Environmental enrichment effects in social investigation in rats are gender dependent

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Abstract

Environmental enrichment (E) can change neuronal structure, improves learning in various tasks and increases cerebral plasticity. However, no E effects were found in a test measuring social olfactory discrimination memory and, although they have been mainly measured in males, gender differences have been reported in other tests. The aims of the present study were to evaluate gender differential effects of E in the social discrimination paradigm which also involves social olfactory discrimination and in the elevated plus-maze test (EPM) for measuring anxiety. E procedure consisted of a combination of social and physical factors; groups of 11–12 Sprague–Dawley rats were separated by sex in large cages with physical stimulus for a period of 8 weeks starting immediately after weaning. Differential gender E effects appeared in the social exploratory patterns: enriched males showed increased exploratory behaviour towards juvenile rats in comparison to control males, whereas no differences were found in females. No effects of E in social discrimination memory were observed. In the EPM, both enriched male and female rats showed less anxious behaviour than non-enriched animals.

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Environmental enrichment (E) can change neural structure and function leading to enduring improvements in learning and memory abilities. The first studies appeared in the early 60s and other results which appeared later reported that E increased brain weight, cortical thickness, the density of synaptic contacts, the size of neuronal nuclei and the number of dendritic branches [9,19,37,38]. More recently, it has been shown that E enlarges the forepaw representation in somatosensory cortex [7], improves auditory cortex processing [12], accelerates visual system development [40], increases gene expression [34,35] and protein levels of brain neurotrophins [33], and increases cellular plasticity in terms of hippocampal neurogenesis, survival of newly generated neurons [5,21,27], levels of synaptic proteins [28] or reduced spontaneous apoptosis [48].

With regard to cognitive function, it has been consistently shown that E improved spatial learning since enriched rats or mice performed shorter swim distances and/or decreased latencies to reach the platform in the Morris water maze in comparison with control animals [21,27,32]. Better performance in the radial- or T-maze [2,29,30], enhanced long-term memory in the novel-object recognition test [5,36], and more rapid habituation of locomotor activity [10,46] have also been observed in enriched animals in comparison with non-enriched ones. However, in other tasks based on olfactory social discrimination learning negative results have been reported [35,36]. To know how social discrimination could be affected by social enriched
housing was one of the aims of the present study and, in relation to that, we investigated the effects of E on the social discrimination memory test based on the ability of animals to recognise other animals by following chemosensory cues [11]; usually, rodents spend more time investigating an unfamiliar juvenile conspecific than a familiar one [8].

The effects of E on emotional reactivity remain controversial since inconsistent effects have been reported [6,13,15,16,23]. Thus, in the elevated plus-maze (EPM) test: (i) E produced an increase in the time spent and the number of entries into the open arms of the EPM [1,15,18,42], (ii) no effects on those measures have also been reported [39], and (iii) others have recently found that enriched mice made fewer entries into, and spent less time in the open arms as compared to control mice, thus suggesting that enriched animals showed signs of increased anxiety [49]. In the later work, it was also shown that enriched males made more total arm entries than control males whereas no effect was observed in enriched females, thus indicating that E could produce differential effects in males as compared with females [49]. A few studies investigating differential effects of E in male and female animals have been reported, indicating some differential morphological and behavioural changes such as: (a) increased dendritic length in the occipital cortex in enriched males and in the somatosensory cortex in enriched females [22]; (b) activity in the open field was increased in males and decreased in females as a result of E [10]; (c) E treatment after traumatic brain injury enhanced spatial memory performance in males but not in females [47].

Thus, the principal aim of the present work was to evaluate the differential gender effects of E in the social discrimination memory and in the EPM tests in Sprague–Dawley rats.

