Cogn Process (2016) 17:15–25
DOI 10.1007/s10339-015-0746-1

RESEARCH REPORT

An enriched environment and 17-beta estradiol produce similar pro-cognitive effects on ovariectomized rats

A. Ortiz-Pérez[1] · J. Espinosa-Raya[1] · O. Picazo[1]

Received: 30 April 2015 / Accepted: 11 December 2015 / Published online: 12 February 2016

Abstract Estrogen depletion due to aging, surgery or pathological events can cause a multitude of problems, including neurodegenerative alterations. In rodents without ovaries, 17-beta estradiol (E2) has been shown to produce beneficial effects on cognition, stimulating brain regions (e.g., the neocortex, hippocampus and amygdala) related to cognition and learning. Another treatment that stimulates these brain regions is an enriched environment (EE), which is a complex set of external factors in the immediate surroundings that facilitates greater stimulation of sensorial, cognitive and motor circuits of the brain. The aim of the present study was to test, using an animal model of ovariectomy-induced impairment of memory, the relative effect of E2 (with a time-released pellet; 1 μg/rat/day), EE exposure and a combination of both treatments. Experimental and control groups were submitted to two memory tests 18 weeks post-surgery: the autoshaping learning task (ALT) for measuring associative learning and the novel object recognition test (NORT) for evaluating short- and long-term memory. To assess potential motor impairments caused by treatments, all rats were tested after the ALT in an automatic activity counter. Results from ALT show that the ovariectomy blocked the conditioned responses displayed, an effect rescued by chronic treatment with estrogen or EE exposure. The combination of both treatments did not improve the results obtained separately. In the NORT, the exploration time for recognizing a novel object was similar in the short run with all groups, but greater in the long run with hormone administration or EE exposure. As with the ALT, in the NORT there was no improvement shown by the combination treatment. These data were not masked by changes in spontaneous activity because this parameter was not modified in the rats by either treatment. Possible action mechanisms are proposed, taking into account the role of corticosterone and BDNF on cognition.

Keywords Estradiol · Cognition · Learning · Enriched environment · Ovariectomy

Introduction

There is evidence that various neurodegenerative alterations are more frequent in hypoestrogenic women (Gibbs 1998; Henderson 2008). According to some authors, estrogen depletion due to aging, surgical or pathological events is partially responsible for the cognitive decline associated with neurodegenerative disorders such as Alzheimer's disease (Craig and Murphy 2010; Zhao and Brinton 2006) and other forms of dementia (Vegeto et al. 2008). Estrogens, besides regulating endocrine processes, stimulate brain regions related to cognitive and learning integration, such as the neocortex, hippocampus and amygdala (Paris and Frye 2008). In fact, hormonal replacement therapy has been proven to be capable of attenuating some symptoms of Alzheimer's (Gibbs and Aggarwal 1998; Ryan et al. 2008; Wu et al. 2008).

Handling Editor: Elsa Addessi, ISTC-CNR, Rome, Italy.

Reviewers: Susanna Pietropaolo, University of Bordeaux 1, France, and Robert Gibbs, University of Pittsburgh, USA.

✉ O. Picazo
rifo99mx@gmail.com

[1] Graduate and Research Section, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Col. Sto. Tomás, 11340 Mexico, DF, Mexico

Since menopause is defined as the permanent cessation of menstruation resulting from the surgical or natural loss of ovarian follicular activity (Utian 2004), the removal of ovaries (OVX) in rats has been proposed, with restrictions, as a model for the study of some disorders associated with human postmenopause (Bosse and Di Paolo 1995). Rats under this hormonal condition have shown high levels of anxiety and depression (Estrada-Camarena et al. 2011; Picazo et al. 2006; Rodríguez-Landa et al. 2009), alterations in body temperature (Li and Satinoff, 1996), bone loss (Gurkan et al. 1986; Kalu 1991), a low threshold of pain (Kobayashi et al. 2000; Okada et al. 1997) and a deficit in memory function detectable with an autoshaping learning task (ALT) 18 weeks after surgery (Espinosa-Raya et al. 2011). Regarding the latter, replacement therapy with E2 or the synthetic steroid tibolone (Espinosa-Raya et al. 2011, 2012), initiated immediately after OVX, seems to overcome this cognitive impairment. Contrarily, when the hormonal treatment is initiated a relatively long time after the removal of ovaries or cessation of their activity, such protective actions are not observed (Daniel et al. 2006; Frick 2009; Gibbs 2010; Maki 2006). This is referred to as the critical period hypothesis (Resnick and Henderson 2002; Zhang et al. 2011) and is currently under discussion.

Another treatment that has reportedly produced improved cognitive abilities is an enriched environment (EE), a complex set of external factors in the immediate surroundings (Nithianantharajah and Hannan 2006). Different schedules of EE induce an improvement in learning and memory through the development of new nerve cells in the hippocampus and enhanced dendritic growth (Bruel-Jungerman et al. 2005; Diamond 2001; Kempermann et al. 1997; Leggio et al. 2005; Saito et al. 1994). Compared to standard conditions, EE facilitates a greater stimulation of sensorial, cognitive and motor circuits of the brain. For instance, non-spatial (Sun et al. 2010) and spatial memory impairment produced by cerebral ischemia or scopolamine can be restored when rats are exposed to a complex environment (Dahlqvist et al. 2004; Lima et al. 2014; Sun et al. 2010; Wadowska et al. 2014).

These findings have been confirmed by clinical studies showing that EE increases the dendrite branching complexity (Jacobs et al. 1993), reduces the risk of Alzheimer's disease (Wilson et al. 2002) and protects against hippocampal lesion atrophy in the chronic stages of traumatic brain injury (Miller et al. 2013; White et al. 2013). In the same sense, it has been shown that for aging humans, aerobic exercise training increases brain volume (Colcombe et al. 2006), reduces brain tissue loss (Colcombe et al. 2003) and improves cognitive function (Colcombe and Kramer 2003).

Even though the majority of studies have reported positive results with EE, some studies have found contradictory data. This could be explained by differences in the schedule of EE exposure, aging and gender of the animals, housing conditions, and so on (Gresack et al. 2007; Lambert et al. 2005). For instance, compared to control animals housed under standard conditions, a few hours of EE exposure improves spatial working memory but not spatial reference memory of female mice (Redolat and Mesa-Gresa 2012), while a 4-week EE exposure of aged, but not young, OVX mice improves object recognition memory (Gresack et al. 2007). These discrepancies about the estrogens–EE interaction are difficult to interpret due to the methodological variables involved, but also because this issue has not been extensively explored so far. To our knowledge, this is the first report using a noninvasive method of hormonal administration for studying the role of E2 and EE on cognition.

