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# Enduring effects of environmental enrichment from weaning to adulthood on pituitary-adrenal function, pre-pulse inhibition and learning in male and female rats

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**Summary** Environmental enrichment (EE) increases stimulation and provides richer sensory, cognitive and motor opportunities through the interaction with the social and physical environment. EE produces a wide range of neuroanatomical, neurochemical and behavioural effects in several animal species. However, the effects of EE have mainly been studied shortly after the treatment, so its long-lasting effects remain to be elucidated. Thus, we studied in male and female Sprague—Dawley rats the enduring effects of EE on tasks that measured emotional reactivity, social exploration and memory, sensorimotor gating and learning. After weaning, rats reared in EE were housed in single-sex groups of 12—14 in enriched cages during 12 weeks, whereas control rats were housed in single-sex groups of 2—3 animals in standard cages. Then, all rats were housed in pairs and successively exposed to different tests between 4 and 60 weeks post-EE. The results indicated that animals of both sexes reared in EE gained less weight during the enrichment period; differences disappeared in females during the post-EE period, but were maintained intact in males. Rats reared in EE showed an altered daily pattern of corticosterone and a lower hormone response to a novel environment (hole board, HB), although no differences in ACTH were found. EE resulted in more exploratory behaviour in the HB and higher number of entries in the open arms of the elevated plus maze (with no changes in the time spent in the open arms), suggesting a greater motivation to explore. Unexpectedly, rats reared in EE showed reduced pre-pulse inhibition (PPI), a measure of sensorimotor gating, suggesting lower capability

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to filter non-relevant information compared with control rats. EE increased social exploratory behaviour towards juvenile rats and social discrimination in males, but decreased social discrimination in females. Finally, in the Hebb-Williams maze, rats reared in EE showed better performance in terms of reduced number of errors and shorter distances travelled in the mazes. It is concluded that EE exposure from weaning to adulthood has important and long-lasting consequences on physiological and behavioural variables, most of them similar in both sexes, although sex differences in response to the EE are also reported.


## 1. Introduction

The development of the nervous system is highly dependent on the interactions between the organism and its environment. Environmental manipulations at early critical periods of development in rodents have a long tradition in behavioural and brain research as a tool to study biological mechanisms underlying behaviour and to model symptoms of human psychiatric disorders. Thus, early exposure of animals to environmental enrichment (EE) can change neural structure and function leading to enduring improvements in learning and memory. The first studies on this subject appeared in the early 1960s and reported that EE increased brain weight and cortical thickness (Bennett et al., 1969; Diamond et al., 1964). From those years until now a host of studies have shown that EE increased dendritic branching, the number of dendritic spines and neurogenesis (Renner and Rosenzweig, 1987; Diamond, 1988; Kempermann et al., 1997), produced neuronal changes at specific sensorial areas of the brain (Coq and Xerri, 1998; Engineer et al., 2004; Sale et al., 2004), improved cellular plasticity (as measured by levels of neurotrophins, expression of synaptic proteins, synaptogenesis or synaptic strength), reduced spontaneous apoptosis and was neuroprotective (Rampon et al., 2000a; Van Praag et al., 2000; Nithianantharajah and Hannan, 2006).

With regard to cognitive functions, it has been consistently shown that EE improves spatial learning in the Morris water maze (Nilsson et al., 1999; Pham et al., 1999), the radial-maze and in T-mazes (Pacteau et al., 1989; Paban et al., 2005; Bennett et al., 2006), enhanced long-term memory (Rampon et al., 2000b; Bruel-Jungerman et al., 2005), and accelerated habituation (Varty et al., 2000; Elliott and Grunberg, 2005). Increased exploratory activity, improved performance in labyrinths and conflict tasks (Renner and Rosenzweig, 1987; Escorihuela et al., 1994) and some positive effects of EE on emotional reactivity have also been reported in rats (Fernández-Teruel et al., 1992, 1997; Escorihuela et al., 1994; Francis et al., 2002) and mice (Benaroya-Milshtein et al., 2004).

However, there are inconsistent results in regard to the effects of EE on the hypothalamus-pituitary-adrenal (HPA) axis. Some studies found that resting levels of corticosterone and/or ACTH did not differ among enriched and control animals (Van de Weerd et al., 1997; Roy et al., 2001; Schrijver et al., 2002; Morley-Fletcher et al., 2003; Martinez-Cue et al., 2005), whereas others reported reduced (Belz et al., 2003) or higher resting levels of ACTH and corticosterone in animals reared in EE as compared with animals reared in control conditions (de Jong et al., 2000; Marashi et al., 2003; Benaroya-Milshtein et al., 2004; Moncek et al., 2004). In mice, higher resting corticosterone levels after EE had been observed in DBA/2J but not CBA/J strains (Haemisch and Gartner, 1994). After a superimposed stressor, some authors reported an attenuated ACTH or corticosterone response in animals reared in EE (Roy et al., 2001; Moncek et al., 2004), whereas other authors found no differences (Schrijver et al., 2002; Morley-Fletcher et al., 2003).

Although there are important discrepancies in the literature on the physiological and behavioural consequences of EE exposure that are likely to be related to particular differences among species, sex, and age at which animals are exposed to enriched housing, it is clear that EE exerts profound biological effects, most of them are beneficial. However, one critical question is the persistence of the effects caused by exposure to EE in a particular life period. There are only a few studies focusing on this topic. Persistent effects (weeks to months) of a previous exposure to an EE has been observed in rats with enhanced synaptic transmission to granule cells through the medial perforant pathway (Green and Greenough, 1986) and improved two-way active avoidance and spatial learning (Escorihuela et al., 1994, 1995). In mice, a long-lasting enhancement of exploratory activity and altered pattern of social behaviour has been reported (Pietropaolo et al., 2004), but in other cases the effects of an EE vanished after the animals were returned to standard housing conditions (Kempermann and Gage, 1999).

On the other hand, the differential impact of EE in males and females has not been extensively studied. In some of the studies the influence of sex has been specifically addressed, Juraska (1984) found that EE caused an increase in dendritic structures of pyramidal and stellate neurons of the visual cortex of male rats and a greater dendritic complexity in the neurons of the dentate gyrus of females. Diamond (2001) described a greater increment in the thickness of the occipital cortex of males reared in EE, whereas females reared in EE had a thicker somatic cortex. Klein et al. (1994) reported that EE (lasting 30 days starting at 25-days old) affected female rats more than males in the predator stress test, and increased the resting levels of corticosterone in females but not males (Teather et al., 2002). EE improved the performance of TS65Dn female mice in the water-maze, whereas no effect was observed in males (Martinez-Cue et al., 2002). On the contrary, the effects of EE appeared to be greater for males than females in the open field (Elliott and Grunberg, 2005), the Morris water maze (Tees, 1999) and the social exploratory behaviour (Peña et al., 2006). Finally, other reports found an overall significant EE effect regardless of sex in different emotional or learning measures (Elliott and Grunberg, 2005; Peña et al., 2006; Tees, 1999).

Therefore, the overall purpose of the present work was to investigate whether EE after weaning exerts enduring, sex-dependent, effects on emotional and cognitive processes in

rats. To this end, we measured exploration (hole board, HB), emotionality (elevated plus maze, EPM), HPA activity, sensorimotor gating (acoustic startle response and PPI) and social and spatial memory (social discrimination and Hebb-Williams Mazes). We expected the effects of EE to persist for a long-time after the termination of exposure to such environment.

## 2. Materials and methods

### 2.1. Animals and general procedure

Subjects were 52 Sprague—Dawley rats (24 males and 28 females) obtained from 9 different litters, which were bred and raised at the main animal facility of the *Universitat Autònoma de Barcelona*. Animals from each litter were randomly assigned to either control or enriched (EE), single-sex, groups after weaning (21 days of age). Control animals were housed in groups of 2—3 in standard macrolon cages (50 cm × 55 cm × 14 cm.) and rats reared in EE were housed in groups of 12 (males) or 14 (females) in large metal cages (100 cm × 43 cm × 50 cm.). Rats had free access to food and water, except when behavioural testing required a restricted food diet, and were always maintained in standard conditions of temperature (22 °C ± 2), on a 12—12 h light-dark schedule (lights on at 0800 h). All experiments were performed during the light phase between 0830 h and 1600 h. The experimental protocol was approved by the Ethics Committee of the *Universitat Autònoma de Barcelona*, following the 'Principles of laboratory animal care' and was carried out in accordance the European Communities Council Directive (86/609/EEC).

