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Behavior ontogeny in the elevated plus-maze: prenatal stress effects

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Abstract

Prenatal stress is a putative model for studying some psychopathological disorders. Indeed, submitting pregnant animals to stress leads to enhanced anxiety in the adult offspring. However, little is known about how prenatal stress effects interacts with anxiety throughout development. To study this issue, prenatally stressed rats were tested in the elevated plus-maze at different ages. During pregnancy female rats were submitted to uncontrollable electric foot shock sessions every other day or kept undisturbed (controls). After delivery, litters from control and stressed dams were left undisturbed from the 3rd to the 14th postnatal days. Male and female rats were tested in the elevated plus-maze at the ages of 30, 45 or 60 days. The following measures were taken in the elevated plus-maze: number of entries and time spent in the arms (or their extremities) and frequency and time spent in naturalistic behaviors (stretching, rearing, end exploring, grooming and head dipping). Decreases in the percentage of entries into and in the time spent (only females) in the open arms were shown by 60-day-old prenatally stressed rats, but not by 30- and 45-day old. Increased open arm ends exploration was shown by 45-day-old prenatally stressed males. Rearing behavior was found to increase with age, a phenomenon more pronounced in females. Additionally, at the younger ages prenatally stressed rats were heavier than controls, an effect which disappeared at young adulthood. In conclusion, anxiogenic prenatal stress effects in the elevated plus-maze could only be detected at early adulthood, not before. Nonetheless, at late adolescence (45 days of age) prenatal stress leaded to an anxiolytic-like effect which can be interpreted as increased risk-taking behavior.

Keywords: Prenatal stress; Elevated plus-maze; Ontogeny; Anxiety

1. Introduction

Prenatal stress is a putative model for studying behavioral disorders and screening psychopharmacological agents. Indeed, it has been associated with some characteristics of generalized anxiety (De Bellis et al., 2000; Salm et al., 2004; Estanislau and Morato, 2005), depression (Morley-Fletcher et al., 2003; Smith et al., 2004) and schizophrenia (Weinstock, 2001; McClure et al., 2004). In addition, some pharmacological treatments can reverse some prenatal stress effects (Morley-Fletcher et al., 2003; Poltyrev and Weinstock, 2004). A large range of prenatal stress effects have been shown in adult animals (e.g., Rimondini et al., 2003; Morley-Fletcher et al., 2003; McClure et al., 2004; Louvart et al., 2005) and there is evidence indicating that prenatal stress effects are mediated by maternal hormones that pass through the placenta (Zarrow et al., 1970; Barbazanges et al., 1996). However, several of these effects were not evaluated in respect to their ontogenetic manifestation.

The effects of many treatments have been shown to depend on the age when the subjects are tested (e.g., Genn et al., 2003; Park et al., 2003; Marin and Planeta, 2004; Maldonado and Kirstein, 2005). The period of transitions from infanthood to adulthood seems to be marked by discontinuities. Indeed, as compared to younger or older animals, periadolescent rats differ in the manner they behave and respond to psychopharmacological agents (Spear and Brake, 1983). Adolescence is usually associated to sexual maturation but this period is also marked by the emergence of several other skills needed for independence from parental care (Kellogg et al., 1998). Alterations along adolescence have been shown to provoke changes in social behavior occurring in unfamiliar environment (Primus and Kellogg, 1989) and in neural functioning considered to be important to stress response (Kellogg et al., 1993; Choi and Kellogg, 1996; Kellogg et al., 1998). The adolescent individual can, in fact, be seen as a changing system: alterations that take place during this period cannot only lead to the emergence of typical adult behavior but also can contribute

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to the development of behavioral disorders as well (Choi et al., 1997).

The elevated plus-maze is an anxiety test based on the exploration of an unfamiliar open environment. Indeed, the elevated plus-maze is one of the most popular models currently used in the study of animal anxiety (Rodgers et al., 1996; Wall and Messier, 2000; Bertoglio and Carobrez, 2002). It was validated for rats (Pellow et al., 1985) and mice (Lister, 1987) and is routinely used in the screening of putative anxiolytic compounds and in the study of neurobiological mechanisms of anxiety. Although even younger animals show a preference for closed over open arms of an elevated-plus-maze (Moreira et al., 2001), anxiety-related behavior changes over development in this test (Imhof et al., 1993).

The present work was aimed at investigating (1) whether prenatal stress can interfere with the emergence of typical adult anxiety-related coping behavior and (2) the ontogeny of such interference. The elevated plus-maze was used for this purpose. Given that in many studies rats are tested in this apparatus slightly heavier than 200 g, animals around this body weight (which is attained approximately at 45 days of age) were used in the experiment. Younger (30-day old) and older (60-day old) animals were also studied. The mentioned ages correspond (Spear, 2000) to early (30 days of age) and late adolescence (45 days of age) and to early adulthood (60 days of age).