Since a clear cognitive deficit in both tests used here has previously been shown following ovariectomy (Bastos et al. 2015; Fonseca et al. 2013; Espinosa-Raya et al. 2012), here only OVX females were used, sham controls being not necessary to demonstrate the impaired performance induced by estrogen depletion. Thus, taking into account the advantages represented by noninvasive procedures and considering the critical period idea, the current study explored whether or not an 18-week protocol of EE alone or in combination with E2 treatment improved this ovariectomy-induced impairment of memory.

Rats with E2 treatment initiated immediately after OVX were exposed to an EE following a modified protocol reported by Leal-Galicia et al. (2008) and then submitted to two memory tests: the ALT and the novel object recognition test (NORT). ALT has been proposed for measuring associative learning (Meneses 2003) and requires an intact neuronal system in the hippocampus, septum and cortex, as well as the participation of the cholinergic signaling transduction pathways in the integration of the acquisition–consolidation process (Steckler et al. 1993; Meneses 1999; Meneses and Terrón 2001; Espinosa-Raya et al. 2007, 2011). On the other hand, NORT has been used for the study of short- and long-term memory (Taglialatela et al. 2009) and requires participation of the perirhinal cortex for acquisition, consolidation and retrieval of object information. Accordingly, glutamatergic, serotoninergic and cholinergic neurotransmission is necessary for the integration of these processes (Dere et al. 2007). Finally, to discard that changes in motor activity could influence performance on these behavioral tasks, all rats were evaluated with an automatic activity counter immediately after the ALT test.

Materials and methods

Animals

The Animal Care and Use Committee of the Escuela Superior de Medicina at the Instituto Politécnico Nacional approved all experimental protocols. Forty female Sprague–Dawley rats (200–250 g) were purchased from Harlan Laboratories (Harlan Mexico, S.A. de C.V.). Before beginning the treatments, the animals were housed in groups ($n = 10$) in polycarbonate cages for 2 weeks under an inverted 12/12-h light–dark cycle (lights on at 9 p.m.) in a temperature-controlled (22 °C) room. All animals had free access to food (Purina Rat Chow) and water throughout the experiments. Animal care and handling were in accordance with internationally accepted procedures and approved by our Institutional Committee (NOM-062 ZOO, 1999). Special care was taken to minimize animal suffering. All experiments were carried out between 11:00 and 15:00, and independent groups were used.

Environmental enrichment

This procedure was carried out following a modified version of the conditions proposed by Leal-Galicia et al. (2007). It has been reported that exposure to this EE schedule elicits an increase in the hippocampal neurogenesis, modifies rat emotionality and improves the recognition memory in old rats (Leal-Galicia et al. 2007, 2008). Briefly, this apparatus consists of an exploration chamber (1.5 × 1.5 m; illuminated by red light) containing different elements such as plastic balls, tunnels, nesting materials, mesh wire ladders and running wheels. For EE exposure, rats in the EE and E2 + EE groups (kept under standard housing conditions) were placed in this chamber 2 h daily (for 2 weeks). To avoid behavioral habituation, for the remaining of the 18-week treatment period exposure was carried out only on Saturdays for 3 h. All moveable objects used for enrichment were placed in a different arrangement inside of the exploration chamber before each session.

Treatments

At the time of surgery, rats were randomly assigned to one of the following groups ($n = 10$ each): (a) a control group not treated with E2 nor exposed to enrichment (CONT); (b) a group chronically treated with E2 but not exposed to enrichment (E2); (c) a group exposed to enrichment but not treated with E2 (EE); and (d) a group treated with E2 and exposed to enrichment (E2 + EE).

Animals in the E2 and E2 + EE groups were subcutaneously implanted with a pellet that time-releases E2 for 60 days. This pellet, containing 0.05 mg of E2 (1 µg/rat/day) (Innovative Res. America, FL, USA), was placed at the dorsal portion of the neck during the ovariectomy. In order to complete 18 weeks of treatment, the pellet was replaced 9 weeks after implantation. It has been continuously confirmed that these pellets supply physiological hormonal levels beginning 2 weeks after their insertion (Singh et al. 2008; Strom et al. 2008a, b; Nordell et al. 2005; Aubele and Kritzer 2012). In order to eliminate variations due to daily handling, animals in the control group were manipulated in a similar manner as those in the experimental groups.

Surgery

Animals were bilaterally ovariectomized (OVX) under 2,2,2-tribromoethanol anesthesia (0.2 g/kg, i.p.), by a well-trained technician, through a dorsal incision that allows for the location and removal of the ovaries (Picazo et al. 2006) and whose complete extraction was corroborated by visual inspection. Immediately after surgery, rats were placed for approximately 2 h in a harm platform until their complete recovery. Rats were then gently transported and housed in plastic cages and randomly assigned to the different treatments.

ALT

The ALT is used to measure associative learning (Meneses 2003). Briefly, it consists of a standard Skinner box (Med Associates Inc., USA) with an illuminated lever in the middle of one wall (4 cm above the floor) and a food tray located 5 cm to the right of the lever. Animals submitted to this test are previously fasted for 24–36 h. On the training day, each rat is placed in the experimental chamber and given time to acclimate to it (∼15 min). During this time, animals find and eat 20 food pellets (45 mg Mach) that are in the tray. Afterward, the rat can obtain another pellet each time the illuminated lever is pressed. An inter-trial interval of 5 s is used as the standard. Thus, an increase or decrease in the number of conditioned responses (CRs) is considered an enhancement or impairment in the consolidation of learning, respectively. The protocol consists of three sessions, one every 24 h, with the first and second sessions comprising 20 trials and the third session only 10. As it is usual practice for this paradigm, data are expressed as the mean number of CRs (i.e., times the lever is pressed) during the last session (Espinosa-Raya et al. 2011).

Spontaneous activity test

To assess the potential effects of the drugs on motor activity, all rats were tested in an automatic activity counter, which consists of an acrylic cage measuring

51.1 × 9.5 × 69.2 cm with two arrays of 15 infrared beams (each spaced 2.5 cm apart) that are perpendicular to each other. The interruption of a beam generates an electric impulse, which is processed and presented as a count (Opto-Varimex; Columbus Instruments, OH, USA). This test was carried out immediately after the third session in the ALT. Ambulation, vertical activity (climbing) and total activity are automatically registered during a 5-min session.

NORT

The NORT is used to study short- and long-term memory. This test, described by Ennaceur and Delacour (1988), is based on the natural tendency of rodents to explore new objects and compare them to ones with which they are already familiar. Thus, when a rat is exposed to a new object, it is expected that the animal will take longer exploring it than when exposed to a familiar one.