Control animals were briefly handled once a week for cage cleaning. The animals reared in EE cages were not handled for cage cleaning since the floor was a metal grid that had underneath two removable cases with sawdust that were replaced twice a week for cage cleaning. The internal configuration of the EE cages was changed every week, creating different spaces with several types of stairs, ropes, tunnels and exercise wheels. Novel objects (playthings like balls, rings and bells) made of metal, plastic or wood were provided in the cages and changed 2—3 times per week, with all cages receiving the same assortment of objects each time. Animals were maintained in these environmental conditions for 12 weeks. Since experimental subjects were housed in the same cage during the enrichment period, the average data from all animals to a cage may be considered as a one single value, but this would require a huge number of animals, which is unapproachable for both ethical and practical reasons.

After EE period, all animals were housed in pairs of the same sex in standard macrolon cages. The animals remained always in this condition except during the period between the 3 days before the first social discrimination test and the day after the second social discrimination test (after which they were again housed in pairs). The groups formed were: control males (CM, $n = 12$), control females (CF, $n = 14$), males reared in EE (EM, $n = 12$) and females reared in EE (EF, $n = 14$).

Male juveniles (23—30 days old) of the same rat strain were used as the social stimulus in the social discrimination memory tests (experiments 4 and 5). They were housed in the same vivarium in standard macrolon cages (groups of 6 animals).

Animals were weighed immediately after weaning, just after the enrichment period and then before each behavioural test. All procedures (see Table 1) were conducted in a dark room illuminated with dim light, except for the acoustic startle response (ASR) and PPI tests that were done under non-illumination conditions. The whole procedures of the hole board (HB), elevated plus maze (EPM), social discrimination memory (SDM) and Hebb-Williams tests were videotaped and analysed by a computer-assisted data acquisition system analysis (version 2.0.14, Panlab, Barcelona, Spain). After testing, the apparatuses were cleaned with a 20% ethanol solution.

**Table 1** Experimental sequence followed during the study. It shows the chronological order of the tests and the age of the animals.

| Experimental sequence | Age in weeks |
|---|---|
| Environmental enrichment | 4—16 |
| Hole board and HPA axis response | 20 |
| Elevated plus maze | 21 |
| Startle response and pre-pulse inhibition | 26 |
| Social discrimination memory 30 min | 27 |
| Social discrimination memory 15 min | 28 |
| Hebb-Williams maze | 60 |

### 2.2. Behavioural testing

#### 2.2.1. Hole board test as a novel environment

The HB apparatus was a white wooden box (66 cm × 66 cm × 47 cm), with four equidistant holes (3.7 cm diameter, 18 cm deep) on the floor that was divided into 16 equal squares with red lines. The four holes contained identical objects (i.e., coloured sand and a plastic container) strange to the animals. The two rats of each home-cage were tested on different days. Number of ambulations, rearings and head dips, time spent head-dipping and doing self-grooming, and number of boluses (defecation) were scored during a 5-min period.

#### 2.2.2. Elevated plus maze

The EPM consisted of four arms made of black formica, extending from a 10 cm square centre positioned 90° from each other to form the shape of a plus sign. Each arm was 50 cm long and 10 cm wide. Two of the opposing arms had wooden walls (enclosed arms, 40 cm high) whereas the other two were the open arms that had only a 0.5 cm ridge to provide additional grip. The whole maze was elevated 50 cm above the floor. Only one animal of the home cage was tested at a time and the cage mate was tested next day. The rat was placed in the centre of the maze facing an open arm and during the 5 min test, the following measures were taken: latency to enter into the open arms, number of arm entries, distance travelled and time spent in each part of the maze. An entry was defined as placing all four paws into a given arm. Risk assessment behaviours were also registered as the number and location of stretched attend postures, defined as flat back postures combined with slow forward movements in the form of a flat back/stretched approach towards the distal parts of the maze. We measured the number of stretched attend postures from the enclosed arms towards the centre and open arms (protected zone) and, from the open arms zone near the centre towards the distal part of the open arms

(unprotected zone; Rodgers and Dalvi, 1997). The number of rearings in the enclosed (protected) and open (unprotected) arms was also registered.

### 2.2.3. Acoustic startle response (ASR) and pre-pulse inhibition (PPI)

To test ASR and PPI a commercially available system was used (Cibertec S.A., Madrid, Spain). The system comprised one sound-attenuating chamber, equipped with a plexiglas animal enclosure (16.5 cm × 30 cm with an adjustable lid). The chamber was illuminated with a dim light and ventilated by a small electric fan that provided background white noise (50 dB) throughout the test. Broad and tone pulses were presented by a speaker positioned 27 cm directly above the animal enclosure. A piezoelectric accelerometer affixed to the animal enclosure frame was used to detect and to transduce motion resulting from the animals' response. Tone pulse parameters were controlled by a computer, using a software package and an interface assembly that digitalized, rectified and recorded stabilimeter readings. Animals were placed in the plexiglas enclosure for 5 min of acclimatization, and then, a pulse (startling tone of 40 ms, 100 dB) was presented alone in the first 12 trials, during which the increase in the amplitude of the response to the pulse is likely to be related to anxiety. On the subsequent 48 trials, the startle tone was either presented alone or 100 ms after pre-pulses intensities of 60 dB, 70 dB or 80 dB (20 ms duration). The four different types of trials were presented at the animals on 12 blocks of 4, containing 1 trial of every type. The same stimulus condition was never presented more than two consecutive times. The interval between each trial was programmed to a variable time schedule with an average duration of 30 s (range, 20—40 s). Average startle amplitude during the 200 ms following the onset of each startle stimulus was recorded and stored on a computer.

Percent pre-pulse inhibition was calculated for each rat at each pre-pulse stimulus intensity as follows: PPI = 100 − [(pre-pulse/startle alone) × 100], where pre-pulse is the average startle response on trials in which there was a pre-pulse stimulus and startle alone is the average startle response on the trials in which the startle stimulus was presented alone (excluding the first 12 trials of the session). This percent was used as a measure of sensorimotor gating that reflects attentional capabilities. An increase in the pre-pulse inhibition is supposed to be related to an increase of attention. Each animal was tested individually and the cage mate was tested another day.

### 2.2.4. Social discrimination tests

Briefly, the social discrimination tests consisted of a first presentation (trial 1, T1) of a juvenile rat to an adult rat for 5 min. After that first presentation, the rats were removed and kept separately in their individual cages. After a delay period, two juvenile rats (the former and another novel one) were simultaneously presented to the adult rat in the same box for another 5 min (second trial, T2) and the time spent investigating each juvenile was recorded.

In the present experiments we tested the animals for social discrimination twice, with delays of 30 and 15 min between T1 and T2. One week after the startle and PPI test, the animals were housed individually for three days prior to the first social discrimination test. The apparatus consisted of a wooden grey arena (60 cm × 40 cm × 40 cm) and the rats to be tested were habituated for 5 min, 24 h before testing. Juveniles were isolated in individual cages for 20 min before the beginning of the experiments. At the end of T1, the rats were removed and kept in their individual cages. During the delay period the juvenile was kept in the observation room in an individual cage with sawdust, while the adult rat was returned to the animal room. During T2, the social investigation times directed to the same and new juveniles were separately measured by identifying the juveniles using coloured marks on the head and above the tail's base. Social-investigatory behaviour, consisting of being proximally oriented to the juvenile, following it or having a direct contact (sniffing, grooming or inspecting any body surface), was measured.

### 2.2.5. Hebb-Williams maze test

Finally, to use a positive control of the effects of EE and to further analyze the memory functions affected by EE, we also tested the animals in a more complex learning test such as the Hebb-Williams Maze, which has been frequently used to detect changes in cognitive functions as a result of EE treatment (Winocur, 1998; Kobayashi et al., 2002).

The apparatus consisted of a square box (75 cm × 75 cm) and black painted wooden walls (12 cm high), covered with a transparent plastic top divided into 36 squares, which allowed the experimenter to define error zones and to arrange appropriately the removable black walls (12 cm high) used to build up the daily different maze patterns. Start and goal boxes (40 cm × 15 cm × 12 cm) were located in two diagonally opposite corners and equipped with sliding doors. Before pre-training, the body weight of each rat was progressively reduced to 85% of its initial value and was maintained at that level throughout pre-training and testing (body weight was recorded daily). Throughout the process of weight reduction, animals were habituated to eat calibrated food pellets (45 mg; Bioserv, Frenchtown, NJ, USA). Initially, the rats were habituated to the maze for 5 min on 5 consecutive days. During these sessions they were allowed to explore freely the maze containing no barriers and to eat pellets in the goal box. Preliminary training was introduced on the 6th day in which the rats were administered a series of practice problems, using simple maze patterns. Each animal received 6 trials per day in which it was placed in the start box and allowed to run to the goal box where it could eat for 10 s. The rats were pre-trained over a maximum of 15 days in order to get them used to the presence of barriers in the apparatus and to establish the habit of going to the goal box with a minimal amount of exploratory behaviour. The criterion for advancing to the test problems was to run the 6 trials of the practice problems within a total of 90 s during two consecutive pre-training sessions. If this criterion was not met within 15 days of the first pre-training session, the experiment was terminated. The total number of sessions required to meet this criterion was used as the measure of acquisition. Four EM and two CM rats were excluded because they did not reach the criterion. Following pre-training, rats that acquired the criterion were tested using the standard series of 12 problems defined by Rabinovitch and Rosvold (1951), with one problem per day and six consecutive trials per problem. Each test problem was videotaped and the number of entries into the error zones, the latency to enter into the goal box and the distance travelled in each trial were measured. Error zones were bordered by broken lines and an error was scored when both front paws of a rat crossed a broken line.

