Behavioural Processes 144 (2017) 66–71

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Environmental enrichment in the absence of wheel running produces beneficial behavioural and anti-oxidative effects in rats

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ARTICLE INFO

Keywords:
Environmental enrichment
Morris water maze
Thigmotaxis
Hippocampus
Antioxidative measurements

ABSTRACT

The effects of early environmental enrichment (EE) when solving a simple spatial task in adult male rats were assessed. After weaning, rats were housed in pairs in enriched or standard cages (EE and control groups) for two and a half months. Then the rats were trained in a triangular-shaped pool to find a hidden platform whose location was defined in terms of two sources of information, a landmark outside the pool and a particular corner of the pool. As expected, enriched rats reached the platform faster than control animals. Enriched rats also performed better on a subsequent test trial without the platform with the geometry cue individually presented (in the absence of the landmark). Most importantly, the beneficial effects of the present protocol were obtained in the absence of wheel running. Additionally, the antioxidative effects in the hippocampus produced by the previous protocol are also shown.

1. Introduction

Early sensory experience is crucial for brain development and for the capacity for learning in adult life (for reviews see Nithianantharajah and Hannan, 2006; Simpson and Kelly, 2011). For example, environmentally enriched animals perform better on cognitive tests conducted when they are adults, with spatial tasks being frequently employed for this purpose, like the Morris pool (Morris, 1981). It is also well described in the literature that physical activity, like wheel running, can modulate cognitive function (Nithianantharajah and Hannan, 2009; Pang and Hannan, 2013). Moreover, this variable has been considered the most important to explain the beneficial effects of environmental enrichment (Kobilo et al., 2011; Mustroph et al., 2012 – although see Nithianantharajah et al., 2008, for an EE protocol not including running wheels that has been found to have beneficial effects in mice). In fact, it is a matter of debate how EE and physical exercise can enhance experience-dependent plasticity in the brain, as well as their differential impacts on anxiety and cognition (Rogers et al., 2016).

Using a modified Morris pool, in a recent study by Chamizo et al. (2016), the effects of early environmental enrichment (EE) and voluntary wheel running on the preference for using – and in the learning of – a landmark or pool geometry when solving a simple spatial task in adult male and female rats were assessed. After weaning, the rats were housed in same-sex pairs in enriched or standard cages (EE and control groups) for two and a half months. Then, the rats were trained in a triangular-shaped pool to find a hidden platform whose location was defined in terms of two sources of information, a landmark outside the pool and a particular corner of the pool. Enriched rats reached the platform faster than control animals. Following training different test trials, without the platform, were conducted. In two single-cue tests enriched rats performed better than control animals. However, an undeniable weakness of the previous work is that there is not a clear distinction between environment enrichment and physical exercise (i.e. specifically, voluntary wheel-running). Given the increasing importance of environmental enrichment in biomedical research, it seemed crucial to us to know the specific conditions required when conducting EE experiments. Consequently, the aim of the present study, only with male rats, was to separate the beneficial effects of EE from those of wheel-running in our specific protocol (Chamizo et al., 2016), although with a main exception: in the absence of wheel-running. After weaning, the rats were housed in enriched or standard cages (EE and control groups) for two and a half months. Then, the rats were trained in a triangular-shaped pool to find a hidden platform whose location was defined in terms of two sources of information, a landmark outside the pool and a particular corner of the pool (a geometry cue). After training the rats received a final single-cue test trial only with the geometry cue. Under these new conditions, would EE rats reached the platform faster than control animals during training and perform better

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http://dx.doi.org/10.1016/j.beproc.2017.09.009
Received 28 April 2017; Received in revised form 7 September 2017; Accepted 12 September 2017
Available online 14 September 2017
0376-6357/

than control animals on the test trial? (an additional question, presented in the Appendix A, addresses oxidative stress parameters in these rats).

2. Method

2.1. Subjects

The subjects were 12 naive male Long Evans rats, distributed into two groups of 6 rats. They were housed in pairs on postnatal day 22. The groups contained equal numbers of rats from two litters, thereby providing equal matched pairs for comparison.

All animal treatment and care abided by the ethical principles of the University of Barcelona regarding the care and use of animals for scientific purposes, as well as by the corresponding principles of the European Community (EEC Council Directive 86/609/EEC).

