Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze

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Abstract

Prenatal stress and maternal separation are used in a large number of studies on early adversity consequences and present some similarities in their effects. The present work investigates the behavioral effects of these two procedures on two models of anxiety: the elevated plus-maze and the elevated T-maze. During pregnancy, female rats were submitted to uncontrollable electric foot shock sessions every other day or kept undisturbed. After delivery, litters from undisturbed dams were submitted to either 180-min daily periods of maternal separations from the 3–14th postnatal days or maintained with the dams all the time. Litters from the stressed dams were left undisturbed from the 3–14th postnatal days. Only males were tested. In adulthood, rats were tested in the elevated T-maze or in the elevated plus-maze. In the latter procedure half the subjects were submitted to a 60-min period of restraint immediately before being tested. The following measures were taken in the elevated plus-maze: frequency and time spent in entries into the arms, stretching, rearing, grooming and head dipping. In the T-maze measures of avoidance and escape latencies were used. Our data indicated that prenatal stress had more pronounced anxiogenic effects than maternal separation, as judged by reduced exploration of the open arms of the elevated plus-maze, but mainly after the restraint stress, and increase in avoidance latencies in the elevated T-maze. The other measures not directly involved in the elevated plus-maze arm exploration yielded similar results. Our data indicate that prenatal stress causes more anxiogenic effects in adulthood than maternal separation but, in the elevated plus-maze, these anxiogenic effects are better seen immediately after an acute stress.

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Keywords: Prenatal stress; Maternal separation; Elevated plus-maze; Elevated T-maze; Early adversity

1. Introduction

The present investigation was aimed at comparing prenatal stress and maternal separation effects on anxiety-related behavior. Prenatal stress and maternal separation have been proposed as models that mimic human anxiety and mood disorders. Indeed, the alterations resulting from either prenatal stress [19,47] or maternal separation [13,17] parallel some of those found in human patients. In addition, pharmacological and nonpharmacological treatments can reverse some of the effects of both prenatal stress [23,26,32] and maternal separation [9,17,44]. Both early adversities are potentially insightful concerning the etiology and characteristics of the anxiety and mood disorders. Nonetheless, whether both putative models appropriately fit the face, construct and predictive validation criteria which are used to assess the usefulness of a model [11] remains to be further investigated.

Prenatal stress leads to enhanced hypothalamus–pituitary–adrenal axis stress responsiveness [10,45,47] as does maternal separation [16,21,30]. While prenatal stress effects are believed to result from maternal hormones that pass through the placenta [3,50], the effects of maternal separation may result from pup elevated corticosterone levels which in turn result from prolonged intervals away from the dam [2]. Although prenatal stress and maternal separation can mimic some aspects of anxiety and mood disorders, to our knowledge, there is no direct comparison between these...
two models in tests relevant to anxiolytic or antidepressant drugs. The elevated plus-maze is one of the most popular models in the study of animal anxiety [4,28,39,46] and there are several studies using it to investigate early adversity effects. Intriguingly, when one considers either prenatal stress [8,18,32,45] or maternal separation effects [16,24,48] investigated with the use of the plus-maze, there are incongruous results, some reporting increased anxiety while others fail to obtain such results or show only augmented overall activity. The elevated plus-maze has been used for over two decades and the measures obtained with it evolved considerably along this period. Results obtained using this model can vary according to which measures are elected as important. In general, studies of prenatal stress and maternal separation with the elevated plus-maze only analyze conventional measures, such as entries and time spent in the arms, neglecting other more recently studied naturalistic behaviors which have been shown to frequently reveal some effects not seen in the conventional measures [5,36,43].

On the other hand, the elevated T-maze is another animal model used to study anxiety-related behaviors. It has the special feature of making possible the study of conditioned and unconditioned fear in the same rat [12]. Derived from the elevated plus-maze, the elevated T-maze is composed of two opposite open arms joined by a central square to a closed arm. Inhibitory avoidance is evaluated by placing the rat in the closed arm and recording the latency to leave it while escape measure is produced by repeating the process in one of the open arms. The inhibitory avoidance measure has been related to generalized anxiety while the escape measure has been related to panic disorder [12,31,42,49]. The elevated T-maze is certainly not so well known as the elevated plus-maze. It has been mainly used in studies of anxiolytic compounds and anxiety neurobiology (e.g., [31,42]). However, to our knowledge, it was almost never applied in the investigation of early adversities effects, the exception being its use in a study of long term effects of prenatal malnutrition [1]. The latter report suggests the elevated T-maze can be useful in the study of a wide variety of early adversities.