### 2.3. HPA function

To evaluate HPA function, blood samples were taken under specific experimental conditions using the tail-nick procedure. The tail-nick consisted of gently wrapping the animals with a cloth, making a 2 mm incision at the end of the tail artery and then massaging the tail while collecting, within 2 min, 300 μl of blood into ice-cold EDTA capillary tubes (Sarsted, Granollers, Spain). The two cage-mated animals were sampled simultaneously by two experimenters, in a room different from the animal room or the testing room.

To evaluate the HPA response to a mild stressor, the animals were previously exposed for 5 min to the HB in a testing room. Immediately after that, the animals were transported to the animal room and placed in their home cages. Ten minutes later, blood samples were taken by tail-nick. This time-point was chosen because 15 min is the minimum time needed for the adrenal to synthesize and release corticosterone in response to the initial ACTH release (Armario, 2006).

Basal samples were taken four days after the HB exposure in order to prevent possible interference with the behavioural and hormonal responses to the test. Sampling was performed under resting conditions during the morning (09:00—1000 h) and the evening (1700—18:00 h) to study possible changes in the basal circadian rhythm of the HPA axis.

### 2.4. Radioimmunoassays

Plasma ACTH and corticosterone were determined by double-antibody RIAs. The ACTH RIA used $^{125}$I-tyrosil-ACTH (Amersham-Pharmacia Biotek, Cerdanyola, Spain) as the tracer, rat synthetic ACTH (Sigma) as the standard and an antibody raised against rat ACTH kindly provided by Dr. W. Engeland (Dept. Surgery, Univ. Minnesota, Minneapolis, USA). The corticosterone RIA used $^{125}$I-corticosterone-carboximethyloxime-tyrosine-methyl ester (ICN-Biolink 2000, Barcelona, Spain), synthetic corticosterone (Sigma) as the standard, and an antibody raised in rabbits against corticosterone—carboximethyloxime-BSA kindly provided by Dr. G. Makara (Inst. Exp. Med., Budapest, Hungary). We followed the RIA protocol recommended by Dr. G. Makara (plasma corticosteroid-binding globulin was inactivated by low pH). All samples to be compared were processed in the same assay. Intra-assay coefficient of variation was 6.6% for ACTH and 12% for corticosterone.

### 2.5. Statistical analysis

The statistical analysis was performed using the 'Statistical Package for Social Sciences' (SPSS, version 15.0) using ANOVA. The particular factors of each ANOVA are indicated in results for each set of variables. When appropriate, subsequent decomposition of the interaction between factors was conducted.

## 3. Results

### 3.1. Body weight

Body weights were analysed by a repeated measures ANOVA with age as within subject factor, and sex and treatment as between subject factors. The ages studied were: 4 weeks (before placing the animals into the EE cages), 16 weeks (when EE finished), 20 weeks (immediately before hole board test), 21 weeks (immediately before elevated plus maze test), 26 weeks (immediately before startle response and pre-pulse inhibition experiment), 27 weeks (before the first social discrimination memory test), 28 weeks (before the second social discrimination test), and 60 weeks (before starting Hebb-Williams pre-training). Body weights are shown in Fig. 1. The ANOVA indicated significant effects of age ($F(7,336) = 1900.4$, $p < 0.0001$), sex ($F(1,48) = 710.95$, $p < 0.0001$) and treatment ($F(1,48) = 20.20$, $p < 0.0001$). The interactions age × sex ($F(7,315) = 222.64$, $p < 0.0001$), age × treatment ($F(7,336) = 5.7$, $p < 0.01$),) and treatment × sex ($F(1,48) = 9.16$, $p < 0.01$) were significant, and that of age × sex × treatment was marginally significant ($F(7,336) = 2.99$, $p = 0.053$). Decomposition of interactions showed lower weight in enriched females as compared to control females just after the enrichment period, but not later on; in contrast, the lower body weight of enriched males was maintained over all the experimental post-enrichment period ($p$ at least $< 0.05$).

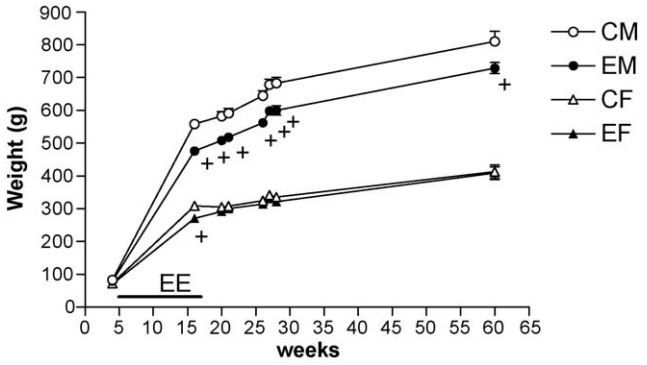


**Figure 1** Effects of enriched environment on body weight gain. Mean ±SE of body weight (g) at different ages for Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups; $^{+}p < 0.05$ comparing EM or EF versus CM or CF (same sex); all CF and EF groups were different from the corresponding CM and EM groups under the same treatment (not shown), except for the first measurement (week 4 after weaning).

### 3.2. Basal and stress levels of HPA hormones

Repeated measures ANOVA was used to analyze the circadian changes in ACTH and corticosterone, with sex and treatment as between subject factors and time of day as within subject factor. The ANOVA of plasma levels of ACTH revealed a significant effect of time ($F(1,43) = 47.7$, $p < 0.0001$), with higher levels in the evening, and sex ($F(1,43) = 7.6$, $p < 0.01$), with higher levels in females. The main effect of treatment was not significant, but interaction time - × treatment was marginally significant ($F(1,43) = 3.92$, $p = 0.054$; Fig. 2A). The ANOVA of plasma levels of corticosterone revealed a significant effect of time ($F(1,43) = 326.6$, $p < 0.0001$) and sex ($F(1,43) = 65.0$, $p < 0.0001$), but not treatment. The overall effects reflect higher corticosterone levels in the evening than in the morning and higher levels in females as compared with males. The interactions time - × sex ($F(1,43) = 47.08$, $p < 0.0001$) and time × treatment ($F(1,43) = 4.95$, $p < 0.05$; Fig. 2B) were significant. To