Briefly, the task consists of an open-field arena (30 × 30 × 15 cm) with two identical objects ($F + F'$) located in opposite and symmetrical corners. During the first phase (acquisition), the rat was placed in the arena for 5 min. To avoid forcing the animal to explore some specific object, it was always placed in the center of the arena. During this phase, animals that did not explore the objects for at least 30 s were not included in the current study. At the end of the session, the rat was returned to its housing cage.

Given that this task is useful for the study of memory at different time points (Taglialatela et al. 2009), animals were tested again 1 h (short-term memory) and 24 h (long-term memory) after the acquisition phase. Before the latter two sessions, one of the familiar objects (F) was replaced by a different one (N). All sessions were videotaped for later scoring by a single observer, who was blind to the treatment conditions.

In this test, data are frequently presented as the preference index (PI), which expresses the difference between the time spent with the familiar object and the novel one [time spent with novel object/(time spent with novel object + time spent with familiar object) × 100] (Wang et al. 2007). A PI above 50 % (i.e., the chance level) indicates a novel object preference and can be interpreted as normal cognitive performance (Navarrete et al. 2008; Walf et al. 2006).

Statistics

Data were analyzed with a one-way ANOVA or with a Kruskal–Wallis analysis (depending on whether populations were normally distributed or not) followed by the corresponding post hoc comparisons. Other statistical analyses were performed using paired t tests. A p value <0.05 was considered significant.

Results

ALT

Hormonal treatment and/or exposure to EE restored cognitive performance in the ALT of OVX rats, compared to the control group. Overall, there was a significant difference across the four groups tested ($H = 15.885$; $n = 40$; $p = 0.001$). Mann–Whitney U tests showed that groups exposed to E2 and EE treatments performed significantly better than the control group (E2 vs C: $U = 7.50$, $n1 = 10$, $n2 = 10$, $p = 0.001$; EE vs C: $U = 24.0$, $n1 = 10$, $n2 = 10$, $p = 0.047$; EE + E2 vs C: $U = 11.0$, $n1 = 10$, $n2 = 10$, $p = 0.003$; Fig. 1). The combination of both treatments did not improve the number of CRs obtained under EE treatment (EE + E2 vs EE: $U = 45.0$, $n1 = 10$, $n2 = 10$, $p = 0.733$). Contrarily, such combination decreased the number of CRs produced only by E2 administration (EE + E2 vs E2: $U = 18.0$, $n1 = 10$, $n2 = 10$, $p = 0.017$).

Spontaneous activity test

As aforementioned, all rats were evaluated with an automatic activity counter immediately after the ALT. It is clear from this evaluation that no treatment altered the motor ability of the animals (Table 1).

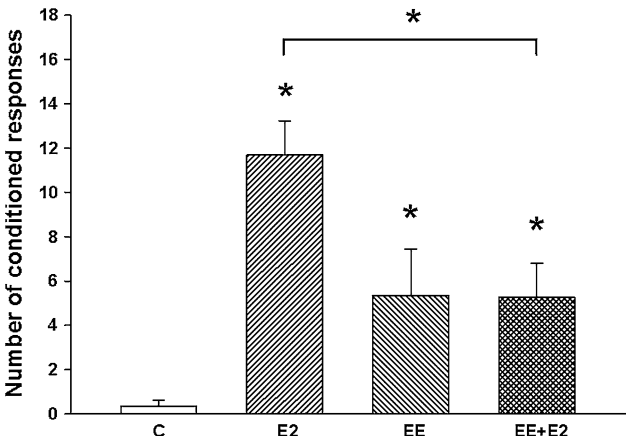

Fig. 1 Number of conditioned responses, in an autoshaping learning task, for rats chronically treated with estradiol (E2), exposed to an enriched environment (EE) for 18 weeks or given a combination of both treatments (EE + E2). *Each column* represents the mean ± SE of ten rats. The *asterisk* denotes a difference versus the control group (*C*) or between those groups indicated by the bracket (Mann–Whitney U test; $p < 0.05$)

Table 1 Spontaneous activity of ovariectomized rats under chronic estrogen treatment (E2) and exposed to an enriched environment (EE)

Experimental groups	Climbing	Ambulation	Total activity
W	270.2 (30.7)	902.7 (80.8)	1084.4 (78.7)
E2	282.2 (26.8)	851.0 (64.8)	1072.5 (64.0)
EE	290.2 (28.7)	903.3 (69.3)	1041.8 (51.6)
EE + E2	320 (24.3)	980.9 (62.8)	1153.6 (67.4)
Kruskal–Wallis	$H = 2.231$; $p = 0.526$	$H = 1.615$; $p = 0.656$	$H = 1.603$; $p = 0.659$

Data are expressed as the mean of counts ± SE ($n = 10$) for 5 min. W—control group without estradiol implanting and without exposition to EE

NORT

All rats in the present study spent a similar amount of time on object exploration during the acquisition phase in the NORT, where F and F' represent identical objects (Fig. 2, upper panel), as reflected by a lack of significant difference in the PI across groups (lower panel; one-way ANOVA; $F = 0.249$; $p = 0.861$).

Although the test carried out 1 h after the acquisition phase showed that rats belonging to each group spent more time exploring the novel object (N) compared to the familiar one (F) (Fig. 3, upper panel; paired t tests; all $ps < 0.05$), the PI did not significantly differ across groups (Fig. 3, lower panel; one-way ANOVA, $F = 1.115$, $p = 0.356$).

In the test carried out 24 h after the acquisition phase, rats belonging to all the experimental groups, but not to the control group, spent more time exploring the novel object than the familiar object (Fig. 4, upper panel; paired t tests, all $ps < 0.05$). Moreover, the PI significantly differed across groups (Fig. 4, lower panel; Kruskal–Wallis; $H = 17.039$; $n = 40$; $p = 0.001$); all experimental groups significantly differed compared to the control group (Tukey's test, all $ps < 0.01$), but did not significantly differ between each other.