Thus, the present investigation was aimed at studying prenatal stress and maternal separation effects on adult male rat behavior in two anxiety tests, the elevated plus-maze and the elevated T-maze. The tests in the elevated plus-maze also included additional pretest restriction stress.

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, considered gestational day 1, the male was removed and the females were moved to individual cages until the occurrence of deliverance. In the day after delivery, all the litters were sexed and, if possible, culled to six males and two females. It is important to notice that, for all groups, routine cage maintenance began only on postnatal day 14. When 21 days old, the pups were weaned and grouped according to treatment. Only the male pups were studied in these experiments. Each cage had either three pairs of male siblings from three different litters or two pairs of siblings and one or two from other litters. Except for cage cleaning, animals remained undisturbed from weaning (21 days old) to early adulthood (60 days), when behavioral tests started (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. All procedures were conducted in accordance with the Institute of Laboratory Animals Resources guidelines for care and use of animals [27].

2.2. Prenatal treatments

In the prenatal stress group, the females were submitted to electric foot shocks at every other day throughout the gestational period. The pregnant females were taken to an experimental 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 Glasco-Stadler generator (model E1064GS, USA). After birth the pups were not handled during the first 2 weeks. In the maternal separation group, pregnant rats were left undisturbed until deliverance. From days 3–14 after birth, the litters were daily submitted to 180-min maternal separation periods. In the separation procedure, the dam was first removed from the cage and then the whole litter was put in a plastic box bedded with paper towel and wood shavings taken from their living cages. After removing the pups, the mother was put back into the living cage. The box with the litter remained in a separate room with controlled temperature (31 ± 0.5 °C) and humidity (40-60%) along the whole separation period. In the control group, the pregnant rats were left undisturbed and, after birth, the litters were not handled until the 14th postnatal day, when routine cage cleaning started.

2.3. Reproductive and developmental parameters

The following reproductive and developmental parameters were evaluated: litter size, number of male and female pups, sex ratio, and male body weight when 21, 60 and 80 days old. Pup mortality was rare and no analysis was carried out on this measure.

2.4. Elevated plus-maze

When 60 days old, the rats were either tested undisturbed or first submitted to a 60-min period of restraint in 17-cm long PVC tubes (7.5 cm in diameter) and then immediately tested in the elevated plus-maze. Thus, control (N=8), maternally separated (N=10) and prenatally stressed rats (N=8) were submitted to a 60-min restraint period immediately before being tested; the remaining control (N=9), maternally separated (N=9) and prenatally stressed rats (N=8) were kept in their cages before testing.
A standard elevated plus-maze described elsewhere [43] was used. To avoid falls, the open arms were surrounded by a 0.5-cm high Plexiglas barrier. The experimental room was lit by a 60-W bulb placed 1.75 m above the central square of the maze (22 lux 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. Observations were done from the TV set screen. An entry was recorded when the rat entered with all four paws into an arm. The frequency and time spent in the following behaviors were also measured [5,37]: head dipping (sniffing the head outside the maze border and below the floor level; as performed in the closed arms it correlates positively while in the open arms it correlates negatively with anxiety [7]); stretching (elongation of the body maintaining the hind paws fixed; closed arm stretching correlates positively with anxiety while open arm stretching correlates with approach-avoidance conflict [7]); 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). Rearing and grooming behavior were performed almost exclusively in the closed arms and no effort was done to distinguish between closed and open arm activity.

2.5. Elevated T-maze

Another set of naïve control (N=11), maternally separated (N=12) and prenatally stressed rats (N=10) was tested in the T-maze when 80 days old. The apparatus was prepared by obstructing the entrance of one of the closed arms of the elevated plus-maze with a cardboard barrier (12 cm × 40 cm). The room was lit by a 15-W bulb (7 lux in the maze central square). A procedure described elsewhere [31] was followed. Briefly, the rats were gently handled by the experimenter for 5 min in the 2 days before the test. On the eve of the test, each animal was also preexposed to one of the open arms for 30 min. An opaque Plexiglas barrier placed at the arm entrance prevented the rats from getting out of the open arm.