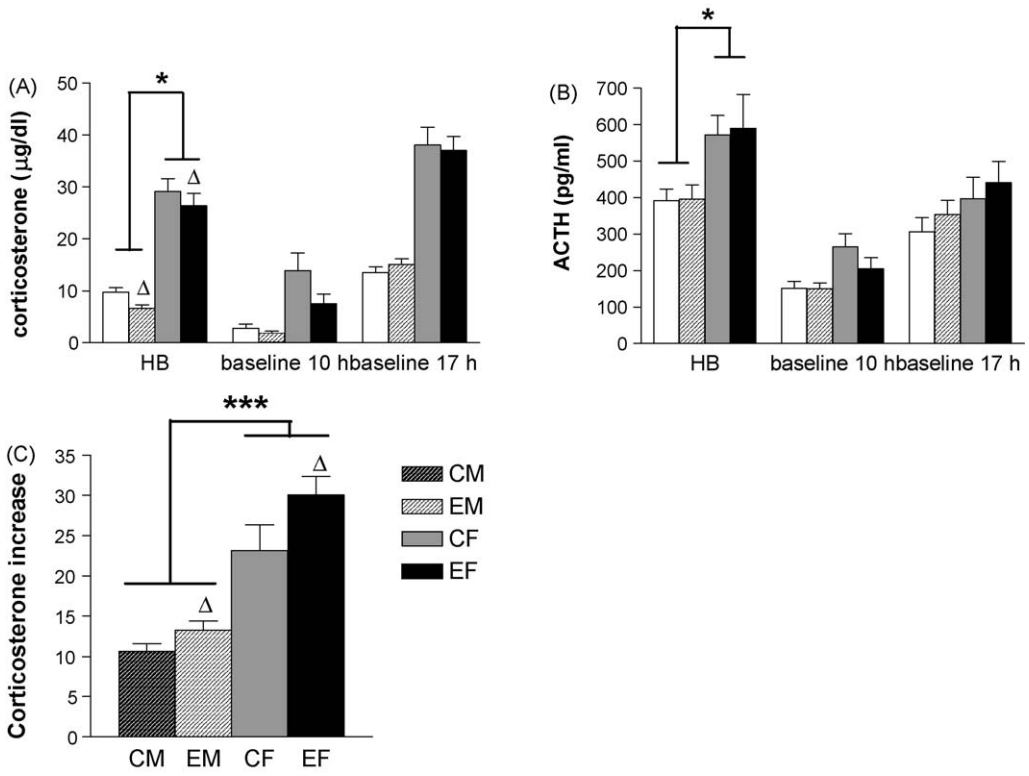


**Figure 2** Effects of enriched environment on basal and stress levels of HPA hormones. Mean ±SE of ACTH (A) and corticosterone (B) are represented. The response to 5 min exposure to the hole board test (HB) was evaluated 15 min after starting exposure to the test. Baseline levels were from a different day and samples were taken at 10 and 17 h. Groups were: Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF). Panel (C) represents the increase of corticosterone from morning to evening levels. $^{*}p < 0.05$, $^{***}p < 0.0001$ overall 'sex' effect; $^{\Delta}p < 0.05$ overall 'treatment' effect (two-way ANOVA).

further analyze such interactions, a new variable reflecting the increase in corticosterone from morning to evening was created (Fig. 2C), with sex and treatment as the main factors. The ANOVA revealed a significant effect of sex ($F(1,46) = 47.1$, $p < 0.0001$) and treatment ($F(1,46) = 5.0$, $p < 0.03$), with no interaction sex × treatment, reflecting a greater increase in female versus males and in enriched versus controls, with no interaction between the two factors.

ACTH and corticosterone levels after the HB were analysed using two-way ANOVA, with sex and treatment as between subject factors. The two-way ANOVA of ACTH showed a significant effect of sex ($F(1,46) = 10.91$, $p < 0.01$), but not of treatment. Regarding corticosterone, significant effects of sex $F(1,46) = 84.17$ $p < 0.0001$) and treatment ($F(1,46) = 4.56$, $p < 0.05$) were found. No significant interaction sex × treatment was observed for any hormone. The results showed that females had higher levels of ACTH and corticosterone after exposure to the hole board and that both enriched male and female rats had lower levels of corticosterone than controls (Fig. 2).

### 3.3. Behavioural response to the HB

Repeated measures ANOVA was used to analyse head-dipping behaviour in the hole board, with sex and treatment as between subject factors and time (five time-bins of 1 min) as the within subject factor. The ANOVA revealed no effect of sex or the interaction of this factor with the other ones in head-dipping behaviour (number of head-dips and time spent head-dipping). It was observed significant effects of time on the number of head dips ($F(4,172) = 4.76$, $p < 0.01$) and the time spent head-dipping ($F(4,172) = 6.51$, $p < 0.001$), as well as time × treatment interactions on the number of head-dips ($F(4,172) = 3.05$, $p < 0.05$) and time spent head-dipping ($F(4,172) = 4.17$, $p < 0.01$). Decomposition of the interaction time × treatment revealed that EM and EF groups performed more head dips and spent more time head-dipping than CM and CF groups only during min. 1 and 2, thus indicating that EE only transiently increased in both sexes exploratory behaviour (Fig. 3A and B).

Ambulation, rearing and defecation in the HB were analysed with two-way ANOVA, with sex and treatment as between subject factors. The ANOVA indicated a significant of sex in ambulations ($F(1,46) = 12,34$, $p < 0.0001$; Fig. 3C), rearings ($F(1,46) = 12.6$, $p < 0.001$; Fig. 3D) and defecations ($F(1,46) = 5.82$, $p < 0.05$; Fig. 3E), reflecting that females showed more ambulations, a higher number of rearings and less defecations than males. In addition, a significant effect of treatment was found on rearings ($F(1,46) = 9.41$, $p < 0.01$; Fig. 3D), with EE increasing the number of rearings. No effect of EE was found on ambulations and defecations. Any significant 'sex × treatment' effect was observed.

### 3.4. EPM

Two-way ANOVA, with sex and treatment as the main factors was used to analyze EPM behaviour. The ANOVA revealed no significant sex × treatment interactions in any variable. The

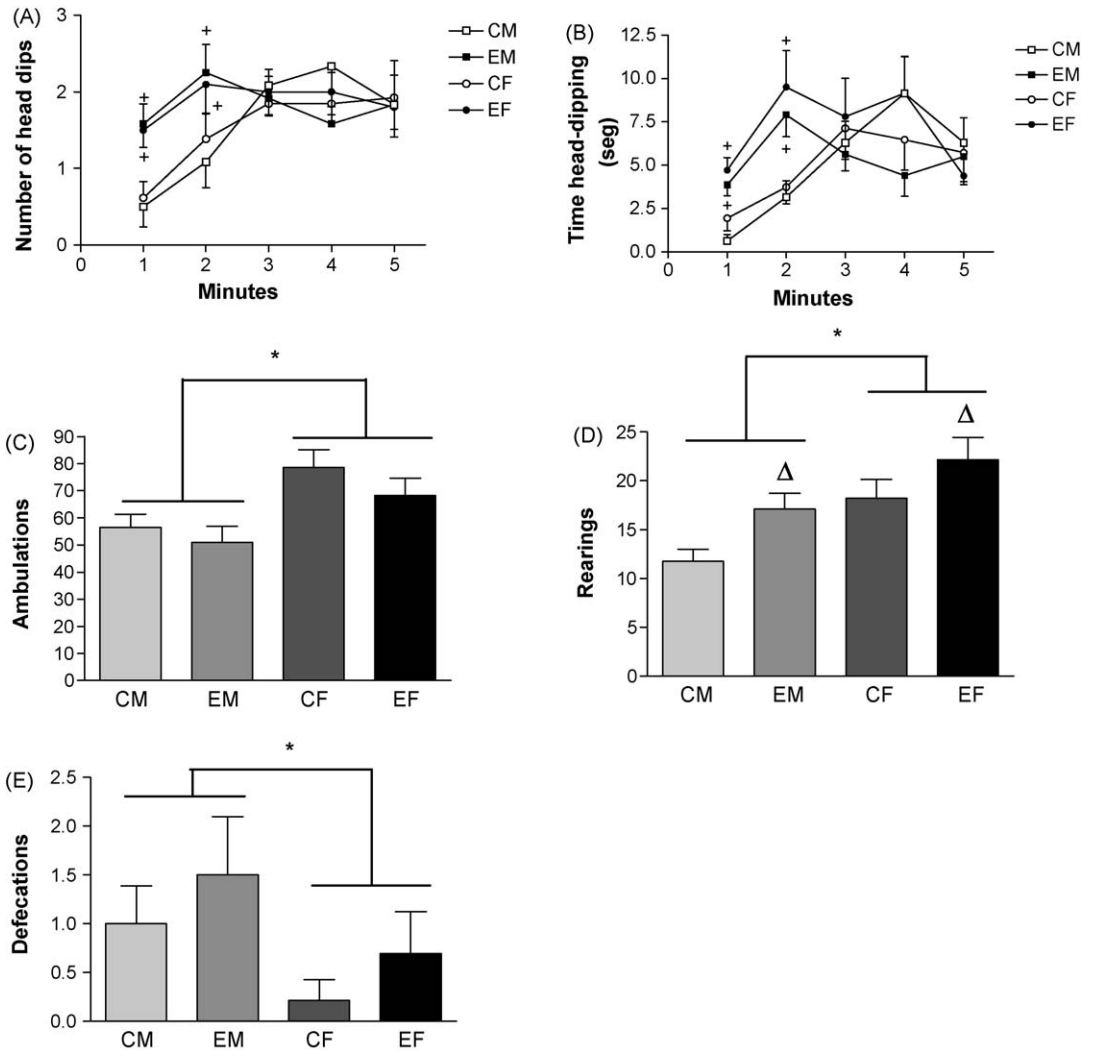


**Figure 3** Effects of enriched environment on hole board behaviour. Mean ±SE are represented. Panels A and B represent the number of head-dips (A) and time spent head-dipping (B) in each of the five min of the test. The other panels represent total ambulations (C), rearings (D) and defecations (D), for Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups. $^{+}p < 0.05$ versus the corresponding CM or CF group (same sex); $^{*}p < 0.05$ overall 'sex' effect; $^{\Delta}p < 0.05$ overall 'treatment' effect (two-way ANOVA).

significance of sex and treatment factors for each variable is indicated in Table 2. A significant sex effect was observed in total distance travelled on all arms and rearings in the closed arms, with higher values in females. EE overall increased the number of entries and the distance travelled into the open arms, whereas the time spent into the open arms was not affected. EE did not affect the total number of entries or the total distance travelled. Significant EE effects also appeared on defensive behaviours and risk assessment behaviours. Thus, EE decreased the number of stretched attend postures from closed arms towards open arms (closed arms returns), the number of stretchings into the open arms directed to more distant parts of the open arms, and the number of rearings in both the open and enclosed arms.