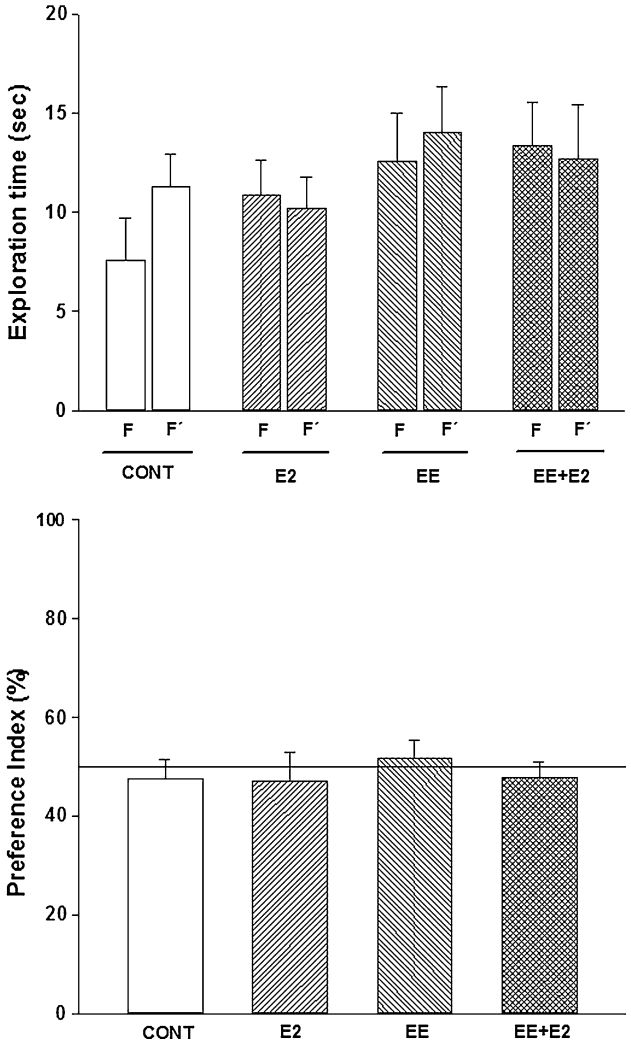

Fig. 2 Behavior of rats chronically treated with estradiol (E2), exposed to an enriched environment (EE) for 18 weeks or given a combination of both treatments (EE + E2) during the acquisition phase in the novel object recognition test. The *upper panel* shows the amount of exploration time spent by rats during a 5-min period, while the corresponding preference index is depicted *below*. In this figure, F and F' denote identical (familiar) objects

Discussion

Data from the current study can be summarized as follows: (a) The impairment of memory, putatively caused by removal of the ovaries, could be overcome by with estrogens or EE; (b) hormonal treatment was better than the combination EE + E2 for improving the performance of rats in the ALT; (c) the combination of these two treatments showed different results depending on the test used for measuring cognition.

As aforementioned, an estrogen deficiency has profound effects on cognition. Accordingly, deterioration of the natural ovarian function or removal of the ovaries has been associated with cognitive deficits (Markowska and Savonenko 2002; Walf et al. 2006). For instance, several studies have reported that during induced or natural proestrus/estrus, rodents perform better on learning tasks than during diestrus or than counterparts without ovaries (Paris and

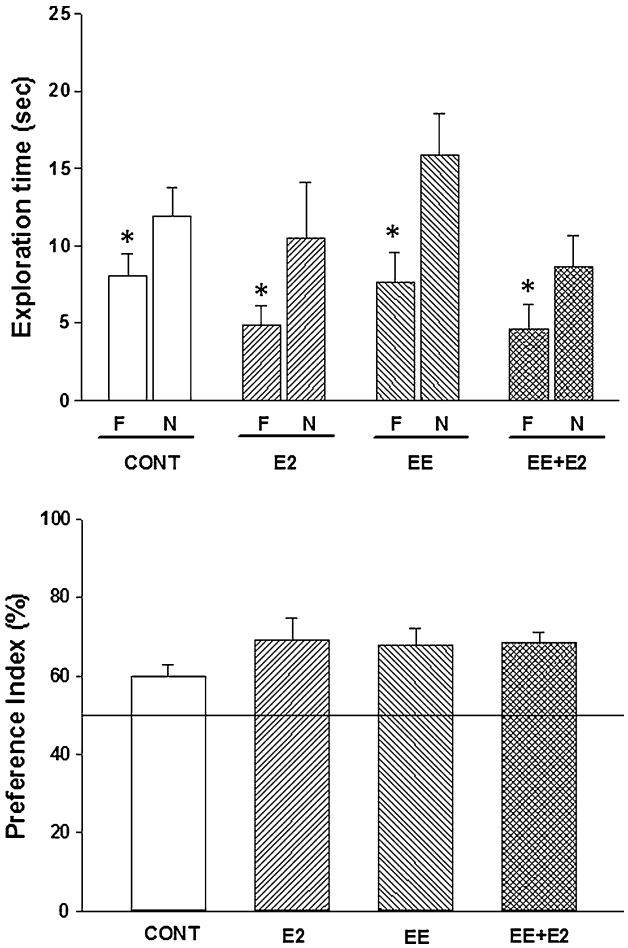

Fig. 3 Exploration time and preference index for rats chronically treated with estradiol (E2), exposed to an enriched environment (EE) for 18 weeks or given a combination of both treatments (EE + E2) 1 h after the acquisition phase in the novel object recognition test. The familiar object is represented by *F* and the novel one by *N*. The *asterisk* denotes the significant difference between the time spent by the rats exploring the novel and the familiar object (paired *t* test; $p < 0.05$)

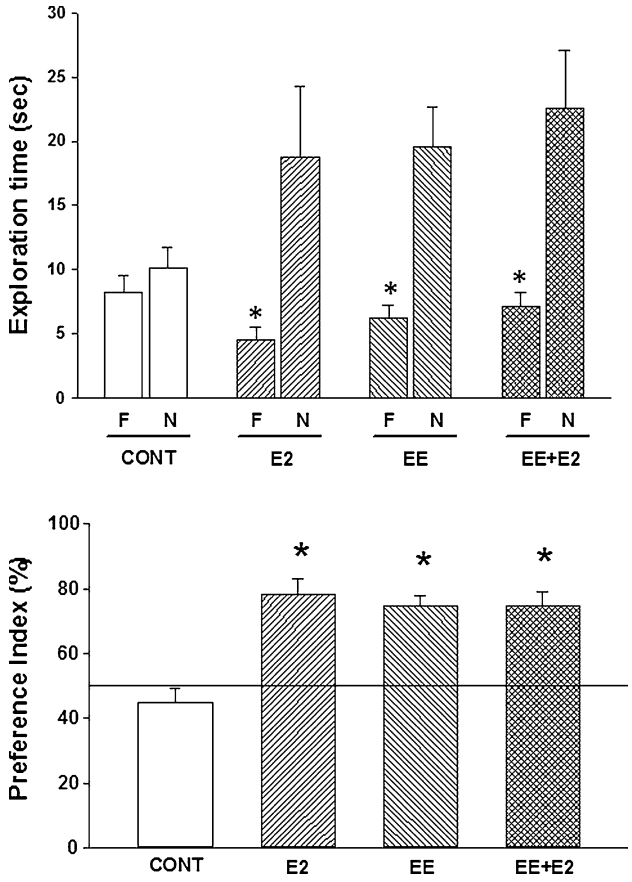

Fig. 4 Exploration time and preference index for rats chronically treated with estradiol (E2), exposed to an enriched environment (EE) for 18 weeks or given a combination of both treatments (EE + E2) 24 h after the acquisition phase in the novel object recognition test. The familiar object is represented by *F* and the novel one by *N*. *Each column* represents the mean ± SE of ten rats. The *asterisk* denotes a significant difference between the time spent by rats exploring the novel and the familiar object (*upper panel*, paired *t* test; $p < 0.05$) and between the control group and each experimental group (*lower panel*, Tukey's test; $p < 0.05$). Paired comparisons among experimental groups did not show a significant difference (Mann–Whitney *U* test; $p > 0.05$)