In the test, each animal was placed at the distal end of the enclosed arm facing the intersection of the arms. The time taken for the rat to leave this arm with the four paws was recorded (baseline latency). The same procedure was repeated in two subsequent trials (avoidance trials 1 and 2) at 30-s intervals. Thirty seconds after the last avoidance trial, the rats were placed at the end of the open arm where they had been preexposed the day before and the latency to leave this arm with all four paws was recorded in three consecutive trials (trials 1–3) separated by 30-s intervals. During all the 30-s intertrial intervals, the animals were kept in a polypropylene cage where they had been habituated before testing. A cutoff time of 300 s was established for the avoidance and escape latencies to occur and the trial was ended if the rat did not exit the arm. A value of 300 was scored as the data for that trial.

2.6. Data analysis

Results (mean ± S.E.M.) referring to litter size, number of male and female pups and male:female ratio were analyzed with the Student’s t-test for independent samples to compare unstressed with prenatally stressed litters. One-way analysis of variance (ANOVA) was used to compare body weight. Body weight in the 21st postnatal day was compared by using the average body weight of male pups from each litter. For body weight at the ages of 60 and 80 days, the animals were plotted individually.

In the elevated plus-maze data, comparisons between the groups were performed using a two-way ANOVA, with perinatal treatment as one factor (three levels: control, maternal separation and prenatal stress) and the pretest condition as the other factor (two levels: unrestrained and restrained). For the elevated T-maze, both avoidance and escape data were analyzed with two-way ANOVAs for repeated measures, with the perinatal treatment as one factor (three levels: control, maternally separated and prenatally stressed) and trials as the repeated measure factor (three levels: baseline, avoidance 1 and avoidance 2, or escape 1, 2 and 3). Whenever appropriate, Duncan’s post hoc test was used. In all cases, significance level was set at P < 0.05.

3. Results

3.1. Reproductive and developmental parameters

The results from reproductive and developmental parameters are shown in Table 1. Comparisons between non-stressed and prenatally stressed animals showed that litter size (t142 = −0.78, P > 0.05), number of male (F142 = 0.31, P > 0.05) and female pups per litter (t142 = −0.99, P > 0.05) and male:female ratio (t142 = 0.13, P > 0.05) were not affected by prenatal stress. As evaluated at the ages of 21 (F240 = 0.189, P > 0.05), 60 (F240 = 0.079, P > 0.05) and 80 days (F230 = 0.977, P > 0.05) neither prenatal stress nor maternal separation altered body weight.

Table 1: Values of some reproductive and developmental parameters (average ± S.E.M.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Prenatal stress</th>
<th>Maternal separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter (24 h after birth)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter size</td>
<td>12.6 ± 0.6</td>
<td>13.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Number of males</td>
<td>7.3 ± 0.5</td>
<td>7.6 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Number of females</td>
<td>5.4 ± 0.5</td>
<td>6.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Body weight (males only): 21 days: 57.8 ± 3.0; 60 days: 356 ± 12; 80 days: 448 ± 20

Litter parameters were taken on day 2 after birth; the maternal separation procedures started on day 3. Control group included 11 litters, later separated into five control and six maternal separation litters; prenatally stressed group included five litters. Male body weight at 21 days was taken from the mean value of each litter. Weight measurements at the age of 80 days was taken before the animals being distributed between unrestrained and restrained: control, N=17; prenatal stress, N=10; maternal separation, N=19. Eighty-day-old groups: control, N=11; prenatal stress, N=10; maternal separation, N=12. No statistical significant differences were found (see text for details).
Table 2