2.2. Environmental enrichment (Housing conditions)

Immediately after weaning, the animals were housed in either an enriched or a control cage, and thus were reared under either enriched or standard conditions (as in Chamizo et al., 2016, although with one main exception). The enriched environment consisted of large aluminium cages measuring 45 × 35 × 50 cm and containing a combination of different objects or 'toys'. Twenty different combinations were used, with two changes every week (i.e. a new combination after every third and fourth day). These combinations included different objects made of wood, metal or hard plastic. One specific object, a running wheel, was present in all combinations although it was manipulated with wire strand so that it was impossible to run in them. The control environment consisted of standard methacrylate and aluminium cages measuring 25 × 15 × 50 cm, identical to those used for housing the animals in the colony room. The two sets of cages (i.e. enriched and control) were provided with a 2 cm layer of woodchip bedding and were kept in the same room, although on different racks. The animals were always maintained on *ad lib* food and water, with a 12:12 h light-dark cycle. The temperature of the home room was kept at 23 °C, with a relative humidity of 56%.

The total duration of the EE procedure was two and half months. During this period all the rats were handled twice a week, coinciding with the change of the combination of objects in the enriched cages. A stereotyped handling procedure was adopted. After the enrichment procedure period the EE animals remained in the large cages, but without objects (with the sole exception of the running wheel, which did not rotate), and the control animals stayed in the standard cages, as usual. The behavioral measures began when the animals were three months old.

2.3. Apparatus

As in the Morris water maze navigation paradigms described by Chamizo et al. (2016) the apparatus was a circular swimming pool made of plastic and fibreglass. It measured 1.58 m in diameter with a depth of 0.65 m, and for the experiment it was filled to a depth of 0.49 m with water rendered opaque by the addition of 1 cl/l of latex. The water temperature was maintained at 22 ± 1 °C. The pool was situated in the middle of a large room and mounted on a wooden platform 0.43 m above the floor. To create the triangular geometry, two acrylic boards forming an angle of 90° were placed inside the pool on top of platforms at the base which supported them vertically. The boards were 39.5 cm high, 0.5 cm thick and 112 cm long. The top of the boards emerged 9.5 cm above the water surface, which was the same height as the outer wall of the pool. The pool was surrounded by black curtains reaching from the ceiling to the base of the pool and forming a circular enclosure 2.4 m in diameter. A single object, landmark X, was suspended from a black false ceiling inside this enclosure, 35 cm above the surface of the water and with its mid-line directly above the wall of the pool. For all rats, landmark X was a ninepin, with blue and yellow segments, 6 cm in diameter at the base and 16.5 cm in height, with the wider part measuring 26 cm in circumference. The single landmark X, as well as the point formed by the corner of the pool with a straight wall to the left and the circular base of the triangle to the right, defined the location of the platform. In order to ensure that the rats used these two sources of information (the landmark and the triangular shaped pool) to locate the platform, rather than any inadvertently remaining static room cues (like noises from pipes and air conditioning), the landmark, the two boards and the platform were semi-randomly rotated with respect to the room (90°, 180°, 270°, or 360°) with the restriction that all four positions of the room were used each day. A closed-circuit video camera with a wide-angle lens was mounted 1.75 m above the centre of the pool inside the false ceiling, and its picture was relayed to recording equipment in an adjacent room. A circular platform 0.11 m in diameter and made of transparent Perspex was mounted on a rod and base which was placed 0.38 m from the point formed by the corner of the pool with a straight wall to the left and the circular base of the triangle to the right, on a line that bisected the centre of the pool, with its top 1 cm below the surface of the water. The hidden platform, P, landmark X, and the geometry of the pool were situated as shown in Fig. 1 (Top).

2.4. Procedure

2.4.1. Circular pool and modified Morris Pool

There were three types of trials: pre-training, training and test trial. Pre-training consisted of placing a rat into the circular pool without the landmark or boards but with the hidden platform present. The rat was given 120 s to find the platform, and once it had been found, the rat was allowed to stay on it for 30 s. If the rat had not found the platform within 120 s, it was picked up, placed on it and left there for 30 s. The