Frye 2008; Van Goethem et al. 2012). The results of the current contribution are in accordance with this evidence. Adult OVX rats evaluated 18 weeks post-surgery showed virtually no response in the ALT, whereas sham-operated females displayed approximately 6–10 CRs/min (Espinosa-Raya et al. 2012). This cognitive deficit due to OVX can be restored by chronic estrogenic treatment initiated post-surgery (Gresack and Frick 2004; Markowska and Savonenko 2002) as long as the beginning of treatment is within 10 weeks post-OVX (McLaughlin et al. 2008). This phenomenon has been observed in several tests including ALT and NORT. In the present study, the NORT showed that E2 treatment reinstated the object recognition memory of castrated females, reaching levels of performance similar to those reported for sexually receptive rats (Fernandez and Frick 2004; Luine et al. 2003; Walf et al. 2006). This evidence is in line with the critical period hypothesis, which considers that estrogen treatment confers optimal performance benefits for women when initiated close in time to the onset of menopause (Resnick and Henderson 2002).

Interestingly, the ALT showed that OVX rats with E2 administration plus EE exposure experienced less cognitive benefits than animals given E2 alone. Contrarily, the NORT demonstrated that OVX rats in these two groups—the combined treatment and the E2 treatment alone—performed in a similar fashion. In this regard, Gresack and Frick (2004) reported that with the NORT, OVX mice administered with E2, but without EE exposure, had a significantly enhanced memory, while the simultaneous application of both treatments led to reduced cognitive

ability, an effect only observed in young but not aged mice (Gresack et al. 2007).

There were obvious methodological differences between the present study and that by Gresack and Frick (2004), including the use of mice instead of rats, acute daily injections of high doses of E2 and a delay in post-OVX hormonal replacement. In such study, the reduced cognitive ability found with the NORT in regard to the combined EE + E2 versus E2 treatment seems to indicate a role of stress factors. Regarding this, we took into account the critical period hypothesis and began the E2 release immediately after the OVX through hormone-releasing pellets instead of injecting rats for 15 days, diminishing in this way the role of stress produced by injections that could have masked the observations of Gresack and Frick (2004).

Some of the main factors that alter the effect of enrichment are the exposure schedule, the size of the enrichment boxes and the number of animals in the housing cages (Diamond 2001; Simpson and Kelly 2011), showing that this manipulation does not always produce the same consequences. For instance, although the majority of studies report a beneficial effect of EE (Costa et al. 2007; Freret et al. 2012; Frick and Fernandez 2003; Hu et al. 2010; Jankowsky et al. 2005; Laviola et al. 2008), some housing conditions can produce stress and as a consequence result in high levels of corticosterone (CORT) (Girbovan and Plamondon 2013). For instance, in intact female rats or mice exposed to EE for 6 weeks, higher levels of plasmatic CORT are found in animals housed four per cage than in those housed individually (Arndt et al. 2009; Konkle et al. 2010; Martin and Brown 2010). It is currently known that this hormone improves performance when found at optimal levels (Gresack and Frick 2004; Sampedro-Piquero et al. 2014), essentially during the consolidation memory phase (Roozendaal 2000), but produces amnesia at high concentrations (Meaney et al. 1988; Schwabe et al. 2012).

Surprisingly, E2 also increases the levels of CORT (Chan et al. 2014; Farid et al. 2013), suggesting that with either E2 administration or EE exposure alone, the mnemonic benefits could be due to the level of CORT produced by each treatment. However, with combined treatment there would seem to be an additive effect of the CORT secretion induced by each individual treatment, which if true would explain the reduction in the CRs produced by this combination. Thus, it is possible that the combined hormone and enrichment treatment result in high levels of CORT and therefore a mnemonic deficit (Schwabe et al. 2012).

In contrast to data derived from the ALT, results from the NORT evidenced the effectiveness of E2, EE and E2 + EE, each producing very similar effects on object recognitive function in the long but not short run. The reason for this finding could lie in the fact that the ALT and NORT stimulate different brain regions: the first excites the amygdala–NAcc–hippocampus pathway (Correa 2007), while the NORT seems to affect hippocampal cortices (Antunes and Biala 2012), specifically the perirhinal cortex (Winters and Bussey 2005).

It is also known that E2 administration and EE exposure, besides producing changes in CORT, increase the expression of the brain-derived neurotrophic factor (BDNF). The role of BDNF in cognition has been well demonstrated (Ozawa et al. 2014), and its increase occurs, under different enrichment protocols, in central amygdala, hippocampus, and/or visual and entorhinal cortices (Farmer et al. 2004; Ickes et al. 2000; Pham et al. 1999; Ramírez-Rodríguez et al. 2014; Ravenelle et al. 2014; Rossi et al. 2006). The increase in this neurotrophin is prevented by an OVX (Berchtold et al. 2001) and can be restored by E2 treatment (Berchtold et al. 2001; Gibbs 1999). When E2 is combined with exercise, as part of EE, the level of BDNF is above the value reached by hormone replacement alone (Berchtold et al. 2001). This evidence suggests that the memory consolidation observed at 24 h, but not at 1 h, after the acquisition phase in the NORT could be due to the BDNF expression induced by either E2 or EE treatment. Finally, although both types of memory, short- and long-term memory, are integrated in hippocampus (Hammond et al. 2004), it is important to address that short-term memory does not require protein synthesis (Balderas et al. 2014; Rossato et al. 2007), an observation that could help to understand the current data.

To sum up, a general hypothesis to explain the present results should include the role of CORT and BDNF as modulators of the learning and memory displayed in the two tests herein used. It should also take into account that recognition memory is integrated in extrahipocampal cortices (Antunes and Biala 2012; Balderas et al. 2008; Spada et al. 2006; Winters and Bussey 2005), where the corticoid receptors are scarce in comparison with the hippocampus (Sánchez et al. 2000) and that autoshaping learning depends on the amygdala–NAcc–hippocampus pathway (Correa 2007), where there is a high density of CORT receptors (Reul and De Kloet 1986; Roozendaal and McGaugh 1997) modulating the actions of CORT on the associative learning (Beylin and Shors 2003; Roozendaal 2000).