<table>
<thead>
<tr>
<th>Behavioral measure</th>
<th>Perinatal treatment</th>
<th>Pretot treatment</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of entries into the open arms</td>
<td>5.911 0.01</td>
<td>32.642 0.00</td>
<td>0.278 0.76</td>
</tr>
<tr>
<td>Percent time spent in the open arms</td>
<td>4.440 0.02</td>
<td>26.963 0.00</td>
<td>0.123 0.88</td>
</tr>
<tr>
<td>Stretching frequency in the open arms</td>
<td>1.972 0.15</td>
<td>0.006 0.04</td>
<td>0.539 0.59</td>
</tr>
<tr>
<td>Time spent stretching in the open arms</td>
<td>3.314 0.05</td>
<td>0.799 0.40</td>
<td>0.008 0.99</td>
</tr>
<tr>
<td>Time spent stretching in the closed arms</td>
<td>1.151 0.33</td>
<td>5.444 0.02</td>
<td>1.759 0.18</td>
</tr>
<tr>
<td>Head dipping frequency in the open arms</td>
<td>3.518 0.02</td>
<td>13.463 0.00</td>
<td>0.437 0.65</td>
</tr>
<tr>
<td>Time spent dipping the head in the open arms</td>
<td>4.665 0.01</td>
<td>14.868 0.00</td>
<td>1.316 0.28</td>
</tr>
<tr>
<td>Head dipping frequency in the closed arms</td>
<td>1.903 0.16</td>
<td>0.164 0.06</td>
<td>0.176 0.84</td>
</tr>
<tr>
<td>Time spent dipping the head in the closed arms</td>
<td>1.618 0.21</td>
<td>0.211 0.65</td>
<td>0.309 0.74</td>
</tr>
<tr>
<td>Closed arm entries</td>
<td>0.726 0.49</td>
<td>7.866 0.01</td>
<td>0.509 0.60</td>
</tr>
<tr>
<td>Percent of time spent in the central square</td>
<td>0.721 0.49</td>
<td>12.014 0.00</td>
<td>0.519 0.60</td>
</tr>
<tr>
<td>Rearing frequency</td>
<td>2.115 0.13</td>
<td>24.700 0.00</td>
<td>1.354 0.27</td>
</tr>
<tr>
<td>Time spent rearing</td>
<td>1.085 0.35</td>
<td>13.433 0.00</td>
<td>0.680 0.51</td>
</tr>
<tr>
<td>Grooming frequency</td>
<td>0.908 0.41</td>
<td>1.364 0.25</td>
<td>0.622 0.54</td>
</tr>
<tr>
<td>Time spent grooming</td>
<td>0.669 0.52</td>
<td>31.642 0.00</td>
<td>0.387 0.68</td>
</tr>
</tbody>
</table>

Subjects were 60-day-old males and included a control group and two other groups (perinatal treatments) submitted either to prenatal stress or maternal separation. These three groups were further divided into two: one tested without any previous restraint, the other tested immediately after a 60-min restraint period (pretot treatment).

3.2. Elevated plus-maze

Table 2 summarizes the statistical ANOVA analysis of the data obtained in the elevated plus-maze. Fig. 1 shows the percentages of entries, time spent and the latencies of the first entry into the open arms. The percentage of entries into the open arms was significantly reduced by restraint but restrained prenatally stressed rats exhibited even lower values than both their control and maternal separation counterparts. In the same vein, the latency to enter an open arm was increased by restraint and, again, prenatally stressed rats exhibited higher latencies than control and maternal separation groups. The percentage of time spent in the open arms was similarly affected by restraint. Unrestrained prenatally stressed rats showed significantly lower measures than maternal separation and control groups while the restrained rats showed no differences between the groups.

Fig. 2 shows the time spent stretching in the closed arms. It can be seen that restrained prenatally stressed rats spent more time stretching than all other groups. Aside from this, no other effect was seen in stretching behavior in the closed arms. Fig. 3 shows the time spent stretching in the open arms. There was only a main effect due to perinatal treatment \( (F_{2,49} = 3.279, P < 0.05) \): both treated groups stretched significantly less than controls independently of being restrained or not.

ANOVA detected no effect in the head dipping behavior performed in the closed arms (Table 3). Fig. 4 shows the frequency and time spent dipping the head in the open arms. It can be seen that restraint had a strong effect on both frequency and time spent dipping the head in the open arms. Unrestrained prenatally stressed rats showed a decrease in both parameters of this behavior as compared to controls.

*Fig. 1. Exploration of the open arms of the elevated plus-maze. Percentage of entries (A), latency of the first entry (B), and percentage of time spent (C). Rats were tested either without any previous restraint or immediately after a 60-min restraint period. C, control; M, maternal separation; P, prenatal stress. a, different from the unrestrained group submitted to the same perinatal treatment; b, different from all the other groups; c, different from the other two groups from the same pretest condition.*
Fig. 2. Time spent stretching in or from the closed arms. Rats were tested either without any previous restraint or immediately after a 60-min restraint period. C, control; M, maternal separation; P, prenatal stress. *, different from all other groups.

Fig. 3. Time (s) spent stretching in the open arms. Rats were tested either without any previous restraint or immediately after a 60-min restraint period. As no differences were found between restrained and unrestrained animals, data were pooled together and only the perinatal treatments are depicted. C, control; M, maternal separation; P, prenatal stress. *, different from C.

A main effect of restraint was also detected in closed arm entries, time spent in the central square, frequency and time spent rearing and time spent grooming (Table 3). Restrained rats exhibited significantly lower measures when compared to unrestrained animals, except for grooming, in which this profile was reversed.