The classical EE procedure developed by Rosenzweig and Bennett [38] consisted of housing groups of 10–12 rats in large cages containing toys and objects, which were replaced daily, giving to the animals the opportunity for learning experiences due to physical, sensory and social interaction with the various components of the environment (enriched condition). Other subjects were either housed in groups of three (standard condition) or isolated (impoverished condition) in standard laboratory cages as control groups. Several studies performed more recently have investigated which of the aforementioned physical versus social components separately contributed to the reported effects of E and they have found that social deprivation was associated with increased activity [43] or impaired reversal learning in the two-choice discrimination task [44], whereas social enrichment increased habituation in the open field [10]. Others have found that exploratory behaviour towards novel objects habituated faster with increasing stimulus complexity of the non-social environment [50] and, that inanimate deprivation delayed acquisition of spatial memory [44]. In the present study, instead of measuring the effects of social deprivation, we were mainly interested in measuring the effects of complex physical and social stimulus (big groups of 10–12 rats) in the social discrimination memory test and in the EPM; for these reason, control animals were housed in pairs in standard macron cages and enriched animals were housed in a very similar way to Rosenzweig and Bennett [38].

Subjects were 44 (24 males and 20 females) Sprague–Dawley rats obtained from nine different litters from the breeding centre of the Autonomous University of Barcelona. The day after weaning they were housed in groups of 3–5 in standard macron cages (50 cm × 55 cm × 14 cm) separated by sexes and, 2 days later, they were weighed and 2–4 pups of each litter and sex were randomly assigned to either control (C) or enriched (E) groups. C animals were housed in groups of 2–3 of the same sex in standard cages and E rats were housed in groups of 11–12 in two large metal cages (100 cm × 43 cm × 50 cm) separated by sexes. Rats had free access to food and water and were always maintained in standard temperature conditions (21 °C ± 2) and on a 12–12 h light-dark schedule (lights on at 8:00 a.m.). All experiments were performed in the light phase between 08:30 and 16:00 h. Male and female juveniles (23–30 days old) of the same rat strain were used as social stimulus in the social discrimination memory test. They were housed in the same vivarium in standard macron cages (groups of six animals, same sex) and maintained in the same standard conditions.

E cages were cleaned twice a week and E rats were not handled except for maintenance till the end of the enrichment treatment. The internal configuration of the E cages was changed every week, creating different spaces with several types of stairs, ropes, tunnels, exercise wheels and so forth. Novel objects (play-things like balls, rings and bells) made of metal, plastic, wood or other materials were provided in the cages and changed two–three times per week, with all cages receiving the same assortment of objects each time. C rats were briefly handled once a week during cage cleaning. Animals were maintained in these environmental conditions for 8 weeks, after which all animals were housed in pairs in standard macron cages until they were 4 months old. The groups formed were: control male (CM, n = 12), control female (CF, n = 11), enriched male (EM, n = 12) and enriched female (EF, n = 9). Animals were weighed immediately post weaning, post enrichment and before each behavioural test to measure the influence of our enriched rearing conditions on that parameter.

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).

