Prenatal Stress Induces High Anxiety and Postnatal Handling Induces Low Anxiety in Adult Offspring: Correlation with Stress-Induced Corticosterone Secretion

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It is well known that the hypothalamo-pituitary-adrenal (HPA) axis is altered by early environmental experiences, particularly in the perinatal period. This may be one mechanism by which the environment changes the physiology of the animal such that individual differences in adult adaptive capabilities, such as behavioral reactivity and memory performance, are observable. To determine the origin of these behavioral individual differences, we have investigated whether the long-term influence of prenatal and postnatal experiences on emotional and cognitive behaviors in adult rats are correlated with changes in HPA activity. To this end, prenatal stress of rat dams during the last week of gestation and postnatal daily handling of rat pups during the first 3 weeks of life were used as two environmental manipulations. The behavioral reactivity of the adult offspring in response to novelty was evaluated using four different parameters: the number of visits to different arms in a Y-maze, the distance covered in an open field, the time spent in the corners

of the open field, and the time spent in the open arms of an elevated plus-maze. Cognitive performance was assessed using a water maze and a two-trial memory test. Adult prenatally stressed rats showed high anxiety-like behavior, expressed as an escape behavior to novelty correlated with high secretion of corticosterone in response to stress, whereas adult handled rats exhibited low anxiety-like behavior, expressed as high exploratory behavior correlated with low secretion of corticosterone in response to stress. On the other hand, neither prenatal stress nor handling changed spatial learning or memory performance. Taken together, these results suggest that individual differences in adult emotional status may be governed by early environmental factors; however, perinatal experiences are not effective in influencing adult memory capacity.

Key words: *prenatal stress; postnatal handling; corticosterone; behavioral reactivity; escape behavior; exploratory behavior; memory performance*

The activity of the hypothalamo-pituitary-adrenal (HPA) axis is altered by both prenatal and early postnatal manipulations. It has been shown previously that prenatal stress induces an increased corticosterone response to stress in weanling rats (Peters, 1982; Takahashi et al., 1988; Henry et al., 1994). In addition, adult rats that have been submitted to a prenatal stress show a prolonged stress-induced corticosterone secretion (Fride et al., 1986; Maccari et al., 1995; Vallée et al., 1996) associated with decreased hippocampal corticosterone receptors (Maccari et al., 1995), indicating a reduced efficacy of the corticosterone feedback mechanism. In contrast, this corticosterone response is reduced in adult rats handled daily for the first 3 weeks of life (Levine, 1962; Meaney et al., 1988; Ogawa et al., 1994; Vallée et al., 1996), and a postnatal manipulation (early adoption) can reverse the effects of prenatal stress on HPA axis activity (Maccari et al., 1995).

The HPA axis can play a role in adaptive mechanisms (emotive and cognitive) and may explain the individual differences occurring naturally in adults (Endroczi and Fekete, 1973; Persky, 1975; Gentsch et al., 1981; Gunnar et al., 1991; Piazza et al., 1991). For example, behavioral reactivity and amphetamine self-

administration are related to HPA activity. It has been shown that those rats having a high locomotor reactivity are also more vulnerable to drug addiction, and they demonstrate a prolonged corticosterone secretion in response to stress (Piazza et al., 1991) associated with a decreased efficacy of hippocampal corticosteroid receptors (Maccari et al., 1991). Furthermore, cognitive performance is also associated with HPA activity. For example, in humans, a negative relationship between stress-induced cortisol levels and memory performance has been reported in healthy adults (Kirschbaum et al., 1996). The administration of glucocorticoid agonists induces memory impairments in humans and in animals (Wolkowitz et al., 1990; Dachir et al., 1993; for review, see McEwen and Sapolsky, 1995). Moreover, glucocorticoids are involved in the regulation of memory storage (McGaugh, 1989). Thus, individual differences in HPA axis activity seem related to individual differences in behavioral reactivity and cognitive performance.

It is well accepted that early environmental experiences have long-lasting effects on adult behavior in humans (Denenberg, 1975; Hillman, 1991; Rutter, 1991; Keelan et al., 1992) and animals (Brunelli et al., 1989; Wainwright et al., 1989; Zimmerberg and Brett, 1992), but many controversies remain in the literature.

Prenatal stress and postnatal manipulations have been associated with an increase (Thompson, 1957; Hockman, 1961; Ader and Belfer, 1962; Thompson et al., 1962; Masterpasqua et al., 1976; Fride et al., 1986) and a decrease (Levine et al., 1967; Fride and Weinstock, 1988; Shiota and Kayamura, 1989; Wakshlak and Weinstock, 1990), respectively, in emotional behavior. In agree-

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ment with these findings, a postnatal manipulation, handling in the first 3 weeks of life, has been reported to prevent the change in behavioral reactivity observed in adult rats previously submitted to a prenatal stress (restraint stress of the dams during gestation) (Wakshlak and Weinstock, 1990). Other authors, however, failed to confirm these results concerning behavioral reactivity (Ader and Conklin, 1963; Meisel et al., 1979; Peters, 1982; Rojo et al., 1985; Moore and Power, 1986; Pfister and Muir, 1992; Ogawa et al., 1994). These differences may be attributable to the use of different early manipulations (Archer and Blackman, 1971; Chapman and Stern, 1979; Suchecki and Palermo Neto, 1991; Ogawa et al., 1994) or different behavioral tests. For example, high locomotor activity in an open field has been interpreted as either high or low emotionality (Levine et al., 1967), depending on the number of sessions of the test. Furthermore, some authors have evaluated behavioral reactivity using a single behavioral measure, often an open field or an elevated plus-maze. In most cases, one measure is not sufficient to assess such a complex response (Hall, 1934), which may reflect, for example, the outcome between conflicting exploratory and escape behaviors (Montgomery, 1955; Archer, 1973; Russel, 1973; Gyertyan, 1992).