### 3.5. ASR and PPI

Startle response was analyzed by a repeated measures ANOVA, with sex and treatment as between subject factors and trial (12 trials) as the within subject factor. The measurement of the intensity of the startle response by the apparatus is related to the strength effect on the piezoelectric accelerometer produced by the animal response, which can be dependent on the body weight, the males being heavier and presenting higher intensities of startle responses than females. We analyzed the startle response over the first 12 trials with a repeated measures ANCOVA with the body weight as a covariate (Young and Cook, 2004) and, no significant effects of sex, treatment, trial or the interaction between these factors were found (data not shown).

Percent PPI was analyzed with sex and treatment as between subject factors and pre-pulse stimulus intensity as the within subject factor (Fig. 4A). The ANOVA revealed a significant effect of pre-pulse intensity ($F(2,96) = 104.62$, $p < 0.001$), no effect of sex, and a significant effect of treatment ($F(1,48) = 8.46$, $p < 0.01$). Only the interaction pre-pulse intensity × treatment was also significant

**Table 2** Effects of sex and enrichment environment on EPM behaviour.

| | CM | EM | CF | EF | F (sex) | *F* (treatment) |
|---|---|---|---|---|---|---|
| TE | 14.1 ± 1.1 | 17.2 ± 1.1 | 16.3 ± 1.3 | 17.6 ± 0.9 | n.s. | 3.46 [a] |
| TD | 1124 ± 78 | 1335 ± 71 | 1368 ± 89 | 1425 ± 93 | 3.86 [b] | n.s. |
| EOA | 5.9 ± 1.1 | 7.7 ± 0.4 | 5.9 ± 0.8 | 7.6 ± 0.6 | n.s. | 4.7 [*] |
| DOA | 370 ± 69 | 492 ± 35 | 378 ± 59 | 503 ± 51 | n.s. | 5.0 [*] |
| TOA | 90.8 ±12.5 | 95.1 ± 12.5 | 77.1 ± 11.5 | 87.1 ± 11.5 | n.s. | n.s. |
| SOA | 1.8 ± 0.4 | 3.5 ± 0.4 | 1.7 ± 0.5 | 3.1 ± 0.7 | n.s. | 6.67 [*] |
| SEA | 6.0 ± 1.0 | 3.2 ± 0.6 | 6.9 ± 1.0 | 4.7 ± 0.8 | n.s. | 7.49 [**] |
| ROA | 0.2 ± 0.1 | 4.7 ± 0.6 | 2.1 ± 0.7 | 5.0 ± 1.0 | n.s. | 22.7 [**] |
| REA | 8.5 ± 1.2 | 13.8 ± 1.7 | 13.6 ± 1.2 | 17.4 ± 1.1 | 10.43 [**] | 11.35 [**] |

Mean ±SE of total entries (TE), total distances travelled (TD), entries in the open arm (EOA), distances travelled into the open arm (DOA), time spent into the open arms (TOA), stretchings in the open arms (SOA), stretchings in the enclosed arms (SEA), rearings into the open arms (ROA) and rearings in the enclosed arms (REA) performed in the Elevated Plus Maze by Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups. Significant 'sex' and 'treatment' factors that appeared in the two-way ANOVA analysis are also shown; n.s: non-significant.

[a] $p = 0.069$.
[b] $p = 0.055$.
[*] $p < 0.05$.
[**] $p < 0.01$.

($F(2,96) = 3.47$, $p < 0.05$). Decomposition of this interaction indicated that, regardless of sex, EE animals showed lower levels of PPI than controls with 60 ($p < 0.004$) and 70 dB ($p < 0.03$), but not with 80 dB.

## 3.6. Social discrimination tests

Repeated measures ANOVAs (between-subjects factors: sex and treatment) were used to assess social discrimination in T2, being the type of juvenile (the same or novel) the within subject factor. When the total exploration time was considered as the variable in the two trials (T1 and T2), the within-subject factor was trial.

In the first social discrimination test, where an inter-trial interval of 30 min was imposed between T1 and T2, we did not observe social memory in any group, since all groups showed a similar exploration time towards the same and the novel juveniles during T2 (no significant effect of treatment or the interaction of treatment with the other factors appeared (Fig. 5A). We then decided to analyze total exploration time in T1 and T2 (the sum of exploration of both juveniles), with repeated measures ANOVA. This analysis revealed no effect of sex or the interaction of sex with the other factors, but a significant effect of trial ($F(1,48) = 11.94$, $p < 0.01$) and the interaction trial × treatment ($F(1,48) = 4.025$, $p < 0.05$). This interaction reflected that control rats showed an enhanced decrease in exploration of juveniles from T1 to T2, whereas enriched animals did not.

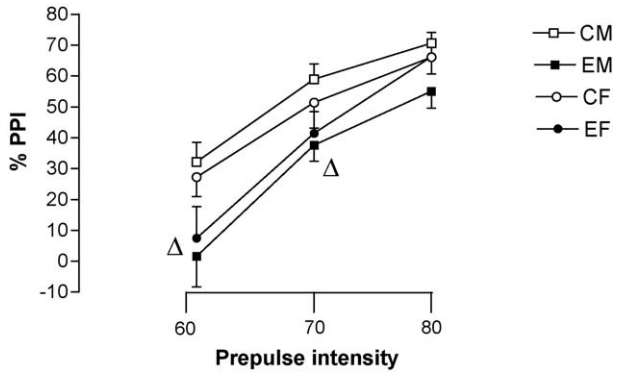


**Figure 4** Effects of enriched environment on ASR and PPI. The mean ±SE of percentage of inhibition to the different pre-pulse intensities (60 dB, 70 dB and 80 dB) for Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups is shown. $^{\Delta}p < 0.05$ overall 'treatment' effect, regardless of sex, at the corresponding pre-pulse intensity (two-way ANOVA).

One week later, we tested again all the animals in a second social discrimination test, with a reduced inter-trial interval of 15 min. In that case, the repeated measures ANOVA of T2 showed a significant interaction type of juvenile × sex × treatment ($F(1,50) = 4.6$, $p < 0.04$; Fig. 5B). The decomposition of this interaction indicated that control females (CF) and enriched males (EM) preferred the novel juvenile, whereas CM and EF animals did not (Fig. 5B). Moreover, the two-way ANOVA of the index obtained calculating the difference between exploration time towards the novel or same juvenile in T2 revealed a significant treatment × sex interaction (ANOVA, $F(1,50) = 4.598$, $p < 0.05$, Fig. 5C), indicating that EE improved social memory only in males whereas a surprising opposite effect was observed in females. When total exploration time in T1 and T2 were analyzed, the ANOVA revealed a significant sex effect ($F(1,48) = 13.37$, $p < 0.01$), indicating that males spent more time investigating juveniles than females during both T1 and T2. No other significant effect was observed.

## 3.7. Hebb-Williams maze task

### 3.7.1. Pre-training

The number of sessions required to meet the acquisition criterion was analysed with a two-way ANOVA, being sex and treatment the between subject factors. No effects of treatment or the interaction treatment × sex appeared in

