did not occur, a 0.8 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). Although an escape failure was defined as failure to escape within 30 to 60 seconds, in most procedures used to assess helplessness, the first seconds following onset of shocks seem to be critical for detecting interference effects in animals preexposed to inescapable shocks, especially under a simple FR1 schedule (26,35). The intertrial interval was 30 s. Avoidance sessions were performed for 3 consecutive days. The number of escape failures was recorded during shock delivery. Escape failure was defined as the failure of the rat to change compartments during the electric footshock.

Statistical analysis
Values of emotivity were expressed as the mean number of boluses excreted+SEM in the open field during the session. Ambulation was expressed as the mean number of squares±SEM entered during the 5 min session. Learned helplessness was expressed as the mean number of escape failures±SEM recorded over 30 trials during each shuttle-box session.

Mean treatment group scores were compared statistically using Kruskall-Wallis non-parametric one-way analysis of variance followed by the Mann-Whitney U-test. In order to simplify presentation and interpretation of the results, only significant values will be described.

Results

ESCAPE AND AVOIDANCE TEST

A first Kruskal-Wallis analysis revealed significant differences between the eight groups during the three daily shuttle box sessions: 1st session $H(df7)=29.07$, $p<0.0002$; 2nd session $H(df7)=26.74$, $p<0.0005$; 3rd session $H(df7)=21.80$, $p<0.003$.

The experiments reported below were conducted simultaneously to avoid replicating control groups, but for the sake of clarity they are presented separately in the figures.

Control (non-treated) animals
As found in previous studies, the post-hoc Mann-Whitney U test revealed that H+SS animals displayed fewer escape failures than did the NH+SS animals (Figs 1, 2, 3). Statistically significant results were observed over the three daily shuttle box sessions (1st session: $U=14.5$, $p<0.05$; 2nd session: $U=21$, $p<0.05$; 3rd session: $U=19$, $p<0.05$).

Effect of prazosin
Post hoc comparisons using the Mann-Whitney U-test showed that prazosin treatment induced a slight but non-significant impairment in the NH animals. However, this drug significantly antagonized the effects of neonatal handling in adult rats (Fig. 1) (H+SS vs H+PRZ: 1st session: $U=7$, $p<0.01$; 2nd session: $U=13.5$, $p<0.01$; 3rd session: $U=6.5$, $p<0.01$. Due to the lack of significant effect of prazosin on non-handled animals, significant differences were also observed between H+SS and NH+PRZ during the three sessions (1st session: $U=4$, $p<0.01$; 2nd session: $U=11$, $p<0.01$; 3rd session: $U=4$, $p<0.01$).

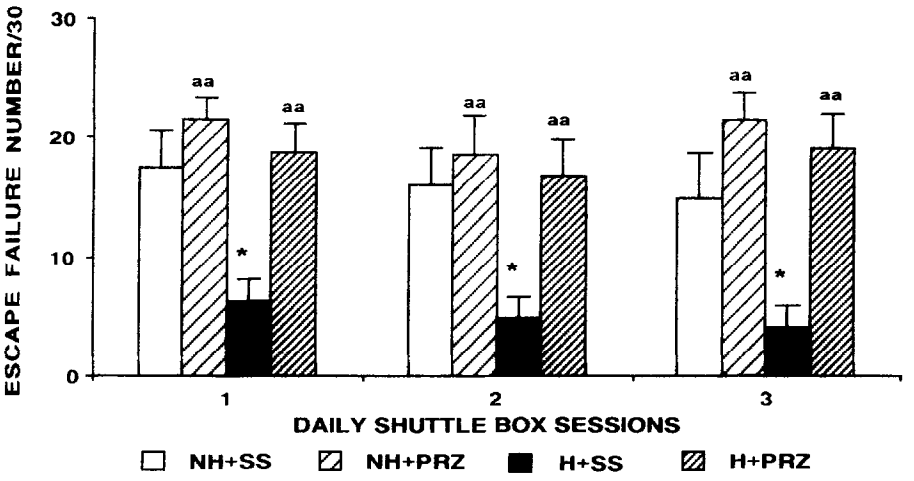

FIG 1.
Effect of prazosin treatment before neonatal handling on learned helplessness. Mean±SEM number of escape failures in neonatal non-handled (NH) and handled (H) rats receiving either saline (SS) or prazosin (PRZ) (2 mg/Kg/day). * $p<0.05$ vs NH+SS; aa $p<0.01$ vs H+SS. (Mann-Whitney U-test).