Another issue that requires clarification is the effect of prenatal and postnatal experiences on cognition, which has been studied to a lesser extent than anxiety. In addition, the behavioral effects reported are not unequivocally related to cognitive functions. For example, avoidance performance, described as a learning capability, is reportedly decreased after a prenatal heat shock (Shiota and Kayamura, 1989), and postnatal handling has no effect on the performance of adult animals in a spatial memory task (the water maze) but improves performance during senescence (Meaney et al., 1988, 1991). However, the reliability of avoidance and water-maze procedures for assessing memory performance has been questioned (Porsolt et al., 1995), given that the motivational or emotional state of the animal can interfere with the memory measure, suggesting that cognitive effects may be confounded by noncognitive factors.

To determine the role of epigenetic factors in behavioral adaptation and the determinism of individual differences in adult behavior, the long-term influence of perinatal experiences on adult emotional and cognitive behavior and their correlation with the stress-induced corticosterone secretion has been studied in this work. To this end, two perinatal environmental modifications were used: a prenatal stress, consisting of repeated restraint of the mother during the last week of pregnancy (Ward and Weisz, 1984), and an early postnatal manipulation, consisting of daily handling during the first 3 weeks of life (Levine et al., 1967; Meaney et al., 1987). In the adult offspring of these perinatal manipulations, anxiety-like behavior has been assessed by evaluating behavioral reactivity in response to novelty, using four different parameters. Furthermore, to discriminate exploratory behavior from escape behavior, a descriptive analysis of these four parameters has been performed using a principal component analysis (PCA). In addition, spatial learning and memory capacities have been analyzed in two different tests. Finally, a correlation between these behavioral responses and stress-induced corticosterone secretion in these rats has been described.

MATERIALS AND METHODS

Subjects

The adult male Sprague Dawley rats (4–7 months old) used in this study were obtained from litters bred on site from Sprague Dawley females and males (Iffa Credo, Lyon, France). Virgin female rats at ~6 weeks of age

(250–300 gm) were housed in groups of 10 for 7 d to coordinate their estrous cycle. After this time, rats were housed individually in the presence of a sexually experienced male rat (450–550 gm). All animals were housed in a temperature- (22°C) and humidity- (60%) controlled animal room on a 14 hr/10 hr light/dark (6 A.M.–8 P.M.) schedule. They had free access to food and water throughout the experiments.

Perinatal manipulations

Pregnant rats were randomly allocated to three groups. Dams in the first group [prenatal stress (PS) group, $n = 15$] were submitted to a restraint stress in an illuminated environment during the last week of pregnancy (Ward and Weisz, 1984). Animals were placed individually in a transparent plastic cylinder (6 cm diameter, 20 cm long) for three 45 min periods per day (9 A.M., 12 P.M., and 5 P.M.). Dams in the second [control (C) group, $n = 31$] and third groups [handling (H) group, $n = 15$] were left undisturbed during pregnancy. After birth, the offspring of all groups were housed in the same animal room, and all pups were kept together with their mothers. The offspring of the first and second groups were left undisturbed, whereas the offspring of the third group were submitted to postnatal handling as described by Meaney et al. (1987). This manipulation was performed daily from postnatal day 1 until postnatal day 21 (day of weaning). Briefly, the pups were picked up and transferred from their home cage to another one containing paper toweling. Separate cages were used for each litter throughout the experiment. The pups from one litter remained together in the cage for 15 min (at 11 A.M. every day) before being returned to their home cage. The mother was taken out of the home cage, kept alone in another cage for the 15 min, and then returned to the home cage after the pups. Handling sessions were always performed in the same room by the same experimenter.

The offspring of all groups were weaned 21 d after birth and left undisturbed until testing at 4 months of age. Only litters of 6–12 pups with approximately half females and half males were kept for the study. According to this condition, one litter was removed from the PS group and one from the H group. Thus, 14 litters were kept in the PS group, 31 in the C group, and 14 in the H group. To prevent litter effects, only two male siblings per litter were tested in adult life. No significant litter effect was observed for the behavioral parameters tested. Male pups of each litter were group-housed after weaning and then housed individually at 1 month of age to increase their sensibility to novelty (Gentsch et al., 1981) and to eliminate the variability in corticosterone levels induced by dominant/submissive relationships established between group-housed animals (Popova and Naumenko, 1972). Three groups of adult animals were thus tested: offspring of mothers stressed during pregnancy (PS group, $n = 27$), undisturbed animals (C group, $n = 61$), and animals submitted to the postnatal handling procedure (H group, $n = 28$). After the control test in the water maze, one prenatal stress rat and one control rat were removed because of motor disturbances.

Corticosterone assay

Plasma corticosterone levels were measured with a radioimmunoassay kit (ICN Biomedicals, Orsay, France) using a highly specific corticosterone antiserum. The minimum level of detection was 0.2 $\mu\text{g}/100\text{ ml}$, and the intra- and interassay coefficients of variation were 5 and 9%, respectively.

Blood sampling

Basal and stress-induced corticosterone secretion was performed in adult animals (4 months). Blood samples (500 μl in heparinized tubes) were taken from the tail vein between 9 and 12 A.M. Restraint stress was performed for a 30 min period using plastic restraint tubes (6 cm wide and 20 cm long). The first sample, for evaluation of basal corticosterone levels, was taken immediately after the animal was placed in the restrainer and <2 min after removal from the home cage. Blood samples were then taken just before releasing animals from the restrainer at 30 and 120 min after the start of the restraint stress.