2. Material and methods

2.1. Subjects

Virgin female Wistar-derived rats weighing 250–300 g were housed three to a cage (40 cm × 34 cm × 17 cm) together with a sexually experienced male for 5 days. In the sixth day, arbitrarily considered gestational day one, the male was removed and the females were transferred to individual cages until the occurrence of deliverance. During pregnancy, cage cleaning procedures were performed three times a week. Births occurred in a 5-day interval. In the day after delivery, all the litters were sexed. Only the litters with 8–13 pups were maintained in the experiment, the remaining ones being discarded. It is important to notice that routine cage maintenance began only on postnatal day 14. When 21 days old, the pups were weaned and grouped by sex and treatment (see Section 2.2) in number of 5 or 6 to a cage. After weaning, except for cage cleaning, animals remained undisturbed. Behavioral tests were performed between 8:00 and 12:00 h. Care was taken so that groups included no more than two pups from the same litter. Throughout the study, the animals were kept in a room with temperature maintained between 24–27 °C and a 12:12 h light/dark photoperiod (lights on at 7:00 a.m.). Commercial rat chow and tap water were available ad libitum throughout the experiment. The experiments reported in this paper were performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior which, in turn, are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Prenatal stress

A prenatal stress procedure reported elsewhere was followed (Estanislau and Morato, 2005). Briefly, females were submitted to electric foot shocks at every other day throughout the pregnancy. The pregnant females were taken to a box with a grid floor that allowed the delivery of 80 electric shocks (0.5 mA, 0.5 s) on a random basis during 100-min sessions carried out between 12:00 and 16:00 h. Electric shocks were delivered by a Grason-Stadler generator (model E1064GS, USA). After birth the dams and pups were not handled during the first 2 weeks. In the control group, the pregnant rats were left undisturbed but for cleaning procedures and after birth, as with the prenatal stress group, the litters were not handled until the 14th postnatal day, when routine cage cleaning started.

2.3. Litter parameters and body weight

Control ($N = 8$) and prenatally stressed ($N = 8$) litters were compared in the following parameters: litter size, number of male and female pups, sex ratio, and body weights at 21 days of age. Pup mortality was rare and no analysis was carried out on this measure. Body weights at the ages of testing were also recorded.

2.4. Elevated plus-maze

Male and female control and prenatally stressed rats were divided into three different groups. Each group was tested in the elevated plus-maze at only one of the following ages: 30, 45 and 60 days. The number of rats in each group can be seen in Table 2.

A standard elevated plus-maze described elsewhere (Setem et al., 1999) was used. Briefly, it consisted of two open arms (50 cm × 10 cm) crossed at right angles with two closed arms (50 cm × 10 cm, surrounded by 40-cm high wooden walls). To avoid falls, the open arms were surrounded by a 0.5-cm high Plexiglas rim. The experimental room was lit by a 60-W bulb placed 1.75 m above the central square of the maze (22 lx in the maze central square). The apparatus was cleaned with a 5%-ethanol solution and dried with a cloth between sessions. All sessions were video recorded by a camera placed 1.90 m above the apparatus. Each rat was gently placed in the maze facing one of the closed arms. Five minutes later, the rat was returned to its home cage. On a transparent mask placed over the TV set screen, lines were traced dividing the maze floor into 21 10-cm squares. This allowed to record a square entry whenever the hind paws entered a square and to locate the exact place where naturalistic behaviors occurred (Garcia et al., 2005). It also allowed to record the number of entries (defined when all four paws were inside a specific area) and time spent in the open and closed arms.

The frequency and time spent in the following behaviors were also measured (Cruz et al., 1994; Rodgers and Johnson, 1995): open arm end exploration (entering the open arm 20-cm distal section from the central square, i.e., the last two squares of the arms); head dipping (sticking the head outside the maze border and below the floor level); stretching (elongation of the body maintaining the hind paws fixed); rearing (rising on the hind limbs both touching and not touching a wall surface); and grooming (friction of any part of the body with the paws and/or the mouth). According to a previous report (Rodgers and Johnson, 1995), head dipping and stretching were further differentiated as a function of where on the maze they were performed. Thus, the closed arms and central square were together designated protected area of the maze, while the open arms were designated unprotected areas. Rearing and grooming behavior were displayed almost exclusively in the closed arms and no effort was done to differentiate these behaviors in function the animals location when performing the activity.

2.5. Data analysis

All results are shown as means ± S.E.M. To compare unstressed with prenatally stressed litter parameters, all the measures were analyzed with the Student's *t*-test for independent samples. Body weight at weaning was evaluated by means of a two-way analysis of variance (ANOVA) with gender as one factor (two levels: male and female) and prenatal treatment as the other (two levels: control and prenatal stress). Body weight at this age was compared by using the average body weight of pups from each litter.

The elevated plus-maze data and body weight at the ages of testing were evaluated by means of a three-way ANOVA with the factors age at testing (three levels: 30, 45 and 60 days of age), gender (two levels: male and female) and treatment (two levels: control and prenatal stress). Whenever appropriate, Fisher LSD post hoc test was used. In all cases, significance level was set at $p < 0.05$.