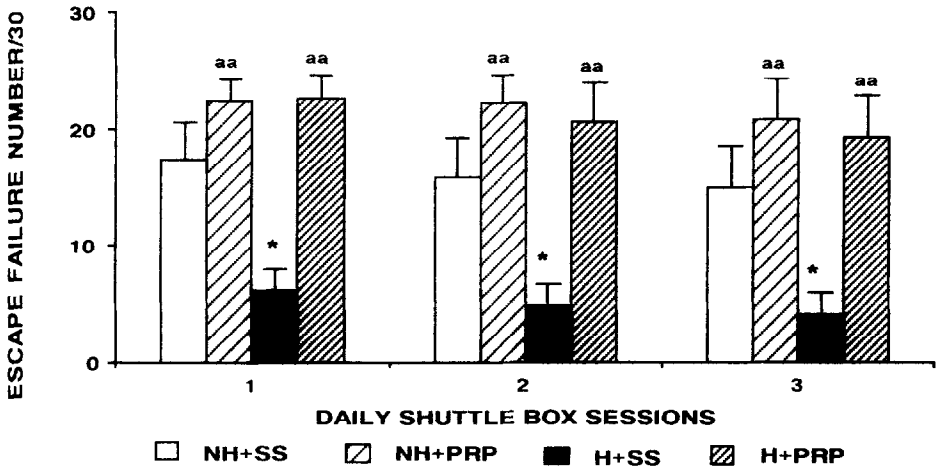

FIG 2.
Effect of propranolol treatment before neonatal handling on learned helplessness. Mean±SEM number of escape failures in neonatal non-handled (NH) and handled (H) adult rats receiving either saline (SS) or propranolol (PRP) (4 mg/Kg/day). * $p<0.05$ vs NH+SS; aap<0.01 vs H+SS (Mann-Whitney U-test).

Effect of propranolol

Post hoc Mann-Whitney U-test indicated that neonatal administration of propranolol increased the number of escape failures either in both NH and H animals. However the propranolol effect was only statistically significant in the H animals. In fact, this drug strongly antagonized the long-lasting effects of handling during the three sessions (Fig 2) (H+SS vs H+PRP: 1st session: U=2.5, p<0.01. 2nd session: U=9.5, p<0.01. 3rd session: U=11.5,p<0.01. The number of escape failures in non-treated H animals was also significantly less than in the propranolol-treated NH rats (1st session: U=3.5, p<0.01; 2nd session: U=0, p<0.01; 3rd session; U=9.5, p<0.01)

Effect of haloperidol

Administration of haloperidol slightly increased escape deficits on both non-handled and handled rats, post hoc analysis of the data using Mann-Whitney U-test failed to detect any significant differences between drug-treated and non-treated rats (Fig 3).As haloperidol did not induced any significant effect on non-handled animals, the reduction of escape failures in H+SS group were statistically as compared to NH+HLP rats during the three sessions (1st session: U=6, p<0.01; 2nd session: U=9.5, p<0.01; 3rd session:U=12.5, p<0.01). Significant difference were also observed between H+HLP vs NH+SS during the first session (U=22, p<0.05) and between H+HLP vsNH+HLP during the first and second sessions (1st: U=15, p<0.01; 2nd: U=16, p<0.01) showing the lack of effect of haloperidol in both handled and non-handled groups.