Adult animals were housed individually 3 days prior to behavioural testing when they were 4 months of age. The social discrimination tests were performed in a grey arena made of wood (60 cm × 40 cm × 40 cm) which was placed in a dark room illuminated with dim light. Adult rats were habituated to the arena during 5 min, 24 h prior to the first trial of testing. All juveniles were isolated in individual cages for 20 min before the beginning of the experiments. Briefly, the social discrimination test consisted of two successive presentations (5 min each) separated by a delay period of 30 min [11]. During the first presentation (T1) a juvenile rat was placed in the box with an adult rat, and the time spent by the adult investigating the juvenile was recorded. Social-investigatory behaviour, consisting of being proximally oriented to juvenile rat or having a direct contact with the other rat by sniffing, following, grooming or generally
inspecting any body surface of the juvenile, were measured. At the end of the first presentation, the rats were removed and kept in their individual cages. The juvenile was kept in the observation room while the adult rat was taken to the vivarium during the delay period. Two juveniles, the same one (which had been exposed previously) and a novel one, were simultaneously presented to the adult during the second exposure (T2). The social investigation times directed to the same and new juvenile were separately measured by identifying the juveniles using coloured marks on the head and above the tail base. Two separate experiments were performed in order to measure social discrimination memory employing male and female juveniles as social stimuli [14]. Thus, male juvenile rats were used in experiment 1 and female juvenile rats were used in experiment 2 performed 1 week after. Four male rats showed sexual behaviour towards the juveniles and were not included in the statistical analysis. We did not observe any aggressive behaviour towards the juvenile rats during the social discrimination tests. Trials were videotaped and social-investigatory behaviour was scored by a trained observer who was blind to the animal’s treatment. After social discrimination experiments were performed the animals were group housed in pairs and 2 months later the EPM test was administered. It 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 only had a 0.5 cm ridge to provide additional grip. The whole maze was elevated 50 cm above the floor. Only one animal 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 behaviour was videotaped and analysed by the computer-assisted data acquisition system analysis (SMART, Panlab). The following measures were scored: latency to enter in 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 [31]. It is interpreted that behaviour in the open arms is indicative of the anxiety level, thus a less anxious animals show increased number of entries and/or time spent into the open arms in comparison with a more anxious one.

In none of these experiments, were different stages of the oestrous cycle controlled for. However, variances in female behaviour did not differ from those in males. The statistical analysis was performed using the ‘Statistical Package for Social Sciences’ (SPSS, version 11.5). To assess social investigation, a mixed ANOVA was used, with two between factors (‘treatment’ and ‘adult sex’) and two within-subject factors (‘juvenile sex’ and ‘type of juvenile’ (same or novel in T2)). Body weights and EPM data were analysed using two-way ANOVAs (between factors ‘treatment’ and ‘adult sex’).

We obtained overall significant effects of E ‘treatment’ and ‘adult sex’ on body weight during the experimental sequence, E animals showing reduced body weights as compared to control rats. This is consistent with other authors [3,17,26,32], although other studies have shown decreased body weight in rats enriched as adults and not in juvenile or aged enriched rats [22]. In our experiment this difference persisted 3 months after the EE treatment finished, thus indicating that those effects on body weight were very long lasting (see Table 1). Interestingly, other treatments such us dietary restriction or physical activity that reduce body weight have been shown to improve performance in spatial learning and to induce neuroanatomical changes in the brain [25].

In the social discrimination tests, adult males spent more time investigating the juvenile during T1 than adult females did (ANOVA, ‘adult sex’: $F(1, 36) = 23.42, P < 0.001$), this difference being dependent of the juvenile sex (ANOVA, ‘juvenile sex’ × ‘adult sex’: $F(1, 36) = 5.45, P < 0.05$). Decomposition of interactions showed that those gender differences were more accused when exploring juvenile females ($F(1, 36) = 23.09, P < 0.001$) than juvenile males ($F(1, 36) = 9.67, P < 0.01$, Fig. 1A and B). Moreover, adult males dispensed similar exploration time in T1 towards male and female juveniles, whereas adult females showed reduced exploration towards juvenile males in comparison with juvenile males ($F(1, 36) = 7.02, P < 0.05$).

In the second exposure (T2), all groups were able to discriminate between the previously exposed juvenile and the novel one. Thus, social memory was maintained in all cases, and this ability was indicated by the significant longer investigation duration towards the novel juvenile compared to the previously exposed (ANOVA, ‘type of juvenile’: $F(1, 36) = 33.17, P < 0.001$, Fig. 1A and B). During T2, males again showed higher exploration times towards juveniles as compared with females (ANOVA, ‘adult sex’: $F(1, 36) = 5.77, P < 0.05$), and this pattern was stronger when the juveniles used were females (ANOVA, ‘juvenile sex’ × ‘adult sex’: $F(1, 36) = 9.67, P < 0.01$). Decomposition of the interaction showed that males dispensed higher exploration times towards female juveniles in comparison to male juveniles ($F(1, 36) = 19.14, P < 0.001$), whereas adult female rats showed similar levels of exploration towards male and female juveniles. Social interactions between both juveniles were also measured and no between group differences were found (experiment 1: CM: 44.03 ± 3.54, CF: 43.54 ± 4.65, EM: 45.07 ± 6.10, and EF: 45.37 ± 4.43; experiment 2: CM: 47.51 ± 5.95, CF: 39.91 ± 3.23, EM: 38.77 ± 4.06, and EF: 36.72 ± 2.35).