3. Results

3.1. Litter parameters and body weight

Table 1 shows litter measurements. Prenatally stressed pups did not differ from non-stressed animals in litter size, number of

Table 1
Parameters (mean ± S.E.M.) analyzed in eight control and eight prenatally stressed litters

Parameter	Control	Prenatal Stress	Statistics[a]
Litter size	12.1 ± 0.5	10.0 ± 1.1	$T_{[14]} = 1.706$
Number of males per litter	6.1 ± 0.7	5.0 ± 0.9	$T_{[14]} = 0.973$
Number of females per litter	6.0 ± 0.9	5.0 ± 0.6	$T_{[14]} = 0.921$
Male:female ratio	1.3 ± 0.4	1.1 ± 0.2	$T_{[14]} = 0.623$

[a] Student *t*-test, non paired samples (*t*-values and degrees of freedom).

males and of females per litter and male:female ratio. At weaning, according to ANOVA, body weight of prenatally stressed (males: 54.4 ± 2.2 g; females: 52.0 ± 2.2) and control rats (males: 47.1 ± 2.7 g; females: 47.7 ± 2.6) showed a main effect of treatment ($F_{[1,28]} = 5.615$, $p < 0.05$), but not of gender ($F_{[1,28]} = 0.141$, $p > 0.05$); no significant interaction between the factors was found ($F_{[1,28]} = 0.368$, $p > 0.05$).

Fig. 1 shows the body weights of male and female control and prenatally stressed rats at the ages of 30, 45 and 60 days. As can be seen in the figure, body weight was affected by age, gender and treatment. An interaction between age and gender was also found. Post hoc comparisons showed, as expected, older animals to be heavier than younger ones for both sexes. Prenatally stressed male and female rats were heavier than age- and sex-matched controls at 30 days of age. Forty five-day-old prenatally stressed males were heavier than their controls; no differences were observed between same age females. Finally, at 45 days of age, prenatally stressed males were heavier than prenatally stressed females, an observation that was not present between controls. At 60 days of age, all females weighted less than the males.

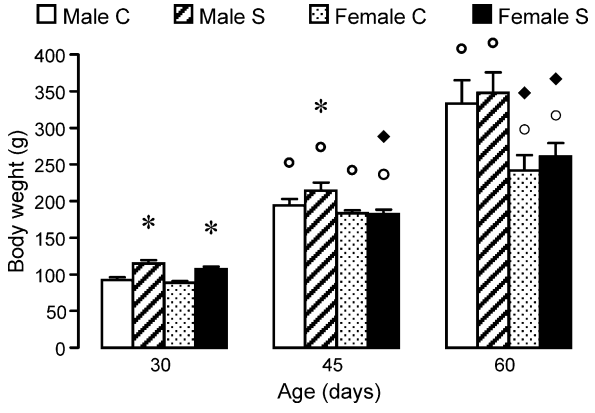

Fig. 1. Body weight of male and female control (C) and prenatally stressed (S) rats at the ages of 30, 45 and 60 days. There were main effects of age ($F_{[2,121]} = 784.28$, $p < 0.05$), gender ($F_{[1,121]} = 94.32$, $p < 0.05$) and treatment ($F_{[1,121]} = 15.45$, $p < 0.05$). There was also an interaction between age and gender ($F_{[2,121]} = 39.70$, $p < 0.05$). *, different from the control group of same sex and age; ○, Different from younger rats of same sex and treatment; ◆, different from males of the corresponding age and treatment (Fisher LSD test, $p < 0.05$).

3.2. Elevated plus-maze

The percentage of entries in the open arms (Fig. 2) is a measure inversely related to anxiety (Cruz et al., 1994; Rodgers and Johnson, 1995). ANOVA showed no differences due to age,