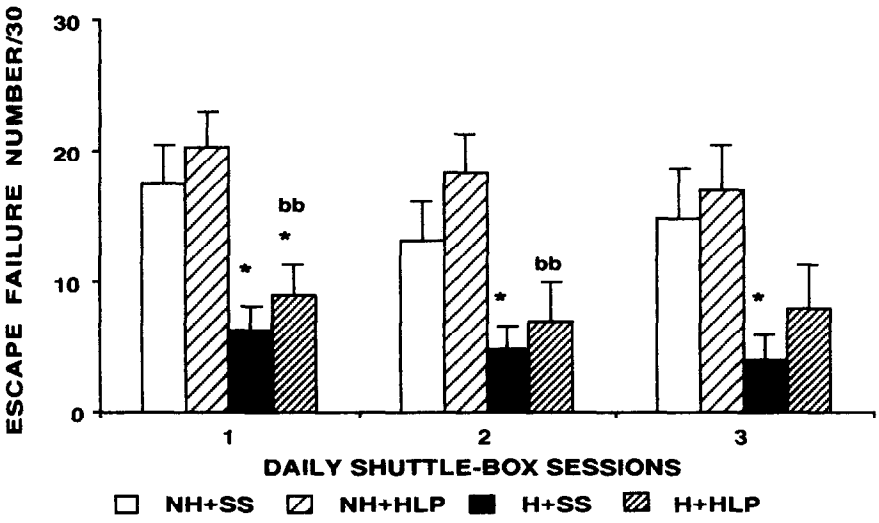

FIG 3.

Effect of haloperidol treatment before neonatal handling on learned helplessness. Mean±SEM number of escape failures in neonatal non-handled (NH) and handled (H) adult rats receiving either saline (SS) or haloperidol (HLP) (1 mg/Kg/day). * p<0.05 vs NH+SS; aap<0.01 vs H+SS; bb p<0.01 vs NH+HLP (Mann-Whitney U-test).

OPEN FIELD

General ambulation
General activity was not affected by handling and/or pharmacological treatments with either prazosin, propranolol or haloperidol (Table I).

TABLE I

Effect of Prazosin, Propranolol and Haloperidol Treatment before Neonatal Handling on Adult Open Field General Activity

Treatment	Saline	Prazosin	Propranolol	Haloperidol
Non-handled	97.3±6.0	95.5±4.2	86.7±3.8	91.3±6.7
Handled	89.7±3.8	91.2±7.8	73.6±11.0	89.1±5.8

Mean±SEM of general ambulation. No significant differences were found between groups (Mann Whitney U test).

Defecation rate
Kruskal-Wallis analysis revealed significant differences between the groups $H(df7)=17.30$, $p<0.05$). As found in previous experiments, untreated H animals exhibited a reduction in defecation rate as compared to non-treated NH animals ($p<0.01$). Post hoc analysis using Mann-Whitney U test showed that prazosin and haloperidol did not induce any alteration in emotional reactivity in either NH or H rats (Fig 4A y 4C). However, propranolol appeared to mimic the effects of infantile handling on emotivity in NH animals, giving rise to statistically significant differences in defecation rate between propranolol-treated and non-treated NH animals ($U=18$, $p<0.05$). Paradoxically, propranolol significantly counteracted the effect of neonatal handling on emotiveness ($U=22$, $p<0.05$) (Fig 4B).

Discussion

As in previous studies (1), the present results provided further evidence for an attenuation of learned helplessness in the adult rat by early postnatal handling. The adult performance of H animals in escape responses were superior to that of the NH animals. They made significantly fewer escape failures. It has been reported that the learned helplessness responses are the results of transient neurochemical changes induced by the uncontrollable stress. Our findings suggest that environmental circumstances during the neonatal period are critical for the establishment of adult responses to stressful environmental situations. These results could be partially attributed to differences in the effect of postnatal treatments on avoidance acquisition (36). However, the effects of pre-exposure to inescapable footshocks cannot be readily discriminated from possible differences in susceptibility to stress.