Conclusions

The current results show that chronic treatment with estrogen or EE exposure has a beneficial effect on two types of memory. Regarding the effect on associative learning, herein measured with the ALT, E2 treatment was

found to be better than certain schedules of EE. Finally, the combination of both treatments did not produce perceptible advantages.

Acknowledgments The authors thank Nielsine Nielsen for reviewing the use of English in the manuscript. This study was partially supported by COFAA and SIP-IPN.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Antunes M, Biala G (2012) The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 13:93–110

Arndt SS, Laarakker MC, Van Lith HA, Van der Staay FJ, Gieling E, Salomon AR, Van't Klooster J, Ohl F (2009) Individual housing of mice—impact on behaviour and stress responses. Physiol Behav 97:385–393

Aubele T, Kritzer MF (2012) Androgen influence on prefrontal dopamine systems in adult male rats: localization of cognate intracellular receptors in medial prefrontal projections to the ventral tegmental area and effects of gonadectomy and hormone replacement on glutamate-stimulated extracellular dopamine level. Cereb Cortex 22:1799–1812

Balderas I, Rodriguez-Ortiz CJ, Salgado-Tonda P, Chavez-Hurtado J, McGaugh JL, Bermudez-Rattoni F (2008) The consolidation of object and context recognition memory involve different regions of the temporal lobe. Learn Mem 15:618–624

Balderas I, Rodriguez-Ortiz CJ, Bermudez-Rattoni F (2014) Consolidation and reconsolidation of object recognition memory. Behav Brain Res. doi:10.1016/j.bbr.2014.08.049

Bastos CP, Pereira LM, Ferreira-Vieira TH, Drumond LE, Massensini AR, Moraes MF, Pereira GS (2015) Object recognition memory deficit and depressive-like behavior caused by chronic ovariectomy can be transitorialy recovered by the acute activation of hippocampal estrogen receptors. Psychoneuroendocrinology 57:14–25

Berchtold NC, Kesslak JP, Pike CJ, Adlard PA, Cotman CW (2001) Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. Eur J Neurosci 14:1992–2002

Beylin AV, Shors TJ (2003) Glucocorticoids are necessary for enhancing the acquisition of associative memories after acute stressful experience. Horm Behav 43:124–131

Bosse R, Di Paolo T (1995) Dopamine and GABAA receptor imbalance after ovariectomy in rats: model of menopause. J Psychiatry Neurosci 20:364–371

Bruel-Jungerman E, Laroche S, Rampon C (2005) New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur J Neurosci 21:513–521

Chan M, Chow C, Hamson DK, Lieblich SE, Galea LA (2014) Effects of chronic oestradiol, progesterone and medroxyprogesterone acetate on hippocampal neurogenesis and adrenal mass in adult female rats. J Neuroendocrinol 26:386–399

Colcombe S, Kramer AF (2003) Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol Sci 14:125–130

Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, Kramer AF (2003) Aerobic fitness reduces brain tissue loss in aging humans. J Gerontol A Biol Sci Med Sci 58A:176–180

Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, Elavsky S, Marquez DX, Hu L, Kramer AF (2006) Aerobic exercise training increases brain volume in aging humans. J Gerontol A Biol Sci Med Sci 61:1166–1170

Correa M (2007) Neuroanatomía funcional de los aprendizajes implícitos: asociativos, motores y de hábito. Rev Neurol 44:234–242

Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H (2007) Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. Neurobiol Aging 28:831–844

Craig MC, Murphy DG (2010) Estrogen therapy and Alzheimer's dementia. Ann NY Acad Sci 1205:245–253

Dahlqvist P, Rönnbäck A, Bergström S-A, Söderström I, Olsson T (2004) Environment enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. Eur J Neurosci 19:2288–2298

Daniel JM, Hulst JL, Berbling JL (2006) Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 147:607–614

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31:673–704

Diamond MC (2001) Response of the brain to enrichment. An Acad Bras Cienc 73:211–220

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats 1: behavioral data. Behav Brain Res 31:47–59

Espinosa-Raya J, Espinoza-Fonseca M, Picazo O, Trujillo-Ferrara J (2007) Effect of a M1 allosteric modulator on scopolamine-induced amnesia. Med Chem 3:7–11

Espinosa-Raya J, Plata-Cruz N, Neri-Gómez T, Camacho-Arroyo I, Picazo O (2011) Effects of short-term hormonal replacement on learning and on basal forebrain ChAT and TrkA content in ovariectomized rats. Brain Res 1375:77–84

Espinosa-Raya J, Neri-Gómez T, Orozco-Suárez S, Campos MG, Guerra-Araiza C (2012) Chronic administration of tibolone modulates anxiety-like behavior and enhances cognitive performance in ovariectomized rats. Horm Behav 61:76–83

Estrada-Camarena E, López-Rubalcava C, Hernández-Aragón A, Mejía-Mauries S, Picazo O (2011) Long-term ovariectomy modulates the antidepressant-like action of estrogens, but not of antidepressants. J Psychopharmacol 25:1365–1377

Farid S, Hussain MM, Asad M (2013) Adrenocortical response to 17-beta estradiol replacement in oophorectomized female sprague dawley rats. J Coll Physicians Surg Pak 23:695–698

Farmer J, Zhao X, Van Praag H, Wodtke K, Gage FH, Christie BR (2004) Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague–Dawley rats in vivo. Neuroscience 124:71–79

Fernandez SM, Frick KM (2004) Chronic oral estrogen affects memory and neurochemistry in middle-aged female mice. Behav Neurosci 118:1340–1351

Fonseca CS, Gusmão ID, Raslan AC, Monteiro BM, Massensini AR, Moraes MF, Pereira GS (2013) Object recognition memory and temporal lobe activation after delayed estrogen replacement therapy. Neurobiol Learn Mem 101:19–25

Freret T, Billard JM, Schumann-Bard P, Dutar P, Dauphin F, Boulouard M, Bouet V (2012) Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan. Neurobiol Aging 33:1005e1–1005e10

Frick KM (2009) Estrogens and age-related memory decline in rodents: what have we learned and where do we go from here? Horm Behav 55:2–23

Frick KM, Fernandez SM (2003) Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. Neurobiol Aging 24:615–626

Gibbs RB (1998) Impairment of basal forebrain cholinergic neurons associated with aging and long-term loss of ovarian function. Exp Neurol 151:289–302

Gibbs RB (1999) Treatment with estrogen and progesterone affects relative levels of brain-derived neurotrophic factor mRNA and protein in different regions of the adult rat brain. Brain Res 844:20–27