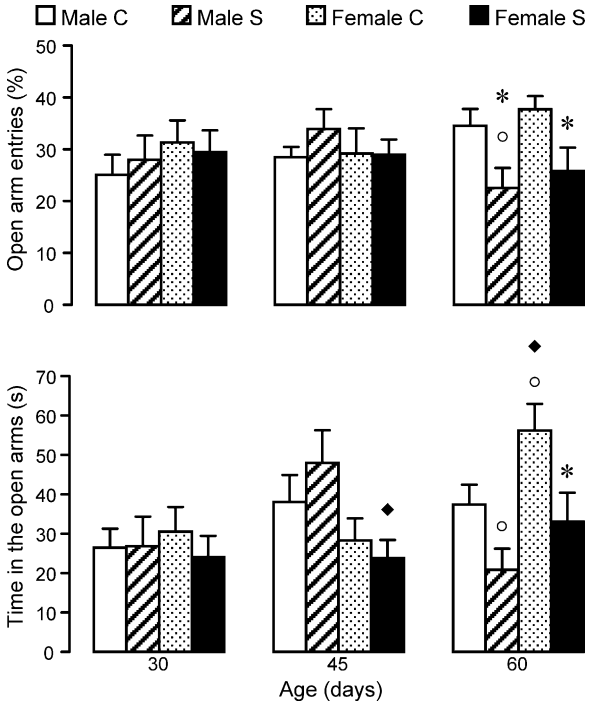

Fig. 2. Percent of entries and time spent in the open arms by male and female control (C) and prenatally stressed (S) rats in the elevated plus-maze at the ages of 30, 45 and 60 days. Percent of entries in the open arms showed an interaction between age and treatment ($F_{[2,121]} = 3.96$, $p < 0.05$). Time spent in the open arms showed interactions between age and gender ($F_{[2,121]} = 6.36$, $p < 0.05$) and between age and treatment ($F_{[2,121]} = 3.21$, $p < 0.05$). *, Different from the control group of same sex and age; ○, different from 45-day-old rats of same sex and treatment; ◆, different from males of the corresponding age and treatment (Fisher LSD test, $p < 0.05$).

prenatal treatment, or gender. On the other hand, ANOVA indicated a significant interaction between age and treatment; all other interactions between factors were not significant. The post hoc test showed 60-day-old prenatally stressed males and females to enter the open arms less than their respective control groups. This effect was not observed at the ages of 30 and 45 days.

Time spent in the open arms (Fig. 2) is also a measure inversely related to anxiety (Cruz et al., 1994; Rodgers and Johnson, 1995). As can be seen in the figure, time spent in the open arms was not significantly altered by any of the three factors, but there were significant interactions between age and treatment and between age and gender. Post hoc comparisons showed that within 60-day-old animals, prenatally stressed females spent less time in the open arms than their controls. In addition, within prenatally stressed males, the 45-day-old group spent more time in the open arms than the other two groups. Within control females, 60-day-old rats spent more time in the open arms than the two other age groups. Also, the following sex differences were found: 45-day-old prenatally stressed male animals spent more time in the open arms than females of the same age and treatment; 60-day-old control females spent more time in the open arms than males of the same age and treatment.

Open arm end exploration (Fig. 3) is yet another measure inversely related to anxiety (Cruz et al., 1994). Entries into the

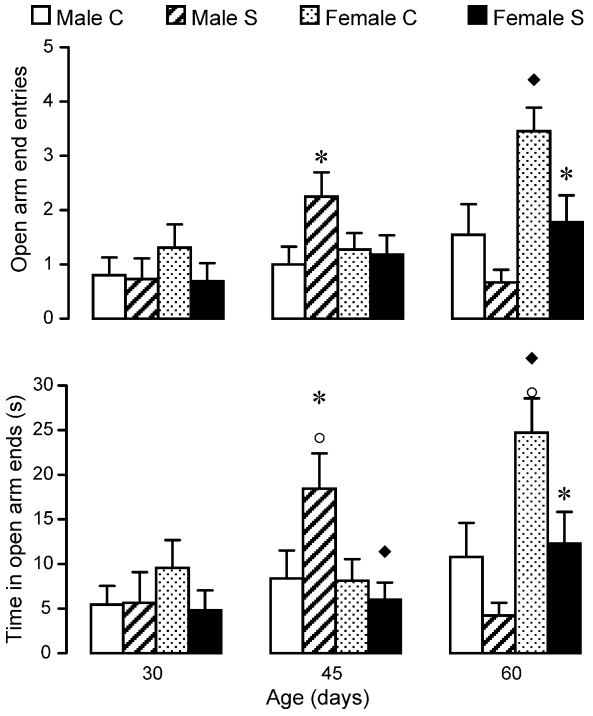

Fig. 3. Entries and time spent in the open arm ends by male and female control (C) and prenatally stressed (S) rats in the elevated plus-maze at the ages of 30, 45 and 60 days. Entries in the open ends showed a main effect of age ($F_{[2,121]} = 5.91$, $p < 0.05$) and interactions between age and gender ($F_{[2,121]} = 5.64$, $p < 0.05$) and between age and treatment ($F_{[2,121]} = 5.23$, $p < 0.05$). Time spent in the open ends showed a main effect of age ($F_{[2,121]} = 4.58$, $p < 0.05$) and interactions between age and gender ($F_{[2,121]} = 7.65$, $p < 0.05$), between age and treatment ($F_{[2,121]} = 4.60$, $p < 0.05$) and between gender and treatment ($F_{[1,121]} = 4.64$, $p < 0.05$). *, Different from the control group of same sex and age; ○, different from the other two groups of same sex and treatment; ◆, different from males of the corresponding age and treatment (Fisher LSD test, $p < 0.05$).

open arm ends showed a main effect due to age. Also, there were statistically significant interactions between the factors treatment and age and between gender and age. No other significant interaction was found. Post hoc comparisons showed 45-day-old prenatally stressed males to enter the open arm ends more than controls of the same sex and age. Sixty-day-old prenatally stressed females entered less than their respective controls. Additionally, it was found that 60-day-old control females entered the open arm ends more than control males of the same age. In respect to the time spent in the open arm ends, again, there was a main effect due to age and significant interactions treatment × age and gender × age. The interaction between the factors gender and treatment was also significant. In the time spent in open arm end exploration, the post hoc comparison showed 45-day-old prenatally stressed males spent more time while 60-day-old prenatally stressed females spent less time than their respective control groups. The 45-day-old prenatally stressed males and the 60-day-old control females spent more time exploring the open arm ends than the other two groups of same sex and treatment. Also, the following sex differences were found: 45-day-old prenatally stressed females stayed less time while 60-day-old controls females stayed longer in the open arm ends than the male group of same age and treatment.