Behavioral apparatus

The equipment was located in a sound-attenuated room, and sessions were recorded automatically on a microcomputer (IBM-PC), allowing the observer to remain outside the experimental room, except in the case of the water-maze experiment. In the Y-maze, interruptions of infrared beams were recorded on the computer. In the open-field, elevated plus-maze, and water-maze tests, the parameters were analyzed by an automatic videotracking system (Viewpoint, Lyon, France).

Behavioral reactivity

Y-maze test. The apparatus was a Y-maze made of gray plastic with three identical arms (50 × 16 cm) enclosed with a 32-cm-high side wall and illuminated by dim light (70 lux). Each arm was equipped with two infrared beams, one at each end of the arm.

Open-field test. The apparatus consisted of a white wooden box (1 × 1 m), as described by Welker (1957) and Denenberg (1969). The field was bordered by 40-cm-high side walls and was well illuminated (550 lux on the floor of the field).

Elevated plus-maze test. The elevated plus-maze was made of wood, according to the specifications of Pellow et al. (1985). The apparatus consisted of two open arms (50 × 10 cm), alternating at right angles, with two arms enclosed by 40-cm-high walls. The four arms delimited a central area of 10 cm². The whole apparatus was placed 1 m above the floor. A 3 cm high wooden rim prevented the rats from falling off the open arms. The level of illumination was 480 lux in the central area, 450 in each open arm, and 300 in the closed arms.

Cognitive performance

Water-maze test. The apparatus, according to the specification of Morris (1984), consisted of a circular swimming pool (180 cm diameter × 60 cm high), which was filled with opaque water at 22 ± 1°C. The pool was arbitrarily divided into four quadrants. The testing room was highly illuminated (350 lux on the liquid surface). Spatial cues were placed in the room and remained in fixed positions throughout the experiment.

Two-trial memory task. The apparatus was a Y-maze made of gray plastic with three identical arms, as described above. The floor of the maze was covered with rat odor-saturated sawdust, and between each session the sawdust was mixed to eliminate olfactory cues. Visual cues were placed in the testing room and kept constant during the behavioral testing sessions.

Behavioral procedure

Four behavioral parameters were included in our working definition of behavioral reactivity: the number of visits to different arms in the Y-maze, the distance covered in the open field, the time spent in the corners of the open field, and the time spent in the open arms of the elevated plus-maze. Cognitive performance was evaluated using the distance and latency to find the hidden platform in the learning and reversal phases of the water-maze test, and the number and duration of visits to the novel arm of the Y-maze measured in the two-trial memory task. The tests were conducted between 9 A.M. and 4 P.M. and were separated by an interval of 7 d, during which time the animals were left undisturbed in their animal room. The rats were submitted alternately to the same test, i.e., the animals of the three groups were mixed within a behavioral session.

Behavioral reactivity

Y-maze test. The animal was placed in one arm and had access to the three arms for 10 min. During this period, the total number of visits to different arms was measured. A visit to one arm was recorded if the two beams of that arm were interrupted consecutively.

Open-field test. At the beginning of the test, the rat was placed in a corner and was allowed to freely explore the field for 15 min. The distance covered in the whole apparatus and the time spent in the corners were recorded.

Elevated plus-maze test. Rats were placed in the central square and allowed to explore the maze freely for 10 min. The parameters measured were the times spent in open and closed arms. The percentage of time spent in open arms with respect to the total time in both open and closed arms was calculated.

Cognitive performance

Water-maze test. Before the behavioral testing, animals were submitted to a 2 d habituation phase, during which they were left for 1 min to explore the pool. During the behavioral testing (learning phase), animals were required to locate a hidden platform submerged (1.5 cm) in the same quadrant throughout the task using only the spatial cues available within the testing room. The animals were given four trials per day for 7 d, and the starting positions changed over trials. Each trial began with the animal in the pool facing the sidewalls and ended either after 90 sec of swimming or after the animals had found the platform; in either case the rat remained on the platform for 20 sec between each trial. Animals then underwent the reversal phase for four trials per day for 3 d, as described previously for the learning phase, except that the platform was in another

quadrant. In a control phase, the platform was visible (2 cm above the liquid surface), and the rats were tested for four trials per day over 2 d. This condition was designed to control the motor and visual demands of the task. In all phases, the parameters measured were the distance and the latency to escape onto the platform. In the reversal phase, the distance covered and the time spent in the quadrant where the platform had been located during the learning phase were measured. The results were analyzed as the individual mean of the four daily trials.

Two-trial memory test. In the first trial, one arm of the Y-maze was closed, and animals were allowed to visit the two other arms for 10 min. During the intertrial interval (ITI), rats were housed in their home cages located in a room different from the test room. During the second trial, animals had free access to the three arms and were again allowed to explore the maze for 10 min. The number and duration of visits in the novel arm (previously closed in the first trial) were calculated as a percentage of the total number or duration of visits in all three arms during the first 2 min of the second trial. This time corresponds to the maximal exploratory activity in the novel arm, which declines thereafter (Dellu et al., 1992). The percentage values were compared to a random level for visits to the three arms, i.e., 33%. The memory performance was tested with progressive ITIs: 6, 8, and 24 hr. A 1 min ITI was conducted to verify that all groups spend more time in the novel arm when no retention is necessary. The different ITIs, separated by 7 d pauses, were tested in different rooms, and the novel arm was changed between each trial.