Interestingly, E treatment increased exploration times towards juveniles during T2 in adult male rats but not in female rats regardless of the type of juvenile (novel or same) and the juvenile sex (ANOVA, ‘adult sex’ × ‘treatment’: $F(1, 36) = 5.77, P < 0.05$). Decomposition of the interaction showed that the difference in social exploration times between adult males and adult females was more pronounced in the E group (C group: $F(1, 36) = 6.03$; $P < 0.05$, E group: $F(1, 36) = 31.91$, $P < 0.001$). Moreover, E increased social investigation in males ($F(1, 36) = 5.75, P < 0.05$) but not in females.

Although it has been shown that adult male rats do not reduce investigation when repeatedly exposed to different social stimuli [11], we compared the total investigation durations in T1 and T2 in order to characterize non-specific, investigatory behaviour-suppressing effects in adult males and females. The ANOVA with repeated measures revealed a significant interaction effect ‘session’ × ‘adult sex’ × ‘treatment’: ($F(1, 36) = 8.35, P < 0.01,$
Fig. 1B). Decomposition of the interaction showed that enriched males were the only group who increased total social exploration times from T1 to T2 ($F(1, 36) = 12.94, P = 0.001$, Fig. 1C and D), whereas no changes were observed in the rest of the groups. Thus, in any case a habituation in social investigation was observed from T1 to T2.

In the EPM, females were more active than males, showing increased total arm entries and more total distance travelled (ANOVA, ‘adult sex’: $F(1, 42) = 5.287, P < 0.05$, Fig. 2A, $F(1, 42) = 8.090, P < 0.05$, Fig. 2B, respectively). E animals also showed a higher percentage of open arm entries ((number of open arm entries/total arm entries) $\times 100$) compared to control animals (ANOVA, ‘treatment’: $F(1, 42) = 4.283$, $P < 0.05$; ‘treatment’ $\times$ ‘adult sex’: n.s., Fig. 2C) which suggested that E treatment decreased anxiety-like behaviour both in male and female rats.

The most remarkable result of the present experiment was that E had gender specific effects on social investigation: enriched males as compared to control males showed increased exploration time towards juveniles in the second encounter whereas no changes in enriched females as compared to control females were observed. Thus, E increased the gender differences appearing in exploration patterns of non-enriched animals, control males being more explorers of social stimuli than control females, which suggests that patterns of social interaction in male rats have greater sensitivity to E than in females. There are no studies evaluating the effects of E treatment on social interaction and, taking into account that E treatment provided enhanced opportunities for social interaction in the E cages, it could be argued that E modified social behaviours towards conspecifics due to the previous experience in those social conditions.

Overall gender differences were also obtained, the males showing longer total exploration times compared to females. This was consistent with other results indicating that females showed less exploration time towards juveniles than males, even though females displayed longer retention intervals to recognise juveniles [4]. Those gender differences in social exploration could be related with the sexually dimorphic vomeronasal pathway for processing non-volatile pheromones, which is involved in the olfactory signals mediating social investigation [14]. Moreover, whereas peripheral vasopressine enhanced social recognition in males and females, vasopressin antagonists blocked those effects only in males, indicating that this neuropeptide could be involved in those gender differences of social investigation [4].