Gibbs RB (2010) Estrogen therapy and cognition: a review of the cholinergic hypothesis. Endocr Rev 31:224–253

Gibbs RB, Aggarwal P (1998) Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer's disease-related cognitive decline. Horm Behav 34:98–111

Girbovan C, Plamondon H (2013) Environmental enrichment in female rodents: considerations in the effects on behavior and biochemical markers. Behav Brain Res 253:178–190

Gresack JE, Frick KM (2004) Environmental enrichment reduces the mnemonic and neural benefits of estrogen. Neuroscience 128:459–471

Gresack JE, Kerr KM, Frick KM (2007) Life-long environmental enrichment differentially affects the mnemonic response to estrogen in young, middle-aged, and aged female mice. Neurobiol Learn Mem 88:393–408

Gurkan L, Ekeland A, Gautvik KM, Langeland N, Rønningen H, Solheim LF (1986) Bone changes after castration in rats A model for osteoporosis. Acta Orthop Scand 57:67–70

Hammond RS, Tull LE, Stackman RW (2004) On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol Learn Mem 82:26–34

Henderson VW (2008) Cognitive changes after menopause: influence of estrogen. Clin Obstet Gynecol 51:618–626

Hu YS, Xu P, Pigino G, Brady ST, Larson J, Lazarov O (2010) Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates neuropathology in familial Alzheimer's disease-linked APPswe/PS1DeltaE9 mice. FASEB J 24:1667–1681

Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC (2000) Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. Exp Neurol 164:45–52

Jacobs B, Schall M, Scheibel AB (1993) A quantitative dendritic analysis of Wernicke's area in human. II. Gender, hemispheric, and environmental changes. J Comp Neurol 327:97–111

Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, Younkin LH, Younkin SG, Borchelt DR, Savonenko AV (2005) Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. J Neurosci 25:5217–5224

Kalu DN (1991) The ovariectomized rat model of postmenopausal bone loss. Bone Miner 15:175–191

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493–495

Kobayashi T, Tamura M, Hayashi M, Katsuura Y, Tanabe H, Ohta T, Komoriya K (2000) Elevation of tail skin temperature in ovariectomized rats in relation to menopausal hot flushes. Am J Physiol Regul Integr Comp Physiol 278:R863–R869

Konkle AT, Kentner AC, Baker SL, Stewart A, Bielajew C (2010) Environmental-enrichment-related variations in behavioral, biochemical, and physiologic responses of Sprague–Dawley and Long Evans rats. J Am Assoc Lab Anim Sci 49:427–436

Lambert TJ, Fernandez SM, Frick KM (2005) Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. Neurobiol Learn Mem 83:206–216

Laviola G, Hannan AJ, Macri S, Solinas M, Jaber M (2008) Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis 31:159–168

Leal-Galicia P, Saldívar-González A, Morimoto S, Arias C (2007) Exposure to environmental enrichment elicits differential hippocampal cell proliferation: role of individual responsiveness to anxiety. Dev Neurobiol 67:395–405

Leal-Galicia P, Castañeda-Bueno M, Quiroz-Baez R, Arias C (2008) Long-term exposure to environmental enrichment since youth prevents recognition memory decline and increases synaptic plasticity markers in aging. Neurobiol Learn Mem 90:511–518

Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L (2005) Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 163:78–90

Li H, Satinoff E (1996) Body temperature and sleep in intact and ovariectomized female rats. Am J Physiol 271:R1753–R1758

Lima AP, Silva K, Padovan CM, Almeida SS, Fukuda MT (2014) Memory, learning, and participation of the cholinergic system in young rats exposed to environmental enrichment. Behav Brain Res 259:247–252

Luine VN, Jacome LF, Maclusky NJ (2003) Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology 144:2836–2844

Maki PM (2006) Hormone therapy and cognitive function: is there a critical period for benefit? Neuroscience 138:1027–1030

Markowska AL, Savonenko AV (2002) Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. J Neurosci 22:10985–10995

Martin AL, Brown RE (2010) The lonely mouse: verification of a separation-induced model of depression in female mice. Behav Brain Res 207:196–207

McLaughlin K, Bimonte-Nelson H, Neisewander J, Conrad C (2008) Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: evidence that the duration of hormone deprivation after ovariectomy compromises 17beta-estradiol effectiveness in altering CA1 spines. Horm Behav 54:386–395

Meaney MJ, Aitki DH, Bhatnagar S, Van Berkel C, Sapolsky KM (1988) Postnatal handling attenuates neuroendocrine, anatomical and cognitive impairments related the aged hippocampus. Science 283:766–768

Meneses A (1999) 5-HT system and cognition. Neurosci Biobehav Rev 23:1111–1125

Meneses A (2003) Pharmacological analysis of an associative learning task: 5-HT(1)–5-HT(7) receptor subtypes function on a pavlovian/instrumental autoshaped memory. Learn Mem 10:363–372

Meneses A, Terrón JA (2001) Role of 5-HT (1A) and 5-HT (7) receptors in the facilitatory response induced by 8-OH-DPAT on learning consolidation. Behav Brain Res 121:21–28

Miller LS, Colella B, Mikulis D, Maller J, Green RE (2013) Environmental enrichment may protect against hippocampal atrophy in the chronic stages of traumatic brain injury. Front Hum Neurosci 7:1–8

Navarrete F, Pérez-Ortiz JM, Femenía T, García-Gutiérrez MS, García-Payá ME, Leiva-Santana C, Manzanares J (2008) Métodos de evaluación de trastornos cognitivos en modelos animales. Rev Neurol 47:137–145

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

Nordell VL, Lewis DK, Bake S, Sohrabji F (2005) The neurotrophin receptor p75NTR mediates early anti-inflammatory effects of estrogen in the forebrain of young adult rats. BMC Neurosci. doi:10.1186/1471-2202-6-58

Okada M, Hayashi N, Kometani M, Nakao K, Inukai T (1997) Influences of ovariectomy and continuous replacement of 17beta-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. Jpn J Pharmacol 73:93–96

Ozawa T, Yamada K, Ichitani Y (2014) Hippocampal BDNF treatment facilitates consolidation of spatial memory in spontaneous place recognition in rats. Behav Brain Res 263:210–216

Paris JJ, Frye CA (2008) Estrous cycle, pregnancy, and parity enhance performance of rats in object recognition or object placement tasks. Reproduction 136:105–115

Pham TM, Ickes B, Albeck D, Söderström S, Granholm AC, Mohammed AH (1999) Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. Neuroscience 94:279–286

Picazo O, Estrada-Camarena E, Hernandez-Aragon A (2006) Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioural actions of some anxiolytic agents. Eur J Pharmacol 530:88–94