Data analysis

ANOVA was used to compare the scores among the groups for one variable (two-way ANOVA, treatment effect) and the time course of repeated measures among the groups (two-way ANOVA, interaction treatment × time). This was followed by *post hoc* comparisons using the Newman–Keuls test (NK). Corticosterone values had a log normal distribution, and a logarithmic transformation was therefore applied to the data for statistical analysis. In the two-trial memory test, the percentage values were compared to the random score (33%) by using a Student's *t* test. For the behavioral reactivity data, a multivariate analysis (Lebart et al., 1985) was performed with Statistica 4.5 package (StatSoft, Tulsa, OK). Data from the three groups were subjected to a PCA (Saporta, 1990). The principal factors were selected according to an eigenvalue > 1 (i.e., the variance of individuals on each factor) (Kaiser, 1960). The results of the analysis were shown on a two-dimensional plane built with the first two principal factors. Variables projected onto this plane were considered to contribute significantly to a factor when their factor loading (i.e., the correlation between the variable and the factor) on this axis was at least 0.70.

RESULTS

Basal and stress-induced corticosterone secretion in adult offspring

The time course of corticosterone secretion differed among the three groups (ANOVA; interaction treatment × time; $F_{(2,226)} = 4.56$; $p < 0.001$) (Fig. 1). Comparison among the three groups revealed no effect of the perinatal manipulations on the basal level (T0) (ANOVA treatment effect; $F_{(2,113)} = 1.16$; NS) or the stress-response peak (T30) ($F_{(2,113)} = 0.96$; NS); however, the stress-induced corticosterone response (T120) differed among groups (ANOVA treatment effect; $F_{(2,113)} = 3.90$; $p < 0.02$). Although prenatal stress prolonged this response, postnatal handling reduced it. Corticosterone levels were significantly higher at 120 min in the PS group than in the C group (NK; $p < 0.01$) or the H group (NK; $p < 0.001$). The H group exhibited lower scores than the C group (NK; $p < 0.02$). The return to baseline values of corticosterone after stress was more efficient for postnatally handled rats and was impaired in prenatally stressed rats.

Behavioral reactivity in adult offspring

Y-maze test

The number of visits in the three arms of the Y-maze decreased for all groups between the first 5 min and the last 5 min (ANOVA time effect; $F_{(2,113)} = 25.23$; $p < 0.001$) (Fig. 2). Nevertheless, the

RESPONSE TO RESTRAINT STRESS IN ADULT OFFSPRING

Figure 1. Plasma corticosterone secretion ($\mu\text{g}/100\text{ ml}$) in basal condition (time, 0 min) and in response to a 30 min restraint stress (time, 30 and 120 min) (mean $\pm$ SEM). The basal (T0) and peak stress (T30) levels were similar for the three groups; however, 2 hr after stress, animals of the prenatal stress (PS) group showed a prolonged stress-induced corticosterone secretion, whereas this secretion was reduced in the handling (H) group compared with control (C) group. PS versus C, $**p < 0.01$; PS versus H, $+++p < 0.001$; H versus C, $*p < 0.02$.

time course differed among the three groups (ANOVA interaction treatment $\times$ time; $F_{(2,113)} = 20.81$; $p < 0.001$) because of a group difference in the 0–5 min interval ($F_{(2,113)} = 31.80$; $p < 0.001$), whereas the number of visits in the 5–10 min interval was comparable among groups ($F_{(2,113)} = 1.18$; NS). During the first 5 min, the PS group made more visits than did the two other groups (NK; PS vs C, $p < 0.001$; PS vs H, $p < 0.001$), indicating a higher initial locomotor activity.

Prenatally stressed animals with prolonged corticosterone secretion after stress show the highest number of visits, and postnatally handled animals with reduced corticosterone secretion show the lower visit scores. Indeed, a correlation analysis between the corticosterone level 120 min after stress and the number of visits during the first 5 min in the Y-maze in individual rats, independent of group assignment, indicated a positive correlation (Pearson's correlation; $r = 0.21$; $p < 0.02$) (Table 1).

Open-field test

The overall distance covered in the task was comparable for the three groups in the 0–15 min period (ANOVA treatment effect; $F_{(2,113)} = 2.48$; NS), whereas the time course differed significantly (ANOVA interaction treatment $\times$ time; $F_{(4,226)} = 7.49$; $p < 0.001$) (Fig. 3A). During the first 5 min, there was a significant difference among the groups in the distance covered (ANOVA treatment effect; $F_{(2,113)} = 8.05$; $p < 0.001$), with a higher score

Y-MAZE TEST

Figure 2. Number of visits (mean $\pm$ SEM) to separate arms of the Y-maze over the two 5 min halves of the 10 min test. Although animals of the PS group exhibited a higher locomotor activity than animals of C and H groups over the first 5 min, the scores were identical in the last 5 min. PS versus C, $***p < 0.001$; PS versus H, $+++p < 0.001$.

Table 1. Correlations between post-stress corticosterone values (log $\mu\text{g}/100\text{ ml}$) and behavioral responses to novelty in the three groups

Behavioral parameters	<i>r</i>	<i>p</i>
Y-maze visits	0.21	0.02
Open-field distance	0.19	0.05
Open-field corner	0.13	0.23
Plus-maze open arm	−0.24	0.01

Behavioral parameters are indicated by their corresponding abbreviations (see legend of Fig. 5). The level of corticosterone is positively correlated with the number of visits to different arms in the Y-maze and with the distance covered in the open field. No correlation, however, was found with the time spent in corners of the open field, and in contrast the level of corticosterone is negatively correlated with the time spent in open arms (%) of the elevated plus-maze.

for PS than for C and H groups (NK; PS vs C, $p < 0.01$; PS vs H, $p < 0.001$). The distance scores were comparable at 5–10 and 10–15 min (ANOVA treatment effect; $F_{(2,113)} = 2.70$; NS; and $F_{(2,113)} = 0.01$, NS, respectively). Thus, prenatally stressed rats showed a high initial locomotor activity.