Table 2 shows other behavioral results. The stretching activity we observed corresponds approximately to the category of stretched attend posture of Rodgers and Johnson (1995) although caution is in order because of differences between the two studies: e.g., the species studied and the nature of the measure, absolute value or percentage. In the study above, protected stretch attend posture was found to be directly related to anxiety. In our study, the frequency of stretches in the protected areas was not affected by age, gender or treatment.

Table 2
Results obtained from control and prenatally stressed male and female rats tested in the elevated plus-maze at the ages of 30, 45 and 60 days

Groups	Protected stretching		Protected head dipping		Unprotected head dipping		Grooming	
	Frequency	Time (s)	Frequency	Time (s)	Frequency	Time (s)	Frequency	Time (s)
30 days of age								
Control males (11)	2.6 ± 0.6	2.4 ± 0.6	1.1 ± 0.3	0.6 ± 0.2	2.9 ± 0.6	2.0 ± 0.5	4.1 ± 0.7	18.8 ± 3.2
Stressed males (11)	4.5 ± 0.6	5.3 ± 1.0	1.2 ± 0.3	0.6 ± 0.1	2.0 ± 0.7	1.0 ± 0.4	3.5 ± 0.6	26.8 ± 5.4
Control females (13)	5.4 ± 0.7	2.8 ± 0.7	0.7 ± 0.3	0.3 ± 0.1	3.1 ± 0.7	1.6 ± 0.4	6.5 ± 1.2	27.5 ± 7.3
Stressed females (13)	3.4 ± 0.7	4.0 ± 1.3	0.8 ± 0.4	0.7 ± 0.5	2.5 ± 0.7	1.9 ± 0.7	3.6 ± 0.7[a]	20.5 ± 6.3
45 days of age								
Control males (12)	5.2 ± 1.0[b]	6.1 ± 1.5	1.3 ± 0.4	0.9 ± 0.2	4.5 ± 0.6	3.5 ± 0.7	3.5 ± 0.7	22.7 ± 5.1
Stressed males (12)	3.1 ± 0.8	3.9 ± 1.1	2.0 ± 0.4	1.4 ± 0.3	6.3 ± 1.1[b]	3.4 ± 0.5[b]	6.5 ± 0.6[a,b]	30.6 ± 3.7
Control females (12)	4.0 ± 0.9	6.0 ± 1.7	1.6 ± 0.4	0.9 ± 0.3	4.5 ± 1.3	2.4 ± 0.8	6.3 ± 1.3[c]	29.5 ± 5.5
Stressed females (12)	3.0 ± 0.5	3.4 ± 0.8	1.8 ± 0.6	1.2 ± 0.5	2.8 ± 0.6[c]	1.7 ± 0.4	7.0 ± 1.0[b]	25.8 ± 4.7
60 days of age								
Control males (12)	2.7 ± 0.7[d]	4.3 ± 1.8	2.1 ± 0.7	0.9 ± 0.3	7.1 ± 1.6[b]	3.6 ± 0.7	4.6 ± 0.6	35.4 ± 8.8
Stressed males (10)	3.9 ± 0.8	7.1 ± 2.0	1.2 ± 0.5	1.1 ± 0.4	3.2 ± 0.7[a,d]	2.4 ± 0.8	5.4 ± 1.3	25.9 ± 4.4
Control males (11)	2.3 ± 0.8	2.2 ± 0.9[d]	1.8 ± 0.5	0.9 ± 0.3	9.1 ± 0.9[d]	6.0 ± 0.6[c,d]	4.4 ± 0.8	13.3 ± 3.2
Stressed males (9)	4.3 ± 1.1	5.6 ± 2.0	2.2 ± 0.6[b]	1.4 ± 0.5	4.9 ± 1.5[a]	3.2 ± 1.1[a]	4.2 ± 0.7[d]	19.8 ± 2.1

The number of rats in each group is indicated in parenthesis.
[a] Different from the respective control.
[b] Different from the same sex corresponding 30-day-old rats.
[c] Different from same treatment and age males (Fisher LSD test, $p < 0.05$).
[d] Different from the same sex corresponding 45-day-old rats.

ANOVA showed a significant interaction between age and treatment ($F_{[2,121]} = 4.52$, $p < 0.05$) but not between any other factors. The comparison between group means showed 45-day-old control males to stretch more frequently than the other two age groups. ANOVA also showed that the time spent stretching in the protected area was not affected by age, gender or treatment. As found for the frequency, ANOVA showed a significant interaction between age and treatment ($F_{[2,121]} = 4.73$, $p < 0.05$) but not between any other factors. The post hoc comparisons test showed that 60-day-old control females spent less time stretching than the 45-day-old control females. No effects were found in the unprotected stretching measures (data not shown).