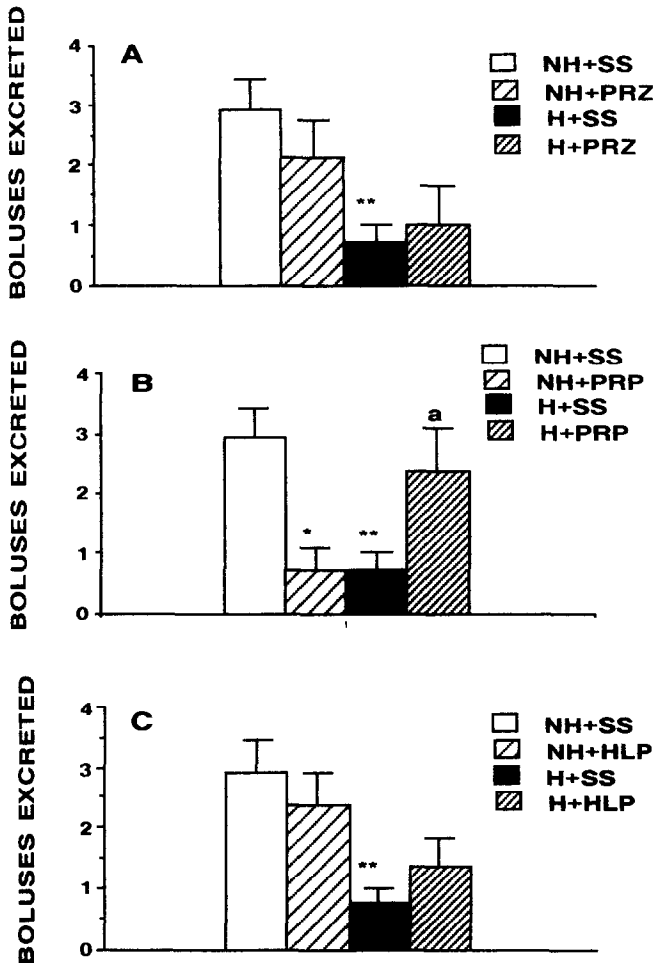

FIG 4

Effect of prazosin, propranolol and haloperidol treatment before neonatal handling on adult open field behaviour. Values represent the average number±SEM of boluses excreted in neonatal non-handled (NH) and handled (H) adult rats treated with either saline (SS), prazosin (PRZ) (2 mg/Kg/day) (A), propranolol (PRP) (4 mg/Kg/day) (B) or haloperidol (HLP) (1 mg/Kg/day) (C). Prazosin and haloperidol did not exert any significant effect on either NH or H animals. Only propranolol treatment modified significantly the number of boluses excreted in both H and NH rats. * $p<0.05$; ** $p<0.01$ vs NH+SS; a $p<0.05$ vs H+SS. (Mann-Whitney U-test).

Although these behavioral effects are thought to involve adaptations in the function of brain neurotransmitter systems, little is known about the long-term neurochemical and behavioral consequences of

interactions between drug administration and the neonatal environment. In the present study, administration of propranolol, a beta-noradrenergic receptor antagonist, or prazosin, an alfal-noradrenergic receptor antagonist had comparable effects. Both drugs antagonized the effect of neonatal handling in the learned helplessness paradigm by increasing the number of escape failures. Since general ambulation in the open field was not affected by exposure to these drugs in the neonatal period, these results could not be attributed to an alteration in motor activity. Neonatal antagonism of noradrenergic systems appears to make adult rats more susceptible to inescapable shocks, by impairing acquisition of the long-lasting benefits of infantile handling.