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Maternal separation</th>
<th>Prenatal stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unrestrained</td>
<td>Restrained</td>
<td>Unrestrained</td>
</tr>
<tr>
<td>Entries into the closed arms</td>
<td>7.8 ± 0.7</td>
<td>6.6 ± 1.3</td>
<td>9.0 ± 1.1</td>
</tr>
<tr>
<td>Percent of time spent in the central square</td>
<td>11.3 ± 1.9</td>
<td>6.6 ± 2.1</td>
<td>15.2 ± 2.7</td>
</tr>
<tr>
<td>Closed arm frequency of head dipping</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Closed arm time dipping head (s)</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Rearing frequency</td>
<td>14.4 ± 1.1</td>
<td>11.1 ± 1.8</td>
<td>19.8 ± 1.4</td>
</tr>
<tr>
<td>Time spent rearing (s)</td>
<td>24.3 ± 3.4</td>
<td>14.1 ± 2.2</td>
<td>26.0 ± 2.8</td>
</tr>
<tr>
<td>Grooming frequency</td>
<td>7.7 ± 1.6</td>
<td>7.3 ± 0.7</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td>Time spent grooming (s)</td>
<td>32.5 ± 6.4</td>
<td>9.9 ± 26.4</td>
<td>34.8 ± 11.0</td>
</tr>
</tbody>
</table>

3.3. Elevated T-maze

Fig. 5 shows the results obtained in the elevated T-maze. ANOVA on the inhibitory avoidance measures showed significant effects of the factors perinatal treatment ($F_{[2,30]} = 4.00$, $P < 0.05$) and trials ($F_{[2,60]} = 19.95$, $P < 0.05$). Interaction between perinatal treatment and trials barely escaped significance ($F_{[4,60]} = 2.38$, $P = 0.06$). Multiple comparisons test showed that the prenatally stressed group exhibited latencies greater than those of control in baseline, avoidance trial 1 and 2. Also, the latencies of the control group in avoidance 2 was greater than that in baseline and avoidance 1 trials. Open arm escape was not affected by perinatal treatments ($F_{[2,30]} = 0.71$, $P > 0.05$), trials ($F_{[2,60]} = 1.27$, $P > 0.05$) neither was there a significant interaction between perinatal treatment and trials ($F_{[4,60]} = 1.88$, $P > 0.05$).

Table 3 Values of some behavioral parameters studied in the elevated plus-maze (average ± S.E.M.).

Control, maternal separation and prenatal stress groups were tested either without any previous restraint or immediately after a 60-min restraint period.

* Different from the respective unrestrained group.
in accordance to a previous study [35]. It seems that stressful
tal stress effects only appear after an acute stressful event is
spent stretching in the closed arms. The fact that some prena-
first entry into one of them, in addition to increases in the time
entries into the open arms and increases in the latency of the
prenatally stressed rats showed decreases in the percentage of
time spent dipping the head. When restrained before the test,
tically stressed group showed decreases in open arm measures:
maze without being previously restrained, rats in the prena-
acutely stressed control rats. When submitted to the plus-
rats, once under acute stress, behave more anxiously than
of the same group; d, different from avoidance trial 1 of the same group.

Fig. 5. Effects of prenatal stress and maternal separation on the latency of
avoidance and escape in the elevated plus-maze. C, control; M, maternal separation; P, prenatal stress. a, different from C in the same
trial. b, different from C and M in the same trial. c, different from the baseline
of the same group. d, different from avoidance trial 1 of the same group.

4. Discussion

Elevated plus-maze results suggest that prenatally stressed
rats, once under acute stress, behave more anxiously than
acutely stressed control rats. When submitted to the plus-
maze without being previously restrained, rats in the prena-
tally stressed group showed decreases in open arm measures:
percentage of time, time spent stretching and frequency and
time spent dipping the head. When restrained before the test,
prenatally stressed rats showed decreases in the percentage of
time stretching in the closed arms. The fact that some prena-
tal stress effects only appear after an acute stressful event is
in accordance to a previous study [35]. It seems that stressful
events lead, in prenatally stressed rats, to responses that are
different in intensity or quality (or even both). In this respect,
it was recently shown [22] that prenatal stress can increase
the long-term effects of a stressor applied in adulthood to
female rats, which resemble post traumatic stress disorder
symptoms. It can be argued that the distinctive response seen
in prenatally stressed animals when a “traumatic-like” event
takes place can be at the origin of alterations seen thereafter.