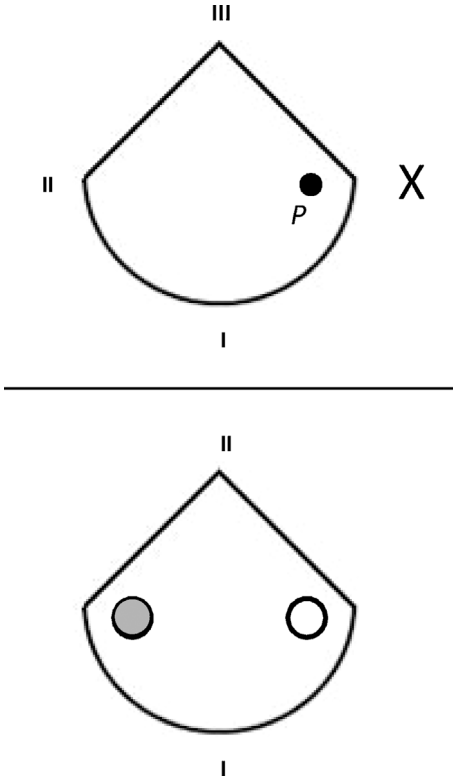

Fig. 1. A schematic representation of the pool and the position of the landmark, X, as well as the hidden platform (*P*) and the starting positions (I, II, III). Top: For acquisition. Bottom: For the learning test (with the geometry of the pool and without the landmark), where the white and the grey circles reflect the target and the control areas, respectively; and I and II the starting positions.

platform was moved from one trial to the next, and the rat was placed in the pool in a different location on each trial; all attempts were made to ensure that rats were placed equally often on the same or opposite side of the pool from the platform, and with the platform to the right or the left of where the rat was placed. Rats performed five of these pre-training trials over two days, with two trials on Day 1 and three on Day 2. Trials were run in groups of eight and rats spent the inter-trial interval (ITI) in small individual compartments. The time (in seconds) to reach the hidden platform, the distance travelled (in mm) and the swim speed were recorded.

The procedure for training was similar to that of pre-training, although with three exceptions. In order to replicate the procedure used in Chamizo et al. (2016), the landmark, X (the ninepin), was always present, as were the two boards forming the unusual triangular pool, as shown in Fig. 1 (Top). As in pre-training, the rat was placed in the pool in a different location on each trial, equating as far as possible the number of occasions with the platform to the right, to the left or in front of where the rat was placed (at positions I, II and III of the previous figure). Rats performed eight trials per day over five days (a total of 40 trials). These trials had an ITI of 8–10 min, and the platform, landmark and triangular geometry were rotated between trials. The time (in s) to reach the hidden platform was recorded.

After the training phase, there was one test day, with eight training trials (identical to the training phase), followed by one test trial, without the platform, 60 s long. On the test trial the rats were tested in the triangular-shaped pool with no landmark. The amount of time that the rats spent in two different but identically sized areas (i.e. the target area close to the previously correct corner and a control area 180° apart, see Fig. 1, Bottom) was recorded. The reason for measuring the time spent in the control area as well as the target area was to check whether rats could discriminate between the two similar corners of the triangle. Each rat was placed in the pool from one specific position (at positions I and II of Fig. 1, Bottom).

2.4.2. Thigmotaxis

Following Harris et al. (2009) – see also Chamizo et al. (2016), behavioral anxiety was measured in terms of thigmotaxis (i.e. wall hugging, rats' natural proclivity to stay near the perimeters of a novel environment). The proportion of time that a rat spent swimming within 15 cm of the wall of the maze was recorded. Such a measure was used on all escape trials: during pre-training in the circular pool, and throughout all the escape trials and the test trial in the triangular pool.

All measures in the different trials were automatically registered by a computer. An alpha level of 0.05 was adopted for all statistical analyses.

3. Results

3.1. Circular pool and modified morris pool

Fig. 2(A) shows the mean latencies for finding the platform by the two groups over the course of the five pre-training trials in the circular pool. An ANOVA conducted on these data taking into account the variables trials (1–5) and group showed that only the variable trials was significant, $F(4,40) = 6.51$ ($p < 0.001$, $\eta_p^2 = 0.39$). Nothing else was significant [group, $F(1,10) = 1.82$ ($p = 0.207$, $\eta_p^2 = 0.15$) and the interaction trials x group, $F(4,40) = 0.56$ ($p = 0.690$, $\eta_p^2 = 0.05$)]. All animals reduced their latencies as pre-training trials progressed.