A variety of endocrine, metabolic, immune and neural responses have been identified in the organism's defensive repertoire to stress. In this regard, the effects induced by noradrenergic antagonism on neonatal handling be mediated by interactions with the HPA axis. Neonatal H animals have been shown to have an increase in hippocampal glucocorticoid receptors (37), which enhances negative feed-back on the hypothalamo-pituitary-adrenal (HPA) axis, protecting adult animals from the damaging effects of glucocorticoids and its related cognitive impairments. This is significant for our findings as the hippocampus is a brain region which receives a dense noradrenergic innervation from the locus coeruleus (38). The effects of glucocorticoids are thought to involve an interaction of several central neurotransmitter systems, and it is of interest that CRF is considered as a neurotransmitter in the locus coeruleus, a noradrenergic nucleus proposed as a site for integrating corticotrophin-releasing factor and noradrenergic modulation of stress responses (39). In this view, corticotrophin-releasing-factor has been postulated to be hypersecreted in depression. Dinan (40) has suggested that stress mediated through the hypothalamic-pituitary-adrenal axis produces the disturbances in monoamine functioning seen in depression. Hypersecretion of cortisol as well as the presence of cortisol receptors in the brain represent possible links with monoaminergic mechanisms. Taken together these observations point to a relationship between the HPA and noradrenergic systems, and it may be that propranolol and prazosin attenuated the effects of handling by interacting with the HPA axis.

Another alternative is that neonatal administration of noradrenergic antagonists induce long-term plastic modifications in the noradrenergic system preventing neonatal handling from exerting its effect. With respect to alfal-adrenoceptors, chronic administration of tricyclic antidepressants has been shown to induce an increase in alfal-adrenoceptors in the geniculate nucleus, and activate transmission of synapses rich in alfal-adrenoceptors in the hippocampus. Other studies point more to an involvement of beta-adrenergic systems. Thus, modification of beta-adrenoceptors has been shown to participate in the behavioural effects of several antidepressants (23,24) and a down-regulation of beta-adrenoceptors has been postulated as a marker of antidepressant efficacy (19,25). It is noteworthy that electroconvulsive therapy have been shown to induce a reduction in the number of beta-adrenoceptors (19).Neurochemical studies have revealed that neonatal environmental manipulations in rats induce long-lasting effects on beta-adrenoceptors linked to adenylate cyclase activity in cortical and hippocampal structures (7). From the behavioural point of view, administration of clenbuterol and salbutamol, two beta-adrenoceptor

stimulants, reversed the escape deficits in the learned helplessness paradigm (26). In this context, one study found evidence for an increased density of beta-adrenoceptors in the hippocampus of rats showing an escape deficit in the shuttle-box after being exposed to uncontrollable electric shocks (41). On the other hand, Hilakivi et al. (42) found that administration of propranolol to rats from the 7th to the 20th postnatal days raised noradrenaline concentrations in the adult limbic forebrain and cerebellum, two brain regions with a high density of beta-adrenergic receptors (43). Neonatal handling might induce a long-term alteration in noradrenergic system in the opposite direction to that induced by propranolol. This effect would be expected to be antagonized by propranolol. Moreover, the neonatal period is a critical period for beta-receptor development. For instance, Bruinink and Lichtensteiger (44) observed a proliferation of beta-receptors from the 15th gestational day to the 31st postnatal day. Taking these data together, the effects of neonatal handling could be attributed to a long-term modification in the beta-receptor population such as density, affinity, and coupling to second messengers. However, Hilakivi-Clarke et al. (45) failed to find any differences in the density or affinity of beta-receptors in the frontal cortex and the hippocampus between handled and non-handled rats. Long-term modifications of neurotransmitter systems induced by handling have yet to be identified and the current data are still inconclusive.