The time spent in the corners of the open field differed between the three groups (ANOVA treatment effect; $F_{(2,113)} = 4.81$; $p < 0.01$), and the time course was also significantly different (ANOVA interaction treatment $\times$ time; $F_{(4,226)} = 3.83$; $p < 0.01$) (Fig. 3B). Although no difference was observed for the three groups in the first 5 min of the test ($F_{(2,113)} = 1.33$; NS), significant differences appeared over the subsequent periods (5–10 min: $F_{(2,113)} = 6.08$, $p < 0.01$; 10–15 min: $F_{(2,113)} = 5.02$, $p < 0.01$). The animals of the H group spent less time in the corners than did animals of the C and PS groups in these periods (NK; 5–10 min: H vs C, $p < 0.01$; H vs PS, $p < 0.01$; 10–15 min: H vs C, $p < 0.02$; H vs PS, $p < 0.001$), indicating that postnatally handled rats looked less for a place of refuge than did control and prenatally stressed rats.

The prenatally stressed animals that had the higher corticosterone levels in response to stress covered a greater initial distance

Figure 3. Distance covered (m) (*A*) and time spent in corners (sec) (*B*) in the open field in the three 5 min intervals of the 15 min test (mean $\pm$ SEM). The PS group covered more distance than did the other two groups over the first 5 min, indicating a high initial activity. PS versus C, $**p < 0.01$; PS versus H, $+++p < 0.001$. Differences were observed for the time spent in corners in the 5–10 and 10–15 min periods. Postnatally handled rats exhibited a lower score than did control and prenatally stressed rats, indicating that they searched less for a place of refuge. 5–10 min period: H versus C, $**p < 0.01$; H versus PS, $+++p < 0.01$. 10–15 min period: H versus C, $*p < 0.02$; H versus PS, $+++p < 0.001$.

in the open field compared with control and postnatally handled animals. Corticosterone levels 2 hr after stress were positively correlated with the distance covered in the first 5 min of the open-field test in the PS, C, and H groups (Pearson's correlation; $r = 0.19$; $p < 0.05$). In contrast, corticosterone levels and the time spent in corners of the open field were not correlated (Pearson's correlation; $r = 0.13$; NS) (Table 1).

Elevated plus-maze test

The percentage of time spent in the open arms of the elevated plus-maze differed significantly among the three groups (ANOVA treatment effect; $F_{(2,113)} = 10.73$; $p < 0.001$). During the 0–10 min period, rats of the PS group spent less time (%) in the open arms than rats of the C group (NK; $p < 0.01$), whereas handled rats spent more time (%) with respect to the control rats (NK; $p < 0.01$). Furthermore, postnatally handled and prenatally stressed

Figure 4. Percentage of time spent in open arms (open/open + closed) measured in the elevated plus-maze over the 10 min test (mean $\pm$ SEM). Prenatally stressed rats had a lower score than that of control and handled rats, whereas the score of the handled rats was higher than the one of control rats, indicating that prenatally stressed rats took refuge more than the other two groups. PS versus C, $**p < 0.01$; PS versus H, $+++p < 0.001$; H versus C, $**p < 0.01$.

Table 2. Characteristics of each factor for the PCA analysis

Factor	Eigen	% tot var	Cumul%
1	1.74	43.64	43.64
2	1.23	30.85	74.50
3	0.57	14.38	88.88
4	0.45	11.11	100

Eigen, Eigenvalue; % tot var, percentage of total variation explained by each factor; Cumul%, cumulative percentage. Two factors were extracted (eigenvalue > 1).

rats differed markedly (NK; $p < 0.001$) during this period. Thus, handled rats explored the open arms, whereas prenatally stressed rats avoided these arms and took refuge in the closed arms.

Correlation analysis indicated a negative correlation between corticosterone levels 120 min after stress and the time spent in the open arms of the elevated plus-maze (Pearson's correlation; $r = -0.24$; $p < 0.01$) (Table 1). Thus, prenatal stress animals, which had the higher corticosterone levels after stress, spent less time in the open arms (Fig. 4).

Characterization of behavioral reactivity in response to novelty

Behavioral reactivity was assessed using four parameters: the number of visits to different arms in the Y-maze, the distance covered in the open field, the time spent in the corners of the open field, and the time spent in open arms of the elevated plus-maze. To characterize different components of behavioral reactivity, a PCA has been performed. With this analysis the four parameters can be associated in two principal components, which have been called factor 1 and factor 2 of behavioral reactivity, as indicated by their eigenvalues, which must be > 1 , and by their total variance [factor 1 (F1), 43.6%, and factor 2 (F2), 30.8%] (Table 2). We have defined the first factor as a representation of exploration

Table 3. Score of factor loadings of each variable (correlation between the variable and the factor) in the PCA analysis

Variables	Factor 1 (exploration)	Factor 2 (escape behavior)
Y-maze visits	−0.28	0.85 ^a
Open-field distance	0.50	0.70 ^a
Open-field corner	−0.85 ^a	−0.07
Plus-maze open arm	0.80 ^a	0.08

^aLoadings > 0.70. Variables are indicated by their corresponding abbreviations (see legend of Fig. 5). Open-field corner was negatively correlated, whereas Plus-maze open arm was positively correlated with Factor 1 (exploration). Y-maze visits and Open-field distance were strongly positively correlated with Factor 2 (escape behavior).