Fig. 2(B) shows that the latencies for finding the platform by the two groups decreased over the course of the training days in the modified pool. A repeated measures ANOVA conducted on these data taking into account the variables days (1–5) and group showed that the variables days, $F(4,40) = 18.92$ ($p < 0.001$, $\eta_p^2 = 0.65$), and group, $F(1,10) = 24.78$ ($p = 0.001$, $\eta_p^2 = 0.71$), were significant, as well as the interaction days x group, $F(4,40) = 2.67$ ($p = 0.046$, $\eta_p^2 = 0.21$). The analysis of the interaction revealed that the groups differed on days 2–5, $Fs(1,10) = 8.71$ ($p = 0.015$, $\eta_p^2 = 0.47$), 13.77 ($p = 0.004$, $\eta_p^2 = 0.58$), 7.75 ($p = 0.019$, $\eta_p^2 = 0.44$), and 9.05 ($p = 0.013$, $\eta_p^2 = 0.48$), days 2–5 respectively. Moreover, both groups almost differed on day 1, $F(1,10) = 4.91$ ($p = 0.051$, $\eta_p^2 = 0.33$). EE animals reached the platform faster than Controls. This was also the case on the escape trials of the test day, $F(1,10) = 11.12$ ($p = 0.008$, $\eta_p^2 = 0.53$). EE rats reached the platform faster than Controls.

Fig. 2(C) shows the time spent in the target and control areas by the two groups during the geometry test trial. Student *t* tests were used to compare rats' performance in the target area with the control area. Both groups spent significantly more time in the target than in the control area [minimum $t(5) = 2.94$]. The implication of these results is that all rats had learned about the correct corner. An ANOVA conducted on these data, taking into account the variables area (Target, Control) and group, showed that only the variable area was significant, $F(1,10) = 18.38$ ($p = 0.002$, $\eta_p^2 = 0.65$). However, the variable group was nearly significant, $F(1,10) = 4.59$ ($p = 0.058$, $\eta_p^2 = 0.31$). The Bayes Factor for this comparison is [$pBIC(H0|D) = 0.26$, $BF = 0.36$], which is not conclusive support for the null hypothesis. Therefore, it seems sensible to accept that EE animals spent more time in the target area than Controls on the geometry test trial.

3.2. Thigmotaxis

Fig. 2(D) shows the time spent swimming in the thigmotaxis area for the two groups over the course of the five pre-training trials in the circular pool. A repeated measures ANOVA conducted on these data taking into account the variables trials (1–5) and group showed that only the variable trials was significant, $F(4,40) = 3.73$ ($p = 0.011$, $\eta_p^2 = 0.27$). The time spent swimming in the thigmotaxis area declined with repeated trials in all rats. Nothing else was significant [group, $F(1,10) = 2.23$ ($p = 0.166$, $\eta_p^2 = 0.18$) and the interaction trials x group, $F(4,40) = 0.32$ ($p = 0.865$, $\eta_p^2 = 0.31$)]. All animals reduced their time swimming in the thigmotaxis area as pre-training trials progressed.

Fig. 2(E) shows the time spent swimming in the thigmotaxis area for the two groups during the escape trials of the training phase in the modified pool. A repeated measures ANOVA conducted on these data taking into account the variables days (1–5) and group showed that the variables days, $F(4,40) = 19.51$ ($p < 0.001$, $\eta_p^2 = 0.66$), and group, $F(1,10) = 33.74$ ($p < 0.001$, $\eta_p^2 = 0.77$), were significant, as well as the interaction days x group, $F(4,40) = 4.12$ ($p = 0.007$, $\eta_p^2 = 0.29$). The analysis of the interaction days x group revealed that the groups differed on days 1–4, $Fs(1,10) = 9.09$ ($p = 0.013$, $\eta_p^2 = 0.48$), 7.96 ($p = 0.018$, $\eta_p^2 = 0.44$), 9.58 ($p = 0.011$, $\eta_p^2 = 0.49$), and 9.09 ($p = 0.013$, $\eta_p^2 = 0.48$), days 1–4, respectively. The groups did not differ on day 5, $F(1,10) = 3.13$ ($p = 0.107$, $\eta_p^2 = 0.24$). These results showed that the time spent swimming in the thigmotaxis area declined with repeated trials, being EE rats less thigmotactic than controls. A further ANOVA conducted on the escape trials of the test day revealed that the groups did not differ ($F < 1.0$).

Fig. 2(F) shows the time spent swimming in the thigmotaxis area for the two groups during the test trial. An ANOVA conducted on these data revealed that the visual difference between the groups did not reach statistical significance ($F < 1.0$). The implication of this result is that all rats were equally thigmotactic during the test trial.