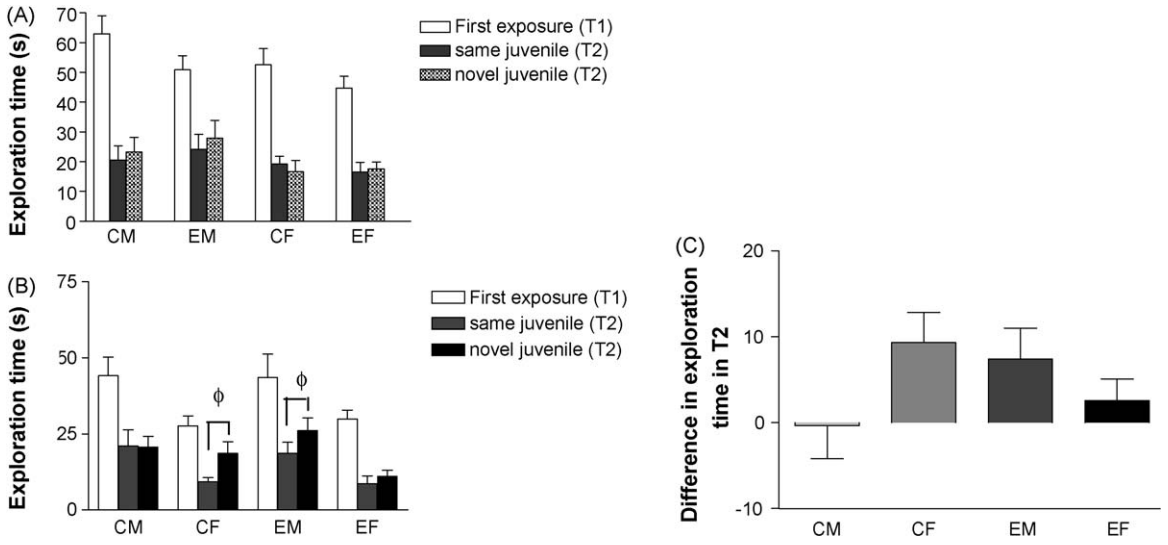


**Figure 5** Effects of enriched environment on social exploration. Mean ±SE of exploration times in the first exposure (T1) and in the second exposure (T2) towards the same juvenile (filled bars) and the novel juvenile (hatched bars) in the first experiment (ITI 30 min, panel A) and second experiment (ITI 15 min, panel B) of social discrimination. (C) Difference between the exploration time directed to the novel juvenile and the exploration time directed to the same juvenile during T2. $^{\Phi}p < 0.05$ between the signalled groups (decomposition of the interaction).

the analysis of the number of sessions to reach the criterion (running the 6 trials of the session within 90 s over two consecutive sessions). The effect of sex was significant ($F(1,39) = 22{,}41$, $p < 0.0001$) in that females reached the criterion and advanced to the maze problems in fewer sessions than males. Mean ±SE of the experimental groups were the following: CF: 8.78 ±0.84; EF: 7.69 ± 0.41; CM: 12.13 ± 0.74: EM: 11.14 ± 0.77.

### 3.7.2. Performance over the 12 problems

A repeated measures ANOVA (between-subjects factors: sex and treatment), with problem as a within subject factor, was applied to the accumulated number of errors, latencies and distances travelled in trials 1—6 for each problem.

The ANOVA indicated an overall significant effect of treatment on number of total errors and total distance travelled ($F(1,36) = 22.8$, $p < 0.0001$; $F(1,36) = 30.6$, $p < 0.0001$, respectively). No effect of sex or the interaction of sex with the other factors was found on total errors or distance. The repeated measures ANOVA indicated a significant effect of maze on number of total errors ($F(11,396) = 58.3$, $p < 0.0001$, Fig. 6A), total distance travelled ($F(11,396) = 69.3$, $p < 0.0001$, Fig. 6B) and total latency ($F(11,396) = 43.4$, $p < 0.0001$, Fig. 6C), showing that performance differed over the 12 mazes. However, on total errors and total distance travelled a significant maze × treatment interaction ($F(11,396) = 3.4$, $p < 0.003$; $F(11,396) = 2.4$, $p < 0.03$, respectively) were found. Further comparisons showed a lower number of errors in enriched animals as compared to controls in problems 2 and 6—11 (Fig. 6A), as well as a lower distance travelled in problems 2, 4 and 6—12. For latencies, the ANOVA revealed significant effects of sex $F(1,36) = 18.5$, $p < 0.0001$) and treatment ($F(1,36) = 12.7$, $p < 0.001$), and the repeated ANOVA revealed a significant maze × sex interaction ($F(11,396) = 2.9$, $p < 0.02$). Further decomposition of the interaction indicated that females were faster than males in mazes 4—12 ($p < 0.05$; Fig. 6C).

### 3.7.3. Performance over the easy, intermediate and difficult mazes

The total number of errors performed in each maze for both treatments and sexes was used to classify the mazes in three categories (mean number of total errors performed in each maze for all animals is showed in Table 3): easy (less than 15 errors per problem: mazes 1, 3, 4 and 12), intermediate (between 16 and 30 errors: mazes 2, 6, 8, 9, 10 and 11) and difficult (more than 30 errors: mazes 5 and 7). A similar classification was performed by Stanford and Brown (2003). The only difference between our classification and that of those authors concerns mazes 6 and 8, which were intermediate in our classification whereas they were considered as difficult problems by those authors. The total number of errors, the total distance travelled and the total latency of each level of difficulty was averaged by the number of mazes included in each category and a repeated measures ANOVA (between-subjects factors: treatment and sex) with level of difficulty as the within subject factor was applied to these variables.

The ANOVA showed significant effects similar to those observed with the analysis of each individual problem and therefore only particular results would be commented. Overall significant effects of level of difficulty on the mean number of total errors ($F(2, 72) = 198.2$, $p < 0.0001$; Fig. 7A), the mean distance travelled ($F(2,72) = 45.1$, $p < 0.0001$; Fig. 7B) and the mean latency ($F(1,72) = 11.5$, $p < 0.0001$; Fig. 7C) were found. No effect of sex or the interaction of sex with the other factors were found for errors and distance, but the interaction level of difficulty × sex was marginally significant regarding latencies ($F(2,72) = 2.89$, $p = 0.073$). This was due to the fact that males increased the latency to reach the goal box as the level of difficulty increased whereas females did not (Fig. 7C). Significant level of difficulty × treatment interactions appeared on the mean number of errors and the mean distance travelled ($F(2,72) = 5.45$, $p < 0.05$ and $F(2,72) = 3.92$, $p < 0.05$,

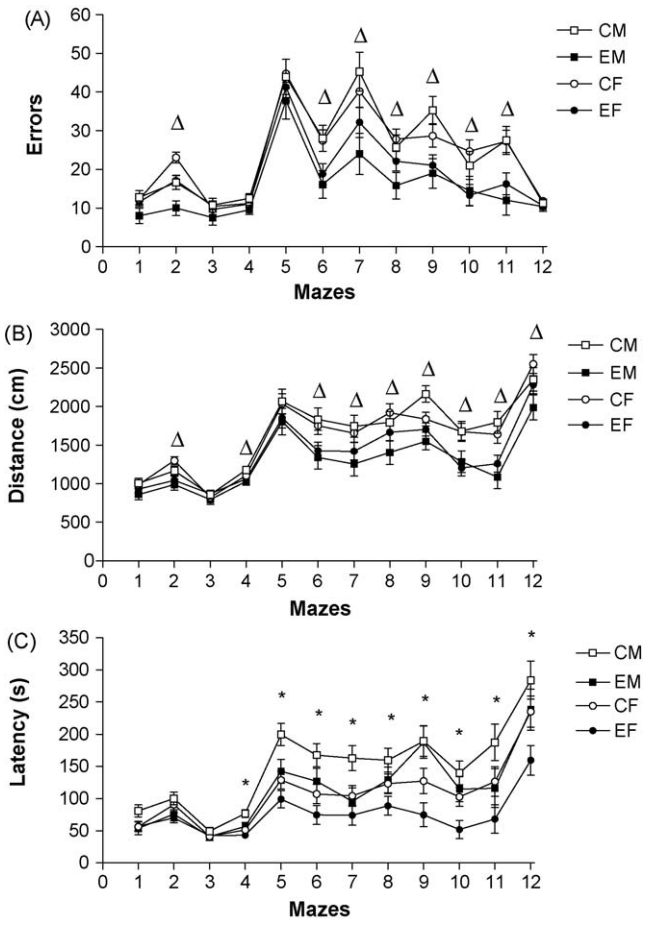


**Figure 6** Environmental enrichment effects on performance in the Hebb-Williams maze. Mean ±SE of total errors (A), distance travelled (B) and latency (C) over six trials of all 12 Hebb-Williams mazes for Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups is shown. $^{*}p < 0.05$ overall 'sex' effect, and $^{\Delta}p < 0.05$ overall 'treatment' effect for each maze (two-way ANOVA).

respectively, Fig. 7A and B), thus indicating that the treatment was more effective in diminishing the number of errors and the distances travelled in the intermediate problems, probably because enriched animals differed more significantly from controls with this level of difficulty, than with the easy and difficult problems (Fig. 7A and B). In addition, non-enriched groups increased the number of errors and distance travelled in the intermediate mazes in comparison with the easy mazes, whereas enriched animals did not show those differences between easy and intermediate mazes; in the difficult mazes, all groups made more errors and travelled more distance in comparison with the easy mazes (Fig. 7A and B).

## 4. Discussion

The present results indicate that exposure of male and female rats to an enriched environment, from weaning to adulthood, exerts long-lasting effects on relevant physiological and behavioural aspects, although some unexpected results appeared in sensorimotor gating. The effects of EE were similar in males and females, with the notable exceptions of body weight gain and social discrimination.