Our plus-maze results also point to the importance of
some measures not often reported in early adversity stud-
ies. When one considers the elevated plus-maze studies on
prenatal stress effects, the reports fail to converge. Some re-
port pro-aversive effects based on reduced open arm explo-
ration [18,33,45] or elevated time spent in the closed arms
[32], while others only report increased overall activity [8].
Differences in procedure and the species or strains studied
may account for some of the discrepancies found [14]. It is
important to notice, indeed, that the parameters chosen as
indicators of anxiety can undoubtedly influence the conclu-
sions reached. Data presented here suggest that not only the
time spent and entries into the open arms but also the latency
to the first entry into them and stretching and head dipping
behavior are useful as alternative anxiety indexes.

Prenatal stress as well as maternal separation resulted in
decreases in the time spent stretching in the open arms in
our plus-maze experiment. Indeed, decreases in stretching
behavior performed in the open arms are pointed as indica-
tive of increased approach-avoidance conflict [7]. Decreased
stretching in the open arms suggests, therefore, that prena-
tal stress and maternal separation may lead to an anxiogenic
effect, which, was seen only in this measure in maternally
separated rats. Its is important to notice that the control group
litters were not handled for the first 2 weeks (except when they
were culled). Similarly to the present work, earlier compa-
isons between nonhandled controls and maternally separated
male rats also did not find any differences in the conven-
tional plus-maze measures [24] or found increased overall
activity, as judged by the entries into the closed arms [16].
There are some reports of maternal separation anxiogenic ef-
ects [15,40,48]. The control groups of these studies were,
however, submitted to standard facility rearing with its non
controlled handling. The latter treatment seems to lead to
anxiolytic effects, similar to those of early handling [15,34],
a well known anxiolytic procedure [20,25,29]. Thus, this im-
portant procedural dissimilarity may account for the differ-
ences between our results and the marked anxiogenic effect of
maternal separation seen in the literature mentioned above.

Stretching behavior seems to be a highly sensitive mea-
sure since in our study it was able to indicate not only prenatal
stress effects but also those of maternal separation. Indeed,
this behavior correlates with the corticosterone response in
the plus-maze test [38]. In the last decade, an increasing num-
ber of laboratories have shown the advantages of a naturalis-
tic approach to the elevated plus-maze in the elucidation of
pharmacological and neurobiological mechanisms involved
in anxiety [5,36,43]. Stretching behavior, thus, is a useful
measure with the potential to indicate effects that cannot be
detected by conventional measures.

The elevated T-maze was developed in an attempt to pro-
vide information on the different types of anxiety (that may
underlie many pathological anxiety disorders) by analyzing
avoidance and escape from the open arms, the first being asso-
ciated with generalized anxiety, the latter with panic [49]. The
increase in inhibitory avoidance found in the present study
can be interpreted [49] as an increase in anxiety, suggesting
prenatal stress can be a model of generalized anxiety. In this
respect, similarities such as increased amygdala volumes ob-
tained by stereological measures in prenatally stressed rats
[41] and functional magnetic resonance in patients with gen-
eralized anxiety disorder [6] are remarkable.
When tested under the same conditions, rats submitted to prenatal stress were more likely to exhibit an anxious profile than those submitted to maternal separation. The similarities reported elsewhere [24] between maternally separated and non-handled controls were replicated in our study. According to the data presented here, maternal separation seemed to lead only to slight anxiety-related behavioral effects. On the other hand, prenatal stress resulted in the increase of several anxiety indexes, mainly after challenging with the restraint. The latter behavioral alterations recorded after the exposure to a stressor suggests that prenatally stressed rats have, while stress hormones are circulating, a comparatively increased activity of brain structures related to anxiety.

Some studies report that maternal separation affects the hypothalamus-pituitary-adrenal axis stress responsiveness [16,21,30], suggesting that it may appropriately model neuroendocrinological correlates of anxiety and/or depression [13,17]. We suspect that maternal separation does not cause such strong behavioral effects, as judged by the data we obtained in the elevated plus-maze and in the T-maze as well. On the other hand, prenatal stress causes more definite behavioral effects, which are accompanied by strong neuroendocrinological correlates [10,19,47] and may constitute a useful model to study the neurobiological, physiological and behavioral facets of some anxiety disorders. Our data on the T-maze suggest prenatal stress may be a model for investigating generalized anxiety. But that remains to be further investigated.

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References


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