It is evident in Fig. 2 that EE animals performed better than control rats, both during training and on the test trial. Moreover, during training EE rats were less thigmotactic than control animals.

4. General discussion

The present results support and expand previous results from Chamizo et al. (2016) suggesting that environmental enrichment alone (i.e., in the absence of voluntary wheel running) can produce beneficial effects at a behavioral level. The implication is that wheel-running does not seem to be the critical variable (as suggested by Mustroph et al.,

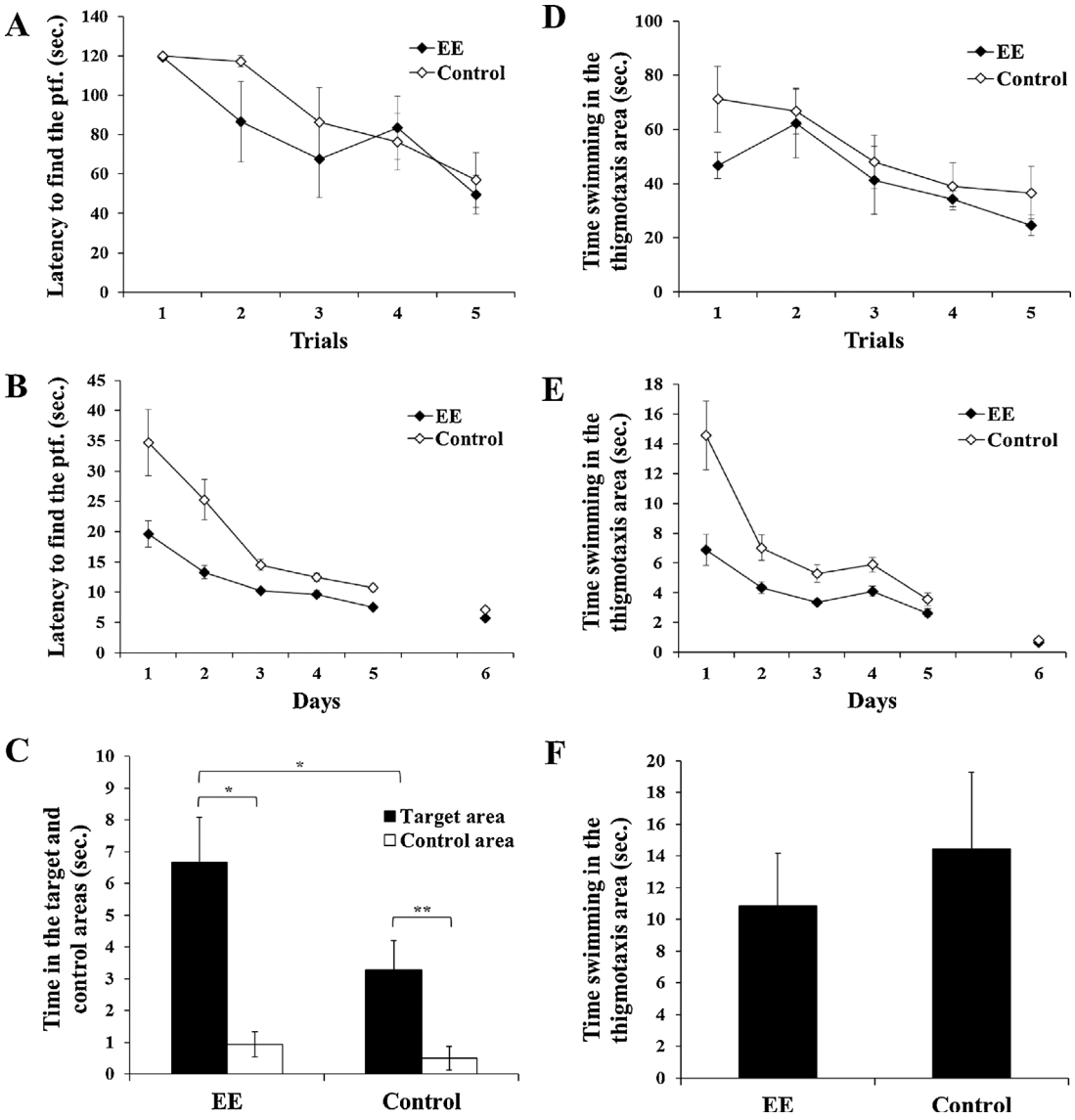

Fig. 2. Behavioural measures for the two groups (Enriched and Control): A, Mean escape latencies during the pretraining trials. B, Mean escape latencies during the training (days 1–5) and test phase (day 6). C, Mean time spent in the two recording areas (geometry and control) during the geometry test trial. D–F, Mean time spent swimming in the thigmotaxis area (i.e., within 15 cm of the wall of the maze): D, during the pretraining trials. E, during the escape trials of the training (days 1–5) and test (day 6) phases. F, during the geometry test trial. (Error bars always denote standard errors of the means. In the test trial, statistical significance of main effects is indicated using an asterisk-based system representing * $p < 0.05$, and ** $p < 0.01$).