As regards social stimuli, our results indicated that gender differences in exploratory patterns were even more evident when juvenile female rats were used. In this regard, Ferguson et al. [14] reported that it was possible to use other social stimuli such as intact males or females in the habituation–dishabituation paradigm to evaluate social recognition. In the present work, we used juveniles of both sexes to evaluate social exploration of adult rats. The data showed that all groups explored the novel juvenile more than the known juvenile independently of the juvenile’s gender, and that there were no difficulties in the recognition task in the second encounter. In addition, male rats displayed a greater overall social interest in different-sex conspecifics than females did, although other experiments have reported an opposite result [45]. Moreover, effects in the order of presentation cannot be discarded since we used male juveniles in the first experiment and females in the second. Thus, although males and females differ in their motivation for social interaction, further
### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weaning 12 weeks</th>
<th>15 weeks (experiment 1)</th>
<th>16 weeks (experiment 2)</th>
<th>25 weeks (EPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>78.05 ± 2.1</td>
<td>494.98 ± 12.3</td>
<td>284.6 ± 6.4 ab</td>
<td>287.03 ± 6b</td>
</tr>
<tr>
<td>EM</td>
<td>76.33 ± 1.3</td>
<td>433 ± 13.6 a</td>
<td>254.6 ± 6.2 ab</td>
<td>297.43 ± 6b</td>
</tr>
<tr>
<td>CF</td>
<td>71.52 ± 2.8</td>
<td>240 ± 6.6 b</td>
<td>254.6 ± 6.2 ab</td>
<td>297.43 ± 6b</td>
</tr>
<tr>
<td>EF</td>
<td>69.57 ± 4.4</td>
<td>44.3; P &lt; 0.05</td>
<td>45.4 ± 4.3; P &lt; 0.05</td>
<td>45.4 ± 4.3; P &lt; 0.05</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Treatment</td>
<td>n.s.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sex × treatment</td>
<td>n.s.</td>
<td></td>
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</table>

Sex: (a) P < 0.05 vs. control rats of same sex, (b) P < 0.05 vs. male rats of same treatment (Duncans’ test).

Fig. 2. Effects of EE treatment on behaviour in the elevated plus-maze. (A) Bars represent the mean (±S.E.) number total entries, (B) total distance travelled, (C) number of open-arm entries, and (D) percent open arm entries (open arm entries/open arm entries + enclosed arm entries) over a 5 min test period. *P < 0.05, **P < 0.01, vs. male animals of the same treatment. +P < 0.05 vs. control group of the same sex.

studies using different social stimuli are needed to understand the meaning of those differences.

In the EPM, enrichment increased the percentage of open arm entries without appearing any effect in the total arm entries or the distance travelled. This result indicates an overall emotionality-reducing effect which is consistent with other works [20,41], although other authors have reported increased activity in the EPM as a result of E [1,38]. We also measured the distance travelled during the social discrimination trials and no significant effects of E were found (data not shown), thus suggesting that the administration of our E procedure for 8 weeks does not increase general activity in those behavioural tests. Concerning gender effects, we obtained that females showed an increased
number of arm entries and longer distance travelled in comparison with males, thus suggesting they were more active overall (Fig. 2A and B). This is consistent with a previous work reporting significant gender effects in activity, females being more active than males [24].

In conclusion, the results showed that the effects of E in the pattern of social investigation are gender dependent, and that E treatment increased exploratory behaviour towards conspecifics only in male rats. In the present study enrichment did not increase discrimination in a social olfactory memory task in either sex. Moreover, enriched animals appeared less emotional than controls since they showed increased percentage of open arm entries into the open arms of the EPM. Finally, subsequent experiments investigating the differential gender effects of EE are necessary to clarify whether most of the effects described in males can be also observed in females. Meanwhile, in the present work no E effects have been observed in enriched females in social investigation patterns, even though enriched animals showed decreased emotionality in the EPM.

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