**Table 3** Statistics of the total number of errors in trials 1—6 of the Hebb-Williams maze task.

| Maze problem | Mean ±SE | Median | Classification |
|---|---|---|---|
| 3 | 9.8 ± 0.8 | 8.5 | Easy |
| 12 | 11 ± 0.4 | 11.0 | Easy |
| 4 | 11.1 ± 0.5 | 11.0 | Easy |
| 1 | 11.4 ± 0.8 | 12.0 | Easy |
| 2 | 17.6 ± 1.0 | 17.0 | Intermediate |
| 10 | 18.5 ± 1.7 | 15.0 | Intermediate |
| 11 | 21.1 ± 1.9 | 24.0 | Intermediate |
| 6 | 22.8 ± 1.7 | 20.0 | Intermediate |
| 8 | 23.5 ± 1.5 | 22.0 | Intermediate |
| 9 | 25.8 ± 1.8 | 26.0 | Intermediate |
| 7 | 35.8 ± 2.4 | 33.0 | Difficult |
| 5 | 42.3 ± 2.0 | 41.5 | Difficult |

Mazes were classified as easy if the mean number of errors was less than 15, intermediate if the errors were more than 15 and less than 30, and difficult if the errors performed were more than 30 (see text).

Post-weaning EE reduced body weight gain both in male and female rats as compared to controls, but female weight normalized during the post-EE period, whereas males showed persistently reduced body weight gain during all the post-EE period studied. In another study, we had not observed differences in food intake between male or female EE rats as compared to controls during the post-EE period (Peña et al., unpublished). The reduced body weight just after the EE period is in accordance with previous results in rats (Moncek et al., 2004; Peña et al., 2006), although no changes has also been reported (Laviola et al., 2004). The results in mice are more controversial (i.e. Haemisch and Gartner, 1994; Kempermann and Gage, 1999; Roy et al., 2001; Tsai et al., 2002). Some of the controversial results can be explained by differences in the energy spent to maintain body temperature in function of the number of animals and the structure of the cages, particularly with mice. However, this does not explain the long-lasting maintenance of the differences between animals reared in EE and control animals during the post-EE period. Therefore, the long-lasting effect of EE on body weight gain is surprising and may suggest enduring changes in metabolism, which is quite interesting considering the accepted beneficial effects of reduced body weight gain. Unfortunately, there is no available data on the long-term consequences of an altered food intake during the post-weaning period, but the data suggest the possibility of a post-weaning window to metabolic programming as already observed during prenatal and postnatal periods (i.e. McMillen et al., 2005). Whether or not enhanced morning to evening glucocorticoid signal observed in EE-reared rats plays a role has to be tested, but this is plausible taking into account the role of glucocorticoids in regulating metabolism and circadian rhythms in peripheral tissues (i.e. Balsalobre et al., 2000).

When basal and stress levels of HPA hormones were studied in the present experiment, we observed the well-described differences between sexes, with higher morning and evening levels of ACTH and corticosterone and a higher HPA response to stress in female than male rats (e.g. Rhodes

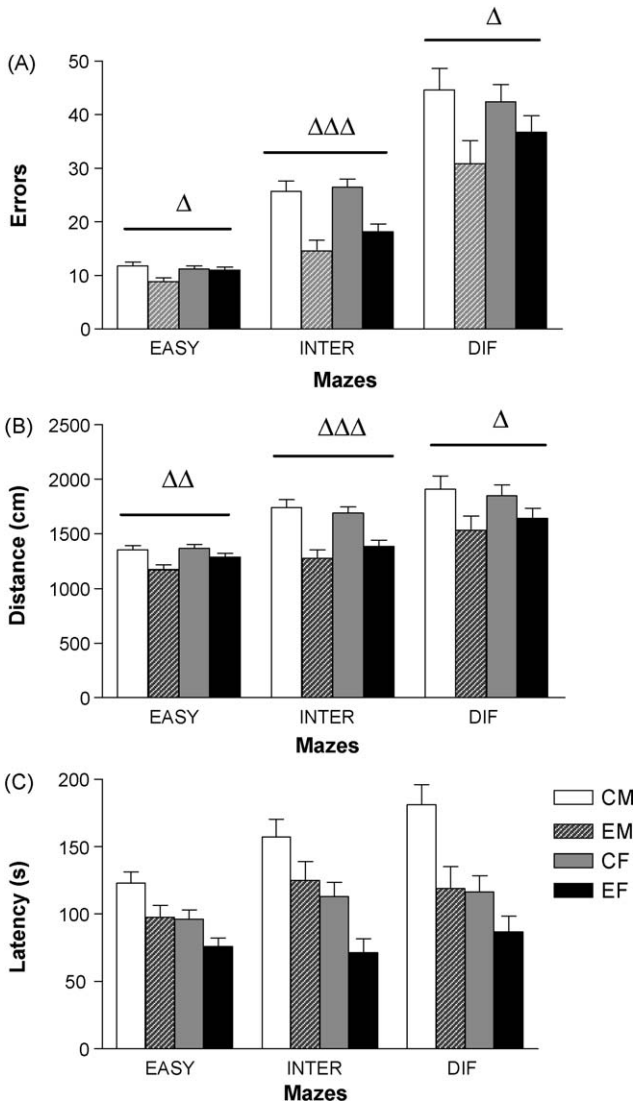


**Figure 7** Environmental enrichment effects on performance in the Hebb-Williams maze. Mean ±SE total errors (A), distance travelled (B) and latency (C) in the 4 easy mazes (1,3,4 and 12), the 6 intermediate (INTER) mazes (2,6,8,9,10 and 11) and the 2 difficult (DIF) mazes (5 and 7) for Control males (CM), Enriched males (EM), Control females (CF) and Enriched females (EF) groups. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.0001$ overall 'treatment' effect (two-way ANOVA).

and Rubin, 1999; Rhodes et al., 2002). However, no specific gender effect of EE was observed. Thus, regardless of sex, EE rats showed normal resting levels of ACTH during the morning and the evening, but a greater increase in corticosterone levels from the morning to the evening. Since circadian corticosterone rhythm involves a neurally mediated amplification of the ACTH-driven signal (Engeland and Arnhold, 2005), EE may have affected such mechanism.

To study the responsiveness of the HPA axis to stress, we used in the present experiment the HB as a mild-stress acute exposure because: (i) it includes a component of emotional reactivity due to the novelty of the situation; (ii) the HPA response to the test has been characterized in our lab (Márquez et al., 2005; Gagliano et al., 2008); and (iii) it is a test that takes advantage of the natural tendency of the rats to explore the holes on the floor so that not only the activity, but the curiosity of the animal is assessed by measuring head-dipping behaviour. Thus, 15 min after a 5 min exposure to the HB, normal ACTH with lower corticosterone levels were found in rats reared in EE. Although, this may suggest a partial dissociation between the two hormones, it is quite possible that corticosterone levels were reflecting presumably lower ACTH levels just after the 5 min exposure to the HB in rats reared in EE as compared with control rats.

The lack of effect of EE on resting morning levels of ACTH and corticosterone is in agreement with most previous results in rats (Pham et al., 1999; Schrijver et al., 2002; Teather et al., 2002; Morley-Fletcher et al., 2003), although Bakos et al. (2004) reported increased corticosterone levels in males reared in EE. The reduced corticosterone response to a novel environment observed in rats reared in EE is in line with other studies measuring HPA and behavioural responses in anxiogenic or stressful circumstances. Thus, Moncek et al. (2004) reported a significantly lower ACTH, but not corticosterone, response to handling stress. Roy et al. (2001) reported normal resting corticosterone levels in mice reared in EE, but a lower corticosterone response to a predator odour (cat) introduced into the home cages, despite no differences in the behavioural response to the situation. In some studies, no altered HPA response to stress was found in rats reared in EE (i.e. Schrijver et al., 2002), although EE appeared to reduce the exacerbated HPA response to stress caused by other early experiences such as prenatal stress and maternal separation (Francis et al., 2002; Morley-Fletcher et al., 2003). These data suggest that beneficial effects of EE on HPA reactivity may be particularly prominent under environmental or genetic conditions that enhance vulnerability to stress.

Enriched housing increased head-dipping behaviour during the first 2 min of exposure to the HB, whereas control animals reduced exploratory behaviour during the first 2 as compared to the latest 3 min. In addition, EE significantly increased the number of rearings, in a way consistent with previous work from our laboratory (Escorihuela et al., 1994; Fernández-Teruel et al., 1992, 1997; Fernández-Teruel et al., 2002) and with results obtained by other authors in the open field test (Larsson et al., 2002). Then, present results confirm and extend the findings from our previous studies reporting enhanced exploration in enriched reared rats in response to novel environments (Escorihuela et al., 1994, Fernández-Teruel et al., 1992, 2002).