2012), at least in this particular protocol, when explaining environmental enrichment. The stimulation produced by the successive experience with the different toys, combined with the reduced thigmotaxis, determined the present results.

In the present study, the results from the five pretraining trials (in the absence of both the landmark and the triangular-shaped pool) revealed that EE and Control rats did not differ in their latencies to find the platform. Considering that the time spent swimming in the thigmotaxis area declined with repeated trials, we conclude that all rats improved their performance as behavioral anxiety (i.e., thigmotaxis) decreased. Therefore, no beneficial effect of early enriched environmental experience was found during pretraining. However on training trials (when the platform was located in one particular corner of the triangular-shaped pool, next to a landmark situated outside the pool), a clear beneficial effect of early stimulation (i.e., environmental enrichment in the absence of voluntary wheel running) was found: EE rats reached the platform faster than Control animals. Considering that EE and Control rats also differed in terms of thigmotaxis during training, it is possible that the superior performance of EE animals reveals the differential behavioral anxiety in the two groups while learning the task, a result initially suggested by Harris et al. (2009) – see also Chamizo et al. (2016).

However the two groups were equally thigmotactic both on the escape trials of the test day and on the geometry test trial. But on the test trial, EE rats performed better than Control animals. Therefore in the geometry test trial, the beneficial effect of the present protocol found in EE rats is difficult to explain by appealing to a differential behavioral anxiety in the two groups (as claimed by Chamizo et al., 2016; see also Harris et al., 2009; Harris et al., 2008). This is an intriguing result possibly due to the small number of animals per group, requiring further research.

This study clearly suggests that the beneficial effect of the present protocol was due to early environmental enrichment in the absence of wheel-running. It encourages to further explore this controversial topic (i.e., the factors that might affect the beneficial effect of environmental enrichment). In conclusion, the present results challenge the notion that the results of the study by Chamizo et al. (2016) could be a consequence of voluntary wheel-running.