Protected head dipping activity (Table 2) is directly related to anxiety (Rodgers and Johnson, 1995), although care should be taken because of the same reasons presented in the presentation of stretching behavior, in the previous paragraph. ANOVA showed that the frequency of head dipping from the protected area (Table 2) was affected by age ($F_{[2,121]} = 4.81, p < 0.05$) but not by gender or treatment; no interactions were detected. Post hoc comparisons showed that 60-day-old prenatally stressed females dipped their heads from the protected areas more frequently than the 30-day-old prenatally stressed females. The time spent dipping the head from the protected area was not affected by any of the three factors and no interactions between factors were detected. In the unprotected area (open arms), however, the frequency of head dippings was altered by age ($F_{[2,121]} = 12.61$, $p < 0.05$) and treatment ($F_{[1,121]} = 7.96$, $p < 0.05$) and there were interactions between these two factors ($F_{[2,121]} = 4.76$, $p < 0.05$) and between age and gender ($F_{[2,121]} = 3.36, p < 0.05$) were detected. Post hoc comparisons showed that 60-day-old prenatally stressed males and females dipped their heads less often than their respective controls. They also showed 45-day-old prenatally stressed males dipped their heads from the unprotected area more frequently than prenatally stressed 30- and 60-day-old males and 45-day-old females. Comparisons between group means also showed 60-day-old control animals to dip the head from the unprotected area more frequently than younger control animals. ANOVA showed the time spent dipping the head from the unprotected areas to be affected by age ($F_{[2,121]} = 11.01$, $p < 0.05$) and treatment ($F_{[1,121]} = 5.89$, $p < 0.05$). ANOVA also detected a significant interaction only between age and gender ($F_{[2,121]} = 5.51$, $p < 0.05$) but not between any other combination of factors. ***Post hoc comparisons showed 60-day-old prenatally stressed females to spend less time dipping the head than their control counterparts. Sixty-day-old control females spent more time dipping the head than the other two control female age groups. Also, 45-day-old prenatally stressed males spent more time dipping the head than 30-day-old males under the same treatment.

Rearing behavior (Fig. 4) is correlated with exploration (Rodgers and Johnson, 1995). ANOVA indicated main effects of age and gender on rearing frequency. It also showed significant interactions between age and gender and between age and treatment. All 45-day-old groups but control males reared more frequently than 30-day-old ones of the same sex and treatment.

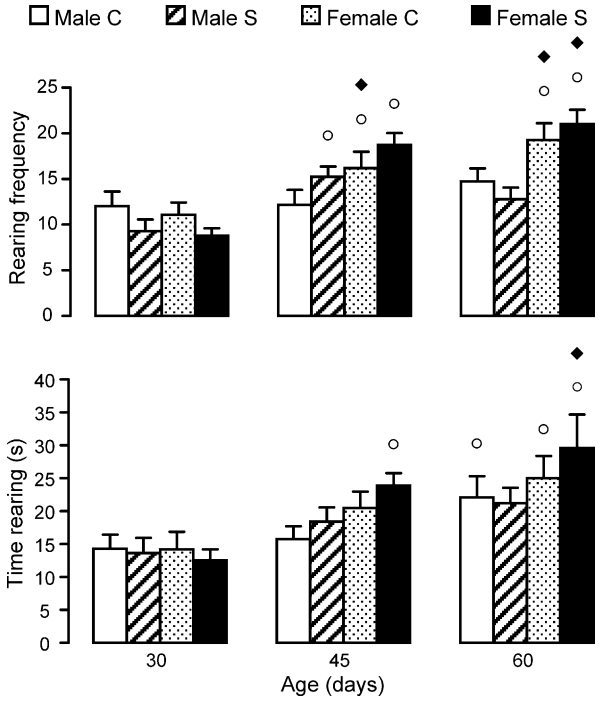

Fig. 4. Rearing behavior by male and female control (C) and prenatally stressed (S) rats tested in the elevated plus-maze at the ages of 30, 45 and 60 days. Rearing frequency showed main effects of age ($F_{[2,121]} = 24.42$, $p < 0.05$) and gender ($F_{[1,121]} = 14.37$, $p < 0.05$) and interactions between age and gender ($F_{[2,121]} = 6.22$, $p < 0.05$) and between age and treatment ($F_{[2,121]} = 3.66$, $p < 0.05$). Time spent rearing showed main effects of age ($F_{[2,121]} = 16.38$, $p < 0.05$) and gender ($F_{[1,121]} = 4.84$, $p < 0.05$). ○, Different from 30-day-old rats of same sex and treatment; ◆, different from males of the corresponding age and treatment (Fisher LSD test, $p < 0.05$).