In the elevated plus maze, our rats reared in EE showed increased number of entries and travelled more distance in the open arms than control rats, although significant differences in the percentage of time spent in the open arms were not found. This specific effect of EE on the distance travelled in the open arms, without changes in time spent into the open arms, was obtained previously in our laboratory (Peña et al., 2006) and is quite similar to the one reported by Roy et al. (2001). The analysis of ethologically relevant variables such as rearings, head-dips and stretched-attend postures (Rodgers and Dalvi, 1997; Wall and Messier, 2001), indicated that animals reared in EE were showing a more daring novelty seeking behaviour in comparison with control rats. Overall, it appears that the reactivity to novel environments of animals reared in EE could be better explained in terms of increased

motivation to explore novel environments rather than in terms of a reduction in fear/anxiety (Fernández-Teruel et al., 1997; Roy et al., 2001). In accordance with that, the EE procedure did not affect ASR over the initial 12 trials of the session, which is consistent with the only previous paper reporting ASR measurements in rats reared in EE (Varty et al., 2000).

EE did not affect differentially males and females, but we did find sex differences in the HB, EPM and ASR, regardless of EE treatment, indicating that females travelled longer distances, showed higher locomotor activity, reared more and defecated less than males, which is consistent with other works (i.e. Gray, 1971; Lehmann et al., 1999a; Aguilar et al., 2003). We did not find sex differences in the magnitude of the ASR, in line with another study in rats tested at 60, 100 and 300 days of age (Borrell et al., 2002), but in contrast with other studies that reported greater startle responses in males than in females (Lehmann et al., 1999b; Aguilar et al., 2003).

Pre-pulse inhibition (PPI) of the startle response provides an operational measure of sensorimotor gating, one of the processes by which an organism filters information from its environment (Braff et al., 1999). The most unexpected result of the present experiments was the decreased PPI observed in male and female rats reared in EE. Control rats showed significant levels of PPI with the three pre-pulse intensities used, whereas rats reared in EE only showed significant levels of PPI with the 80 dB pre-pulse. This result is not in accordance with that reported by Varty et al. (2000), who exposed rats to three different rearing conditions for 8 weeks after weaning: EE (three rats per cage with toys), control (three rats per cage) and isolation (one per cage). The animals reared in EE did not differ from control animals whereas the isolated animals showed a decreased PPI. In another study a relatively short period of EE (2 weeks) did not alter PPI in adolescent and adult male rats but, EE attenuated the marked long-lasting PPI deficit caused in by the administration of valproic acid during gestation (Schneider et al., 2006).

Although in the present work as well as in the two commented studies EE started after weaning, other factors could explain some of the discrepancies. First, our EE rats were reared in groups of 12—14 and housed into big cages for a more prolonged period of time (12 versus 2 or 8 weeks). Second, we tested the rats at an older age (26-week-old in our study and 12-week-old in the other two). Third, in mice, the influence of post-weaning EE on PPI appears to be modulated by exercise (Pietropaolo et al., 2006), making it difficult to compare different EE conditions. Finally, in addition to other minor differences in the PPI test itself, genetic/environmental differences between the animals may also affect the results. In any case, the data regarding PPI would require further studies in order to be confirmed and to investigate whether reduced PPI is always linked to deficits in brain processing of non-relevant sensorial information.

Since dopaminergic activity in several brain regions, including the prefrontal cortex and the nucleus accumbens are involved in the control of PPI (Geyer et al., 2001), it would be important to know the EE-induced neurochemical changes in dopaminergic activity in those brain regions. Unfortunately, most available data (i.e. Zhu et al., 2005) have compared dopaminergic function in enriched versus isolated animals, the later condition having per se important effects on PPI (Cilia et al., 2001, 2005). When group-housed animals were taken as a reference, post-weaning EE exposure in mice reduced the number of dopamine neurons in the substantia nigra pars compacta and the expression of dopamine transporters in the striatum (Bezard et al., 2003), whereas, in rats the dopamine content in frontoparietal cortex, hippocampus and nucleus accumbens were not altered (Bakos et al., 2004; Galani et al., 2007). EE studies more specifically addressing the relationship between PPI and the dopaminergic system are needed.

The decrease of PPI, suggestive of impaired sensorial processing after EE, contrasted with the results observed in the social discrimination test and in the Hebb-Williams maze. Improvement of spatial learning by EE treatment is well documented, but there are few studies on the effects of EE on social memory. Social memory in the rat can be evaluated with the social recognition and social discrimination tests and has been proposed as a valid paradigm for short-term olfactory memory (Thor and Holloway, 1982; Bluthe et al., 1990; Engelmann et al., 1995). When a delay of 15 min was used, EE improved social discrimination in males and, apparently, impaired it in females. However, the fact that the total exploration time did not decrease from the first to the second exposure in any experimental group allow us to rule out that habituation to the social exploration or lack of motivation for social interaction had occurred. With a delay of 30 min, the animals were not able to discriminate between the two juveniles, which is in contrast with a previous result from our laboratory (Peña et al., 2006), likely because shorter intervals are needed in middle-age as compared to young rats (Bluthe et al., 1990; Becker and Grecksch, 2000). Finally, overall sex differences were obtained, the males showing greater amount of exploratory behaviour towards the juveniles in the two encounters. This result is consistent with previous results from our laboratory and with the observation from other authors indicating that females showed less exploratory behaviour towards juveniles than males (Liebsch et al., 1998; Peña et al., 2006).

When we tested the animals in the Hebb-Williams maze, we observed that: (i) females needed fewer sessions of training to reach acquisition criterion than males; (ii) no differences between sexes were obtained in the resolution of the 12 mazes; (iii) EE treatment improved the performance of males and females in comparison with control animals. The fact that females had a faster rate of acquisition during the initial sessions in the Hebb-Williams test in comparison with males cannot be explained by reduced emotionality because no sex effects were found in the EPM and ASR. A more relevant explanation is that acquisition criterion employed was based on latency ($<$90 s) and this would favour females since they travelled faster than males.

We found significant effects of EE in the number of errors and distances travelled, which are two variables traditionally used for analyzing the level of performance associated to the progression in learning. Thus, EE improved the execution of the maze in both male and females as compared with controls, making fewer errors and travelling shorter distances to reach to the goal box. Regarding sex effects, we did not find differences between males and females which is in line with other reports (Das and Broadhurst, 1959; Stanford and Brown, 2003), although significant differences have been reported in other spatial tasks, such as the water maze (Berger-Sweeney et al., 1995; Perrot-Sinal et al., 1996). With

the aim of providing a more functional and practical interpretation of our data we analyzed the performance of the animals taking into account the level of difficulty implicit in the mazes (Stanford and Brown, 2003). Although EE improved learning, the effects of the treatment were more evident in the intermediate category, which is reasonably consistent with other works reporting no effects with relatively simple tasks, but significant effects with more demanding tasks (Escorihuela et al., 1995; Larsson et al., 2002).

Thus, EE affected the subjects in a positive fashion. The EE animals performed fewer errors, travelled less distance and were more accurate solving the problems, particularly those above a certain level of difficulty, in comparison with non-enriched controls. The fact that the animals started the training in the Hebb-Williams 10 months after EE treatment had finished and that the animals were 14-month-old indicated that EE had long-term effects in this complex learning task, even 10 months after living in standard rearing conditions. Furthermore, the results obtained in the Hebb-Williams indicate that the PPI deficits obtained in the acoustic startle response paradigm are unlikely to be related to difficulties in sensorial processing or learning deficits.

Finally, a limitation of the present study is the lack of estradiol measurements in our rats. In only one report using EE, 17-β estradiol was measured, but this was done in brain injured rats and no effect of EE was found (Wagner et al., 2002). Rearing in an EE has been found to reduce the beneficial mnemonic effects of exogenous estrogen administration to ovariectomized rats of different ages (Gresack and Frick, 2004; Gresack et al., 2007a, 2007b), suggesting altered responsiveness to estrogens in females living in EE, that may be related to altered levels of estrogens or of estrogens receptors.

To summarize, the present results showed male and female differences in activity/exploration of novel environments, in the patterns of social exploration, as well as in the rates of learning a complex task. Differential gender effects due to EE were manifested preferably in the social discrimination test, where the treatment seemed to be beneficial for males but not for females. However, in general, common beneficial effects of EE on both sexes were found, such as the decreased emotional reactivity and the improvement of performance in the Hebb-Williams maze. The impairment in the PPI observed in our enriched animals of both sexes represents a paradoxical result that needs further investigation in future studies.

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## Conflict of interest

All the authors declare that they have no conflicts of interest.

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