BEHAVIORAL REACTIVITY IN RESPONSE TO NOVELTY

Figure 5. PCA of behavioral variables. Two factors were extracted. F1 is represented by the horizontal axis and F2 by the vertical axis. F1 accounts for 43.6% of the total variance and F2, 30.8%. The locations of the variables in this analysis are represented by a circle and correspond to the following parameters: *Y-maze visits* = number of visits in the Y-maze during the first 5 min; *Open-field distance* = distance covered in the open field during the first 5 min; *Open-field corner* = time spent in corners in the open field during the 10–15 min period; *Plus-maze open arm* = time spent (%) in open arms of the elevated plus-maze during the 10 min of the test. Factor 1 (F1) has been defined as exploration and factor 2 (F2) as escape behavior.

behavior in response to novelty because the two parameters, *plus-maze open arms*, which is an index of exploration, and *open-field corners*, which is an index of lack of exploration, are correlated positively ($r = 0.80$) and negatively ($r = -0.85$), respectively, with F1. The second factor has been defined as a representation of escape behavior in response to novelty because the two parameters, *open-field distance* and *Y-maze visits*, are positively correlated ($r = 0.8$ and 0.70) with F2 (Table 3).

Figure 5 shows the PCA results and graphically represents the idea that prenatally stressed rats, which show an increased reactivity during the first 5 min in the open field and in the Y-maze, show a high escape behavior. Moreover, these rats show a reduced

WATER-MAZE TEST

Figure 6. Performance scores in the learning phase and reversal phase of the water maze. The parameters measured were the distance (A) and latency (B) to escape onto the hidden platform. The performance improved for all groups, over the 7 d of learning and the 3 d of reversal, for both the distance and latency measures. Prenatal stress (PS), control (C), and handling (H) groups did not differ in cognitive capability.

exploration in the elevated plus-maze, whereas handled rats exhibit high exploratory behavior, given that they spent more time in the open arms of the elevated plus-maze and less time in the corners of the open field.

Cognitive performance in adult offspring

Water-maze test

During the *control test*, when the platform was visible, the three groups exhibited an identical distance score (ANOVA treatment effect; $F_{(2,113)} = 0.34$, NS) (mean $\pm$ SEM; PS group, 2.43 ± 0.19 m; C group, 2.48 ± 0.10 m; H group, 2.61 ± 0.20 m) and latency score (ANOVA treatment effect; $F_{(2,113)} = 0.54$, NS) (mean $\pm$ SEM; PS group, 8.81 ± 0.69 sec; C group, 8.48 ± 0.36 sec; H group, 9.24 ± 0.83 sec). These results indicate that all groups exhibited similar visual and sensorimotor capacities.

During the *learning phase*, as shown in Figure 6, comparison

Table 4. Comparison between the performance scores of the three groups and the chance level (33%) in the two-trial memory task in the Y-maze

		<u>1 min ITI</u>		<u>6 hr ITI</u>		<u>8 hr ITI</u>		<u>24 hr ITI</u>	
df		<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
Number of visits in novel arm (%)									
PS	26	6.47	0.001	2.96	0.01	9.00	0.001	8.40	0.001
C	60	11.05	0.001	4.84	0.001	9.53	0.001	6.66	0.001
H	17	6.47	0.001	2.64	0.02	4.83	0.001	5.95	0.001
Duration of visits in novel arm (%)									
PS	26		0.001	3.00	0.01	4.93	0.001	3.37	0.01
C	60	7.63	0.001	4.04	0.001	4.63	0.001	4.90	0.001
H	27	6.42	0.001	2.32	0.005	3.65	0.01	2.78	0.01

All measures were significantly different from chance, showing that the three groups were capable of discriminating the novel arm in each circumstance. PS, Prenatal stress; C, adult control; H, postnatally handled.

among the three groups across the 7 d of learning for the swimming performance revealed no effect of the perinatal manipulation for the distance (ANOVA treatment effect; $F_{(2,113)} = 0.18$; NS) or the latency ($F_{(2,113)} = 0.72$; NS). All groups improved their performance over the course of the learning phase as revealed by a decrease in the distance ($F_{(6,678)} = 160.73$; $p < 0.001$) and latency ($F_{(6,678)} = 182.50$; $p < 0.001$). Moreover, the interaction (group $\times$ day) was no different for the distance (ANOVA; $F_{(12,678)} = 1.37$; NS) or the latency (ANOVA; $F_{(12,678)} = 1.21$; NS), showing that the three groups did not evolve differently over the course of learning.

In the *reversal phase*, a comparison of performance in the three

groups did not reveal any significant difference in the distance scores (ANOVA treatment effect; $F_{(2,113)} = 0.24$; NS) or latency scores ($F_{(2,113)} = 0.09$; NS). The distance (ANOVA treatment effect; $F_{(2,226)} = 80.39$; $p < 0.001$) and the latency ($F_{(2,226)} = 85.67$; $p < 0.001$) improved across the 3 d of the reversal phase for the three groups. Nevertheless, no difference was found in the interaction (group $\times$ day) for the distance (ANOVA; $F_{(4,226)} = 0.35$; NS) or latency ($F_{(4,226)} = 0.22$; NS). The distance and time scores in the quadrant where the hidden platform had been placed in the learning phase were also not different among groups (ANOVA treatment effect; distance: $F_{(2,113)} = 0.92$; NS; mean $\pm$ SEM; PS, 1.49 ± 0.09 m; C, 1.58 ± 0.09 m; H, 1.74 ± 0.13 m;

TWO-TRIAL MEMORY TEST

Figure 7. Performance scores in the two-trial memory task in the Y-maze at four intertrial intervals (ITI). The parameters measured were the number of visits to the novel arm (%) (A) and the time spent in the novel arm (%) (B). PS, C, and H groups performed identically for the two parameters in each ITI session. The dotted line represents the chance level (33%) of visits in the three arms.