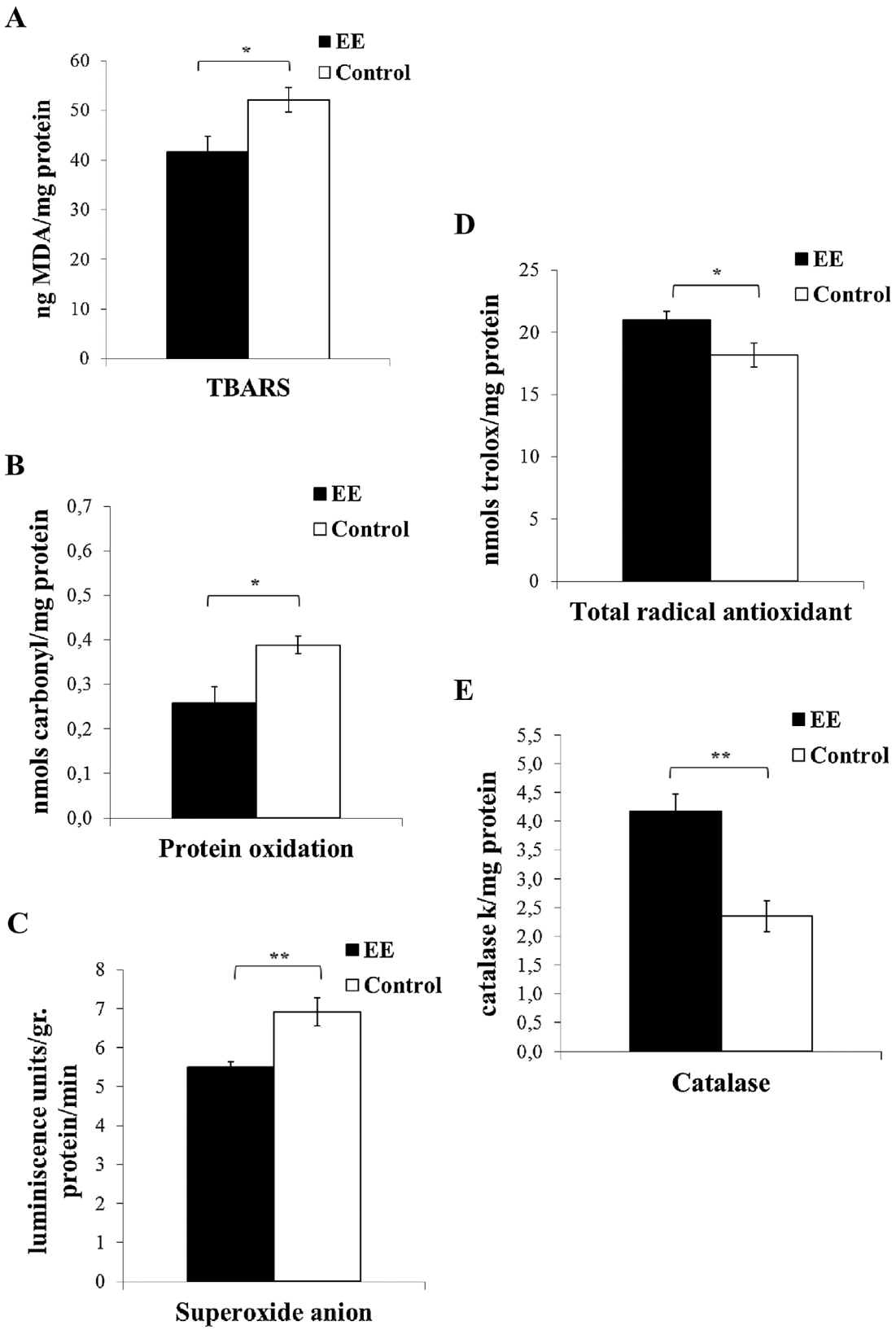

Fig. 3. Measures in the hippocampus for the two groups (Enriched and Control). Prooxidant (left-hand panels): A, TBARS; B, protein oxidation activity; C, superoxide anion activity. Antioxidant (right-hand panels): D, total radical antioxidant parameter; E, catalase. (Statistical significance of main effects is indicated throughout using an asterisk-based system representing * $p < 0.05$, and ** $p < 0.01$).

Acknowledgements

This research was supported a grant from the Spanish 'Ministerio de Ciencia e Innovación' (Ref. PSI2013-47430-P). The authors are very grateful to C. A. Rodríguez for excellent help to run the subjects in the pool. Correspondence should be addressed to V. D. Chamizo, Universitat de Barcelona, Institut de Neurociències, Departament de Cognició, Desenvolupament i Psicologia de l'Educació, Passeig de la Vall d'Hebron 171, 08035-Barcelona, Spain (victoria.diez.chamizo@ub.edu).

Appendix A

Evaluation of antioxidative effects in the hippocampus

Is the hippocampus involved in the absence of wheel-running? After the behavioral measures the animals were sacrificed by decapitation, their brain was removed, and the hippocampus was dissected and frozen in liquid nitrogen (as in Mármol et al., 2015) in order to detect oxidative stress, a neurodegenerative process. We refer to oxidative stress when a disturbance is denoted in the prooxidant/antioxidant balance within the cells in favor of the prooxidants, leading to potential oxidative damage (in other words, when the oxidant activity in a tissue overwhelms its endogenous antioxidative and repair mechanisms). Five biochemical markers have been used in the present study [three of them prooxidant (TBARS, protein oxidation, and superoxide anion), and two antioxidant (TAC – total antioxidant capacity – and catalase)] in order to measure the oxidative stress parameters of EE animals in the absence of wheel running. In EE animals we predicted less oxidative stress as well as higher antioxidant capacity in the hippocampus than in Control rats. Would that be the case?