For all treatments, at the age of 60 days females continued to rear more frequently than 30-day-old females submitted to the same treatment. Sex differences were found at the ages of 45 and 60 days: control females reared more than control males; in the prenatally stressed group a less intense effect was found: 60 day-old females reared more often than their male counterparts. Time spent rearing was also found to be affected by age and gender. All 60-day-old males and females, with the exception of the prenatally stressed males, reared longer than 30-day-old counterparts. At the age of 45 days, prenatally stressed females exhibited longer times rearing than their 30-day-old female counterparts. Finally, at 60 days of age prenatally stressed females reared longer than males under the same treatment.

There is no agreement about the interpretation of grooming activity in the plus-maze literature, though it is sometimes considered displaced activity (Archer, 1973). Table 2 shows the results of grooming behavior. Grooming frequency was not affected by either of the three factors but ANOVA detected a significant interaction between age and treatment ($F_{[2,121]} = 4.21, p < 0.05$) but not between any other combination of factors. Post hoc comparison showed that 45-day-old prenatally stressed males groomed more often than the corresponding control group. On the other hand, 30-day-old prenatally stressed females groomed less often than the corresponding control group. Also, 45-day-old prenatally stressed males and females groomed more frequently than their respective 30-day-old counterparts. At 45

days of age, control females groomed more than control males. ANOVA showed no main effects of either three factors on the time spent grooming; it also showed no significant interactions between factors.

No effects of either three factors were found in the entries in the closed arms and in the time spent in the central square nor were significant interactions found (data not shown).

4. Discussion

The present study showed that prenatal stress led to age-dependent effects on anxiety as evaluated in the elevated plus-maze. Prenatal stress resulted in no anxiogenic effects in the 30- and 45-day-old groups. At the onset of adulthood (60 days of age), on the other hand, prenatally stressed rats presented behavioral differences which indicate increased anxiety: decreases in the percentage of entries and in the time spent in the open arms as well as decreases in the unprotected head dipping measures. The 60-day-old prenatally stressed females also showed reduced exploration of the open arm ends. One cannot claim prenatal stress effects on anxiety only become visible in the early adulthood. As a matter of fact, 45-day-old males showed an unexpected anxiolytic prenatal stress effect: the increased exploration of the open arm ends.

The present work shows that prenatal stress effects on emotional responsivity, as evaluated in the exploratory behavior, emerge at late adolescence. Sparse information is available about the ontogeny of prenatal stress effects (for a review, see Kofman, 2002). The vast majority of studies on prenatal stress effects – either on anxiety-related behavior or on stress neuroendocrinology – explore 'long term effects' in adult animals (e.g., Rimondini et al., 2003; Morley-Fletcher et al., 2003; McClure et al., 2004; Louvart et al., 2005). There is, however, at least one study which explores the very issue of the ontogeny of prenatal stress effects on emotional responsivity (Dickerson et al., 2005). It shows that, differently from our results, prenatally stressed male rats as young as 45 days exhibit increased fearfulness in the defensive withdrawal test. As in our study, on the other hand, 25-day-old rats also failed to show prenatal stress effects. In spite of similarities between the elevated plus-maze and the defensive withdrawal tests (e.g., ethologic validation—the main measures of both are related to the rats' ability to cope with aversive open spaces), the contrasting results can probably be accounted for by procedural differences. For instance, pre-exposure to the open space in the defensive withdrawal procedure has no correspondent in our plus-maze test since we did not adopt pre-exposure. The test may become less aversive as a result of such pre-exposure. In the present work, throughout pregnancy, the female rats were submitted to electric foot shocks, a regimen importantly different from the one used in the cited work (removal to a novel cage and subcutaneous injection of saline during the last week of pregnancy). Probably because of being mild, these procedures, according to the authors, do not alter maternal behavior, contrary to our results, in which the stress regimen may have altered it (see discussion below). It should also be noted that the present work and the above cited study were carried out with different rat strains. Aside from the differences already mentioned, we can think of no other explanation for the contrasting results. However, it is important to bear in mind that both studies agree on the age when prenatal stress effects can firstly be detected: 45 days, an age which correspond to late adolescence (Spear, 2000).

What kind of changes in the organism can be related to the present behavioral results? To our knowledge, the present work is one of the first demonstrating prenatal stress-related ontogenetic changes in exploration of an aversive environment, but we have no clues as to the mechanisms underling such changes. Nonetheless, some studies seem relevant to this matter. It is important to notice that social behavior in unfamiliar environment changes over adolescence (Primus and Kellogg, 1989) and that this change is modulated by gonadal hormones (Primus and Kellogg, 1990). Prenatal stress has been shown to affect the fetal gonadal function in blue foxes (Osadchuk et al., 2003) and the responsiveness to the gonadal hormones in rats (Frye and Wawrzycki, 2003). It has been reported that defensive withdrawal responses of prenatally stressed male rats are age-dependent: from late adolescence on increased fearfulness is found (Dickerson et al., 2005). The authors suggest that such an increase in male fearfulness is due to decreases in testosterone. In the same vein, in females, proestrous has been associated to anxiolytic and antidepressant effects which can result from the estrogen level associated to this phase of the cycle (Fernandez-Guasti and Picazo, 1992; Frye and Wawrzycki, 2003). Prenatal stress is suggested elsewhere to alter the ability of estrogen to alleviate depressive behavior (Frye and Wawrzycki, 2003). One possibility is that the same applies to anxiety-related behaviors.