Dopamine blockade via the administration of haloperidol only slightly reduced the learned helplessness responses in H and NH animals. Although the biochemical basis of depression is generally considered to involve nor- adrenergic and serotoninergic systems, there have been suggestions that dopamine plays a role in this psychiatric disorder. From this standpoint, Petty et al. (21) found that administration of haloperidol induced helpless behaviour. Furthermore, biochemical and autoradiographic studies have identified a nearly linear increase in D2-dopamine and alfa1-noradrenergic receptor binding in rat forebrain tissue from the first to the third neonatal weeks (27). On the other hand, chronic administration of antidepressants such as imipramine, amitriptyline or mianserine enhance dopaminergic function (46), and they have been found to increase the responsiveness of postsynaptic D2/D3 receptors in the mesolimbic system (47) Nevertheless, most antidepressants do not have an immediate effect on dopamine function following acute administration (48), which might account for the lack of activity of haloperidol in our learned helplessness paradigm. It is not known how antidepressants sensitize dopaminergic transmission, although it might be mediated indirectly by a primary action on noradrenergic or serotoninergic pathways (13). Interestingly and in line with our results, Bean and Lee (49) reported that the decrease in density of striatal D2-dopamine receptors induced by social isolation during early development was not reversed by daily handling. These observations along with our results suggest that the effects of neonatal handling are not directly mediated by D2-dopamine receptor systems. Nevertheless, from an ontogenetic point of view, the dopaminergic system also develops markedly during the neonatal period. Thus, the density of striatal D2-dopamine receptors in the rat increases from birth reaching a peak approximately 28 days after birth (50). However, it seems that neonatal handling has little influence on D2-dopamine receptor expression. On the other hand, the apparent lack of effect of haloperidol might have been due to the low dose used, although its sedative action excludes higher doses.

Taken together, the present results, regardless of the specific mechanisms underlying this long-term effect, indicate that noradrenergic systems are involved in the long-term influence of neonatal handling on the acquisition of learned helplessness during adulthood. Early life experiences could contribute to the development of neurotransmitter systems in parallel with genetic programs giving rise to long-lasting modifications. However, it is not known how environmental and pharmacological manipulations in neonatal life modify psychogenetically adult behaviour (2,51). As yet we lack information on the effect of drugs on the stimulus responsiveness of rat pups, especially with respect to sensory stimulation.

A second finding of the present study was that infantile handling exerted a long-term effect on emotional responsiveness as shown by the decrease in defecation rate in the open field. In the present experiments, the reduction in emotivity induced by neonatal handling was only affected by propranolol. Propranolol induced a decrease in NH rat emotiveness, mimicking the effect of handling in this paradigm. However, when animals received both handling and propranolol, no synergistic action was noted. On the contrary, the level of emotivity in these animals was comparable to that of the non-handled, untreated group. It seems that there may be interference between the two manipulations. However, paradoxically, propranolol antagonized the effect of neonatal handling on this parameter. The lack of action of prazosin on emotiveness suggests that noradrenergic systems are only partially involved in this behavioral response. In spite of the finding that the susceptibility to become helpless is directly dependent on the level of emotivity (29), the ability of prazosin to reverse the effects of handling on learned helplessness, but not on emotivity in the open field, suggests that these behavioral effects are not mediating by common mechanisms. High emotivity may have a sufficient but not mandatory role in the development of learned helplessness.

In conclusion, these findings support the hypothesis that the influence of neonatal handling on acquisition of learned helplessness in adulthood is mediated through noradrenergic systems. The handling-induced noradrenergic modifications were thought to impinge on the HPA axis. The effect of neonatal handling did not appear to be mediated by D2-receptor mechanisms. On the other hand, beta-adrenergic receptors may play a part in the improved emotional response induced by handling. Nevertheless, neither alfa1-adrenergic nor D2-dopamine receptor systems appeared to be involved in the diminished emotivity induced by neonatal handling.

Future studies will be directed at gaining more understanding of the neonatal plasticity of these systems and the mechanisms underlying the effects of neonatal handling. Our results point to a role for catecholaminergic systems in the effects of neonatal handling, although a more thorough exploration of these systems will be required before drawing conclusions. As central neurons are closely interrelated and controlled by a host of feed-back mechanisms, the symptoms observed after simple pharmacological manipulations cannot be attributed to a unique system. The different neurobiological hypotheses on the long-lasting effects of handling may well be complementary, constituting an overall system. Studies involving pharmacological or behavioral manipulations in the neonatal period

would benefit from the understanding gained by neurochemical investigations of the various neurotransmitter systems involved.

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