An ANOVA was conducted on these data taking into account the variables measure (TBARS, protein oxidation activity, superoxide anion activity, total radical antioxidant parameter, and catalase) and group. It revealed that the variable measure was significant, $F(4,40) = 398.74$ ($p < 0.001$, $\eta_p^2 = 0.98$), as well as the interaction measure x group, $F(4,40) = 7.72$ ($p < 0.001$, $\eta_p^2 = 0.44$). The variable group was nearly significant, $F(1,10) = 4.06$ ($p = 0.072$, $\eta_p^2 = 0.29$). The analysis of the interaction showed that group was significant on all five measures, $Fs(1,10) = 7.07$ ($p = 0.024$, $\eta_p^2 = 0.41$), TBARS; 10.02 ($p = 0.010$, $\eta_p^2 = 0.50$), protein oxidation activity; 13.83 ($p = 0.004$, $\eta_p^2 = 0.58$), superoxide anion activity, 5.80 ($p = 0.037$, $\eta_p^2 = 0.37$), total radical antioxidant parameter; and 20.44 ($p = 0.001$, $\eta_p^2 = 0.67$), catalase.

Fig. 3 shows the effects of the different prooxidant measures (A-C) and antioxidant measures (D and E).

The decrease in TBARS levels in group EE indicates that free radicals have been successfully removed by endogenous antioxidants, thus preventing oxidative stress. On the other hand, protein oxidation is a clear consequence of oxidative stress. The fact that in the control group the values were higher than in EE rats demonstrate that the present EE protocol had beneficial antioxidant effects. Superoxide anion is one of the most commonly formed free radicals. The higher levels of its activity in the control group support the claim that the present EE protocol minimizes oxidative damage. Likewise, antioxidant compounds play an important role as a health protective factor. This explains that the hippocampus of EE rats shows higher levels of total radical antioxidant parameters. Finally, the higher levels of catalase observed in EE rats, also indicate that EE animals were more protected from oxidative stress than control rats. In conclusion, these results replicate those by Mármol et al. (2015). Thus the absence of wheel running does not appear to have altered the beneficial effects of the previous EE protocol.

References

Chamizo, V.D., Rodríguez, C.A., Sánchez, J., Mármol, F., 2016. Sex Differences after Environmental Enrichment and Physical Exercise in Rats when Solving a Navigation Task. Learn. Behav. 44, 227–238.

Harris, A.P., D'Eath, R.B., Healy, S.D., 2008. Sex differences, or not, in spatial cognition in albino rats: acute stress is the key. Anim. Behav. 76, 1579–1589.

Harris, A.P., D'Eath, R.B., Healy, S.D., 2009. Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing. Anim. Behav. 77, 1459–1464.

Kobilo, T., Liu, Q.R., Gandhi, K., Mughal, M., Shaham, Y., van Praag, H., 2011. Running is the neurogenic and neurotrophic stimulus in environmental enrichment. Learn. Mem. 18, 605–609.

Mármol, F., Rodríguez, C.A., Sánchez, J., Chamizo, V.D., 2015. Anti-oxidative effects produced by environmental enrichment in hippocampal and cerebral cortex of male and female rats. Brain Res. 1613, 120–129.

Morris, R.G.M., 1981. Spatial localization does not require the presence of local cues. Learn. Motiv. 12, 239–260.

Mustroph, M.L., Chen, S., Desai, S.C., Cay, E.B., Deyoung, E.K., Rhodes, J.S., 2012. Aerobic exercise is the critical variable in an enriched environment that increases neurogenesis and water maze learning in male C57BL/6J mice. Neuroscience 219, 62–71.

Nithianantharajah, J., Hannan, A.J., 2006. Enriched environments: experience-dependent plasticity and disorders of the nervous system. Nat. Rev. 7, 697–709.

Nithianantharajah, J., Hannan, A.J., 2009. The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders. Prog. Neurobiol. 89, 369–382.

Nithianantharajah, J., Barkus, C., Murphy, M., Hannan, A.J., 2008. Gene-environment interactions modulating cognitive function and molecular correlates of synaptic plasticity in Huntington's disease transgenic mice. Neurobiol. Dis. 29, 490–504.

Pang, T.Y.C., Hannan, A.J., 2013. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology 64, 515–528.

Rogers, J., Buret, L.S., Pang, T.Y., Meiklejohn, H., Zeleznikow-Johnston, A., Churilov, L., van den Buuse1, M., Hannan, A.J., Renoir, T., 2016. Dissociating the therapeutic effects of environmental enrichment and exercise in a mouse model of anxiety with cognitive impairment. Transl. Psychiatry 6, 1–12.

Simpson, J., Kelly, J.P., 2011. The impact of environmental enrichment in laboratory rats—behavioural and neurochemical aspects. Behav. Brain Res. 222, 246–264.