Changes in the prenatal stress behavioral effects throughout time suggest age-related neurobiological alterations. Important associations between the increased anxiety found in prenatally stressed animals and the functioning of corticotrophin releasing hormone (CRH) systems have been shown. For instance, prenatal stress leads to increases in CRH content and release in amygdala minces (Cratty et al., 1995) and to a higher number of CRH_1 receptors in the amygdala of male rats (Ward et al., 2000). Furthermore, intracerebroventricular administration of the CRH antagonists α-helical-$CRF_{(9–41)}$ and [D-phe^{12}, $Nle^{21,38}$, C^{α}-$MeLeu^{37}$]-$CRF_{(12–41)}$, suppresses the differences in the defensive withdrawal response to restrain between prenatally stressed and control male rats (Ward et al., 2000). The functioning of stress-related neural systems is believed to be modulated by changes associated to adolescence (Kellogg et al., 1993; Choi and Kellogg, 1996; Kellogg et al., 1998). Ontogenetic changes found in the present work suggest prenatal stress may interfere with this modulation.

The present results showed that, before adulthood, the open arm end are more explored by 45-day-old prenatally stressed males than by their corresponding control. This age correspond to late adolescence (Spear, 2000). Open arm end exploration is an important anxiety index (Cruz et al., 1994) and an earlier report associates increased exploration of the open arms at this age with a strong exploratory drive which could be interpreted as a proneness to risk-taking behavior (Macrì et al., 2002). In

human adolescents, elevated scores for risk-taking and sensation-seeking behaviors are associated with drug abuse (Wills et al., 1999). It is worth noticing that problems related to substance abuse are generally more prevalent in males and in late adolescence, as compared with females or with other ages (American Psychiatric Association, 1994). Behavior of 45-day-old prenatally stressed males in the present work showed that they engage in risk-taking behavior more than controls, resembling this aspect of human adolescent drug abusers.

An unexpected finding was that prenatal stress led to increases in body weight, mainly at weaning and at 30 days of age. Papers mostly report that prenatal stress leads either to no effect (Takahashi et al., 1990; McClure et al., 2004) or to decreased body weight (Drago et al., 1999; Fonseca et al., 2002). Only one study reporting increases in body weight was found, and the authors investigated calves (Lay et al., 1997). The ontogenetic perspective can indeed shed some light on apparently conflicting results. Most studies available investigate adult animals. We have no explanation for this prenatal stress effect on body weight but given that the differences found in 30-day-old were not seen at the age of 60 days, it is worth investigating how these effects disappear.

In the present study, as the animals grew up, some behaviors were more consistently observed. For instance, head dipping from the closed arms was deeply affected by age. In fact, 30-day-old animals hardly performed this behavior because their small size did not allow it or made it very difficult. Rearing behavior also tended to increase with age. Given that rearing is seen as an index of vertical exploration, apparently exploration becomes more meticulous along adolescence.

One should bear in mind that alterations in maternal behavior of the dams due to the procedures used in the experiment cannot be ruled out as a factor contributing to the prenatal stress effects found in the present work. Indeed, the effects resulting from some prenatal stress regimens cannot be attributed only to 'prenatal' factors since it has been shown that stress experimented during pregnancy alters future maternal behavior in rats (Patin et al., 2002; Smith et al., 2004) and mice (Meek et al., 2001). In addition, when nursing pups born from stressed pregnant females, even non-stressed mothers exhibit altered maternal behavior in comparison to control dams (Meek et al., 2001). Maternal behavior has been shown to regulate emotional and stress reactivity (Caldji et al., 1998; Francis and Meaney, 1999; Caldji et al., 2000; Calatayud and Belzung, 2001). One may think that in order to avoid the problems mentioned above, prenatally stressed pups could be reared by unstressed dams. In fact, such procedures have already been made (Fujioka et al., 2001; Meek et al., 2001) and the results showed that some prenatal stress effects can be partially or completely reversed by adoption (Maccari et al., 1995; Fujioka et al., 2001), limiting the usefulness of these procedures. An intriguing possibility is, thus, that prenatal stress effects shown in the present work was also influenced by altered maternal behavior by the stressed dams.

In conclusion, prenatal stress effects on anxiety in the elevated plus-maze showed to be age-dependent, since anxiogenic effects were only detected in young adult animals and anxiolytic-like effects were found in males at late adolescence. Developmental changes in prenatal stress effects are important since they can further validate it as a model for some behavioral disorders. For instance, some human behavioral disorders (e.g., depression and schizophrenia) commonly take place at early adulthood. On the other hand, drug abuse is mainly prevalent in male adolescents (American Psychiatric Association, 1994). In fact, changes in prenatal stress effects throughout development can suggest what kind of human behavioral disorders it better fits as a model.

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