Table 5. Summary of results

	C	PS	H
Stress response of HPA axis (2 hr post-stress only)	—	↑ _C	↓ _{C, PS}
Behavioral reactivity in response to novelty	—	↑ _C	↓ _{C, PS}
Escape behavior	—	↑ _{C, H}	—
Y-maze (number of visits)	—	↑ _{C, H}	—
Open field (distance)	—	↑ _{C, H}	—
Exploratory behavior	—	↓ _C	↑ _{C, PS}
Open field (time in corners)	—	—	↓ _{C, PS}
Elevated plus-maze (time in open arms)	—	↓ _C	↑ _{C, PS}
Cognitive performance	—	—	—
Water maze (distance and latency in learning and reversal phases)	—	—	—
Two-trial memory test (number and duration of visits of the novel arm with 4, 6, 8, and 24 h ITI)	—	—	—

Adult rats that have been submitted to prenatal stress (PS) exhibit prolonged stress-induced corticosterone secretion at 2 hr after stress as do adult control (C) and postnatally handled (H) rats, whereas secretion was lower in H rats compared with C and PS rats. Moreover, the behavioral reactivity in response to novelty differed among groups. Prenatal stress increases and postnatal handling decreases this behavior, as has been shown by the analysis of escape and exploratory behaviors. In contrast, no difference was observed in the cognitive performance whatever the test. The arrows indicate in which direction the endocrine and behavioral responses changed, and the subscripts denote those groups for which the difference was statistically significant.

latency: $F_{(2,113)} = 0.61$, NS; PS, 5.72 ± 0.45 sec; C, 6.11 ± 0.33 sec; H, 5.72 ± 0.45 sec). The above results demonstrate that the behavior in the three groups did not evolve differently over the course of reversal.

Two-trial memory test

During the *control test*, with a 1 min ITI, all rats made more visits to, and spent more time in, the novel arm than in the other two arms (Tables 4, 5; Fig. 7). There was no preference between the other two arms, given that they were explored at a similar frequency by all animals (ANOVA arm effect; $F_{(1,113)} = 1.57$; NS). No difference was observed among groups in the number of visits (%) or the duration of visits (%) in the novel arm (ANOVA treatment effect; $F_{(2,113)} = 0.61$; NS; and $F_{(2,113)} = 0.58$; NS, respectively), and all performances were significantly above chance level (33%), demonstrating that the three groups recognize, and spend more time in, the novel situation.

During the *behavioral testing*, for the longer ITIs, the number of visits to the two previously visited arms remained similar for all animals, at each ITI (6 hr ITI: $F_{(1,113)} = 2.40$, NS; 8 hr ITI: $F_{(1,113)} = 2.29$, NS; 24 hr ITI: $F_{(1,113)} = 3.08$, NS). The three groups exhibited identical performance at each ITI for both the number of visits (%) to the novel arm (6 hr ITI: $F_{(2,113)} = 0.78$, NS; 8 hr ITI: $F_{(2,113)} = 2.58$, NS; 24 hr ITI: $F_{(2,113)} = 0.08$, NS) and the length of time spent (%) in the novel arm (6 hr ITI: $F_{(2,113)} = 0.51$, NS; 8 hr ITI: $F_{(2,113)} = 1.12$, NS; 24 hr ITI: $F_{(2,113)} = 0.07$, NS). Moreover, all scores were significantly different from chance, demonstrating that all groups continued to discriminate the novel arm.

DISCUSSION

These experiments demonstrate that prenatal stress and postnatal handling induce opposite behavioral responses to novelty and opposite neuroendocrine responses to stress in adult offspring. Prenatal stress induces a novelty-induced escape behavior and a prolonged stress-induced corticosterone secretion. The results obtained with the PCA show that the behavioral reactions to novelty can be dissociated in two responses: exploratory and escape. Thus, the increased number of visits in the Y-maze test and increased distance covered in the open-field test during the first 5 min were associated in the same factor of the analysis and

may be interpreted as an initial escape behavior in response to novelty (Archer, 1973; Aulich, 1976). Furthermore, prenatally stressed rats spent less time in the open arms of the elevated plus-maze, reflecting an avoidance of anxiogenic places (Montgomery, 1955; Pellow et al., 1985). The postnatal handling manipulation, in contrast, induces an enhanced exploratory behavior in response to novelty and a reduced stress-induced corticosterone secretion. Indeed, handled rats spent more time in the open arms of the elevated plus-maze and spent less time in the corners of the open-field test. These two behavioral responses are interpreted as a unique behavior, because they are associated in the PCA analysis. They may represent an exploration of anxiogenic environments, i.e., the center of the open field (Hall, 1934; Archer, 1973) and the open arms of the elevated plus-maze (Montgomery, 1955; Pellow et al., 1985).

The correlation analysis including all the animals showed that both the number of visits in the Y-maze test during the first 5 min and the time spent in the corners of the elevated plus-maze are positively correlated with the corticosterone secretion after a stress, whereas the time spent in open arms of the elevated plus-maze is negatively correlated with the corticosterone secretion after a stress. In other words, animals with high levels of corticosterone 2 hr after stress, such as prenatally stressed animals, have a high escape behavior, and animals with a reduced corticosterone secretion 2 hr after stress, such as postnatally handled animals, exhibit a high exploratory behavior. In contrast, neither prenatal stress nor handling changed spatial learning and memory performance in adult rats. Indeed, in the water maze, the distance and latency measures did not differ among the groups in the learning and reversal phases. Moreover, in the two-trial memory test, the number of visits and time spent in the novel arm were not different among the groups.