Ramírez-Rodríguez G, Ocaña-Fernández MA, Vega-Rivera NM, Torres-Pérez OM, Gómez-Sánchez A, Estrada-Camarena E, Ortiz-López L (2014) Environmental enrichment induces neuroplastic changes in middle age female Balb/c mice and increases the hippocampal levels of BDNF, p-Akt and p-MAPK1/2. Neuroscience 260:158–170

Ravenelle R, Santolucito HB, Byrnes EM, Byrnes JJ, Donaldson ST (2014) Housing environment modulates physiological and behavioral responses to anxiogenic stimuli in trait anxiety male rats. Neuroscience 270:76–87

Redolat R, Mesa-Gresa P (2012) Potential benefits and limitations of enriched environments and cognitive activity on age-related behavioural decline. Curr Top Behav Neurosci 10:293–316

Resnick SM, Henderson VW (2002) Hormone therapy and risk of Alzheimer disease: a critical time. JAMA 288:2170–2172

Reul JM, De Kloet ER (1986) Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. J Steroid Biochem 24:269–272

Rodríguez-Landa JF, Hernández-Figueroa JD, Hernández-Calderón B, Saavedra M (2009) Anxiolytic-like effect of phytoestrogen genistein in rats with long-term absence of ovarian hormones in the black and white model. Prog Neuropsychopharmacol Biol Psychiatry 33:367–372

Roozendaal B (2000) Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology 25:213–238

Roozendaal B, McGaugh JL (1997) Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. Neurobiol Learn Mem 67:176–179

Rossato JI, Bevilaqua LR, Myskiw JC, Medina JH, Izquierdo I, Cammarota M (2007) On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. Learn Mem 14:36–46

Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006) Brain-derived neurotrophin factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur J Neurosci 24:1850–1856

Ryan J, Scali J, Carriere I, Ritchie K, Ancelin ML (2008) Hormonal treatment, mild cognitive impairment and Alzheimer's disease. Int Psychogeriatr 20:47–56

Saito S, Kobayashi S, Ohashi Y, Igarashi M, Komiya Y, Ando S (1994) Decreased synaptic density in aged brains and its prevention by rearing under enriched environment as revealed by synaptophysin contents. J Neurosci Res 39:57–62

Sampedro-Piquero P, Begega A, Arias JL (2014) Increase of glucocorticoid receptor expression after environmental enrichment: relations to spatial memory, exploration and anxiety-related behaviors. Physiol Behav 129:118–129

Sánchez MM, Young LJ, Plotsky PM, Insel TR (2000) Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. J Neurosci 20:4657–4668

Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS (2012) Stress effects on memory: an update and integration. Neurosci Biobehav Rev 36:1740–1749

Simpson J, Kelly JP (2011) The impact of environmental enrichment in laboratory rats-behavioural and neurochemical aspects. Behav Brain Res 222:246–264

Singh M, Sumien N, Kyser C, Simpkins JW (2008) Estrogens and progesterone as neuroprotectants: what animal models teach us. Front Biosci 13:1083–1089

Spada JA, Galíndez C, Spada A (2006) Lesión de las cortezas extrahipocampales del lóbulo temporal medial: descripción de un caso. Rev Neurol 43:403–408

Steckler T, Andrews JS, Marten P, Turner JD (1993) Effects of NBA lesions with two neurotoxins on spatial memory and autoshaping. Pharmacol Biochem Behav 44:877–889

Strom JO, Theodorsson E, Theodorsson A (2008a) Order of magnitude differences between methods for maintaining physiological 17β-estradiol concentrations in ovariectomized rats. Scand J Clin Lab Investig 68:814–822

Strom JO, Theodorsson A, Theodorsson E (2008b) Substantial discrepancies in 17beta-oestradiol concentrations obtained with three different commercial direct radioimmunoassay kits in rat sera. Scand J Clin Lab Investig 68:806–813

Sun H, Zhang J, Zhang L, Liu H, Zhu H, Yang Y (2010) Environmental enrichment influences BDNF and NR1 levels in the hippocampus and restores cognitive impairment in chronic cerebral hypoperfused rats. Curr Neurovasc Res 7:268–280

Taglialatela G, Hogan D, Zhang WR, Dineley KT (2009) Intermediate-and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. Behav Brain Res 200:95–99

Utian WH (2004) Menopause-related definitions. Int Congr Ser. doi:10.1016/jics200401102

Van Goethem NP, Rutten K, van der Staay FJ, Jans LA, Akkerman S, Steinbusch HW, Blokland A, Van't Klooster J, Prickaerts J (2012) Object recognition testing: rodent species, strains, housing conditions, and estrous cycle. Behav Brain Res 232:323–334

Vegeto E, Benedusi V, Maggi A (2008) Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. Front Neuroendocrinol 29:507–519

Wadowska M, Woods J, Rogozinska M, Briones TL (2014) Neuroprotective effects of enriched environment housing after transient global cerebral ischemia are associated with the upregulation of insulin-like growth factor-1 signaling. Neuropathol Appl Neurobiol. doi:10.1111/nan12146

Walf AA, Rhodes ME, Frye CA (2006) Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 86:35–46

Wang D, Noda Y, Zhou Y, Mouri A, Mizoguchi H, Nitta A, Chen W, Nabeshima T (2007) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunction in beta amyloid25–35 icv-injected mice:

involvement of dopaminergic systems. Neuropsychopharmacology 32:1261–1271

White JH, Alborough K, Janssen H, Spratt N, Jordan L, Pollack M (2013) Exploring staff experience of an "enriched environment" within stroke rehabilitation: a qualitative sub-study. Disabil Rehabil. doi:10.3109/096382882013872200

Wilson RS, Mendes De Leon CF, Barnes LL, Schneider JA, Bienias JL, Evans DA, Bennett DA (2002) Participation in cognitively stimulating activities and risk of incident Alzheimer disease. JAMA 287:742–748

Winters BD, Bussey TJ (2005) Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. J Neurosci 25:4243–4251

Wu J, Zhu YQ, Wu J (2008) Effects of estrogen and estrogenic compounds on cognition in ovariectomized rats. Climacteric 11:212–220

Zhang Q, Han D, Wang R, Dong Y, Yang F, Vadlamudi RK, Brann DW (2011) C terminus of Hsc70-interacting protein (CHIP)-mediated degradation of hippocampal estrogen receptor-α and the critical period hypothesis of estrogen neuroprotection. Proc Natl Acad Sci 108:E617–E624

Zhao L, Brinton RD (2006) Select estrogens within the complex formulation of conjugated equine estrogens (Premarin) are protective against neurodegenerative insults: implications for a composition of estrogen therapy to promote neuronal function and prevent Alzheimer's disease. BMC Neurosci 13:7–24