One factor of high anxiety-like behavior, the escape behavior in adult prenatally stressed rats, has been described previously in the literature. Similar adult prenatally stressed rats show an increased locomotor activity in a circular corridor (Deminière et al., 1992), which is similar to our observations in the Y-maze, given that both environments are characterized by an absence of refuge for the animals. In contrast, some authors report a decreased open-field activity (Ader and Belfer, 1962; Masterpasqua et al., 1976; Fride

et al., 1986; Suchecki and Palermo Neto, 1991) or no effect on this activity (Ader and Belfer, 1962; Ader and Conklin, 1963; Masterpasqua et al., 1976; Meisel et al., 1979; Fride et al., 1986; Alonso et al., 1991; Suchecki and Palermo Neto, 1991) in prenatally stressed rats, whereas the present study shows first a transient increase. These discrepant results may depend on many factors, including the kind of prenatal stress, the period of stress application, the age of the offspring, and the open-field paradigm used.

The second behavioral expression of the high anxiety-like behavior in prenatally stressed rats, the avoidance of anxiogenic environments, is in agreement with a previously reported increase in food-related neophobia, after a prenatal novelty stress, which was interpreted as a decreased exploration in a novel situation (Pfister et al., 1981). Moreover, a decreased exploration in the open arms of the elevated plus-maze has been reported after the application of a prenatal stressor consisting of restraint and an unpredictable noise (Fride and Weinstock, 1988; Wakshlak and Weinstock, 1990). Conversely, increased exploration has also been found after a predictable prenatal stress (Weinstock et al., 1988), indicating the critical importance of the prenatal stress paradigm chosen.

Adult prenatally stressed animals exhibited similar neuroendocrinological and behavioral characteristics of adult high-responder (HR) animals described previously in the literature (Deminière et al., 1989; Piazza et al., 1989, 1990, 1991). Prenatally stressed and HR animals displayed a prolonged stress-induced corticosterone secretion, an increased locomotor activity in a circular corridor, and a higher amphetamine self-administration. Together, these results suggest that early events in life can influence adult animals to become HR animals. Furthermore, we can speculate that the anxiety-like behavior described in this study could be associated with a higher vulnerability to drug addiction.

Concerning the expression of low anxiety-like behavior in adult postnatally handled rats, the increased exploratory behavior in anxiogenic environments is similar to previous findings showing that the exploratory deficit in the open field and elevated plus-maze observed in prenatally stressed rats is reversed by postnatal handling (Wakshlak and Weinstock, 1990). Moreover, handled rats demonstrate increased exploration in the open field (Levine et al., 1967) and in a hexagonal tunnel maze (Fernandez-Teruel et al., 1991, 1992), and they have reduced food-related neophobia (Weinberg et al., 1978; Bodnoff et al., 1987). Taken together, these reports and the results presented here suggest a handling-induced decrease of anxiety-like behavior.

In contrast to the demonstrated changes in anxiety, prenatal and postnatal manipulations did not influence cognitive performance in the adult offspring. This result is in agreement with a previously reported lack of influence of a prenatal stress (ultrasound exposure) on adult memory capacities (Hande et al., 1993); however, decreased adult learning performance after a prenatal heat shock has been reported (Smith et al., 1981; Shiota and Kayamura, 1989). It has been suggested that the intensity of the prenatal stress procedure may be critical for the induction of memory impairments (McEwen and Sapolsky, 1995; Luine et al., 1996). The finding that postnatal handling did not affect cognition is supported by previous studies demonstrating that handling induced no change in the latency performance in the water maze in adult rats (Meaney et al., 1988, 1991); however, it improved memory performance in old rats (Meaney et al., 1988, 1991; Escorihuela et al., 1995). Thus, we can suggest that prenatal and postnatal manipulations induced memory alterations only in old age. In other words, the elevated stress-induced corticosterone

secretion in adult prenatally stressed animals seems to appear before the cognitive impairments in later life.

Our results show that corticosterone secretion 2 hr after stress is correlated positively with escape behavior and negatively with exploration behavior. Memory performance is not changed and thus not correlated with the corticosterone level. It has been shown previously that differences in HPA axis activity are associated with differences in locomotor activity in response to novelty and with differences in susceptibility to drug addiction (Piazza et al., 1991). To our knowledge, however, no relationship between HPA axis activity and escape or exploration behavior, such as those demonstrated in this study, has been confirmed previously. Thus, the alterations of HPA axis activity induced by prenatal stress and postnatal handling may represent a mechanism underlying the changes observed in adult behavioral reactivity.

A dysregulation of the HPA axis has also been associated with cognitive impairments, but only in old rats (Sapolsky et al., 1986; Meaney et al., 1988, 1991; Escorihuela et al., 1995). It may be proposed that in adult rats, different factors can counteract the influence of altered HPA activity on memory capacities. For example, hyperactivity of the HPA axis observed in prenatally stressed rats is accompanied by increased plasma concentrations of glucose (Vallée et al., 1996), which may be able to counteract existing cognitive problems, given that increased plasma concentrations of glucose improve memory in humans (Hall et al., 1989) and rats (Lee et al., 1988; Gold, 1995). Furthermore, only one of the two types of hippocampal corticosteroid receptors is influenced by prenatal stress (Weinstock et al., 1992; Maccari et al., 1995) or postnatal handling (Meaney et al., 1988). It may be hypothesized that modifications of both types of receptors are necessary to noticeably alter cognition, given that both types are involved in the evaluation and consolidation of spatial information (Oitzl and de Kloet, 1992; McEwen and Sapolsky, 1995).

In conclusion, this study shows that the emotional patterns of adults is differentially influenced by perinatal experiences. Prenatal stress induces a hyperanxiety, expressed as an escape behavior, which is positively correlated with post-stress levels of corticosterone, whereas early postnatal handling induces a hypoanxiety, expressed as an exploration behavior, negatively correlated with post-stress levels of corticosterone. We show that prenatal stress and postnatal handling may be two useful models for studying individual differences in stress-induced reactivity that occur naturally in life, although early experiences seem insufficient for altering the cognitive status in adulthood and may be involved only in cognitive alterations during senescence.

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