Neurobiology of Learning and Memory 101 (2013) 19–25

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

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

Object recognition memory and temporal lobe activation after delayed estrogen replacement therapy

Cristina S. Fonseca, Isabela D. Gusmão, Ana C.S. Raslan, Brisa Marina M. Monteiro, André R. Massensini, Márcio F.D. Moraes, Grace S. Pereira *

Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 17 September 2012
Revised 12 December 2012
Accepted 30 December 2012
Available online 5 January 2013

Keywords:
Estrogen replacement therapy
Object recognition memory
c-Fos
Amygdala
Perirhinal cortex
Hippocampus

ABSTRACT

The critical window hypothesis predicts that estrogen replacement therapy (ERT) must be administered early on the menopause or ovariectomy (OVX) to positively affect cognition. However, the neural substrates, underling the time dependent efficacy of ERT, are still not completely known. In order to address this issue, we submitted female mice to 12 weeks of OVX followed by 5 weeks of chronic ERT (OVX_{E2}). Within the first 12 weeks, the OVX animals showed a progressive compromised performance in the object recognition memory (ORM) task. After ERT, OVX_{E2} mice, but not the control group (OVX_{oil}), were able to recognize the new object in the test session. Further, we evaluated the c-Fos expression in hippocampus, perirhinal cortex (PC) and central amygdala (CeA) of OVX_{oil} and OVX_{E2} mice, after context exposure (CTX) or object exploration (OBJ). We observed that ERT increased c-Fos expression unspecifically for CTX and OBJ. In addition, only the OVX_{E2} group showed significantly higher c-Fos expression in the PC and CeA after object exploration. Thus, our results showed that delayed chronic ERT improves ORM (compromised by OVX) and increases constitutive c-Fos expression in temporal lobe regions. Furthermore, we showed for the first time that PC and CeA, but not the hippocampus, present a distinct pattern of activation in response to object exploration in ovariectomized females that underwent delayed-ERT.

1. Introduction

Several reports and clinical observations have indicated that menopausal women may suffer from compromised memory function (reviewed by Hogervorst & Bandelow, 2009). Accordingly, the estrogen replacement therapy (ERT) may counteract some of the deleterious effects caused by the long-term deprivation of estradiol (Phillips & Sherwin, 1992; Robinson, Friedman, Marcus, Tinklenberg, & Yesavage, 1994; Sherwin, 1994). However, if initiated late in life, ERT may be ineffective or even deleterious (Grady et al., 2002; Shumaker et al., 2004; Whitmer, Quesenberry, Zhou, & Yaffe, 2011). In fact, the aforementioned results corroborate to the "critical therapeutic window" hypothesis, which states that after menopause there is a time window in which ERT is effective. Thus, outside this conceived critical window, the long-term effects of estrogen deprivation on brain function become irreversible (MacLennan et al., 2006; Maki, 2006).

The ovariectomy (OVX) is a procedure that reduces the levels of circulating ovarian hormones in rodents, thus mimicking some features of women menopause. Although it is arguable that OVX is a proper animal model for menopause; it is, nevertheless, a very useful procedure to study the effects of ovarian hormones in memory (reviewed by Brinton, 2012). In fact, there are studies showing that OVX impairs object recognition memory (ORM) (Gresack & Frick, 2006; Wallace, Luine, Arellanos, & Frankfurt, 2006), though the duration of the OVX seems important, since short periods may have no effect on ORM (Capettini, Moraes, Prado, Prado, & Pereira, 2011; Fernandez et al., 2008).

The deleterious effects of OVX on memory can be reversed, in the ORM task, by a single injection of estradiol (E2) (Daniel, Hulst, & Berbling, 2006; Fernandez et al., 2008) or progesterone (Frye & Walf, 2008, 2010; Lewis, Orr, & Frick, 2008) immediately after ovariectomy. Interestingly, it seems that in rodents, as in humans, the timing of estrogen therapy onset after OVX is paramount to determine its efficacy. For example, 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 (Daniel et al., 2006).

* Corresponding author. Address: Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, CEP 31270-901, Campus Pampulha, Belo Horizonte, MG, Brazil. Fax: +55 31 3409 2939.
E-mail address: grace@icb.ufmg.br (G.S. Pereira).

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2012.12.016

Despite the amount of behavioral data suggesting the existence of a critical time window for ERT, the neural substrates involved and the underlying mechanisms of its effectiveness is not completely understood. Thus, it is unclear what are the plastic changes triggered by ERT that reverse the memory deficit induced by OVX. To pursuit this question, we first investigated ORM along different periods post-OVX (up to 12 weeks) in adult female mice. After determining at which point OVX-induced memory deficit was evident, we started a long-term ERT (5 weeks); which has been shown to be long enough to reverse the ORM deficit (Heikkinen, Puolivali, & Tanila, 2004). Further, we evaluated the effect of delayed-ERT on the pattern of c-Fos expression in temporal lobe structures. In summary, our results showed that delayed chronic ERT improves ORM (compromised by OVX), increases constitutive c-Fos expression in temporal lobe regions and induces object-dependent activation of the perirhinal cortex and central nucleus of the amygdala.

2. Methods

2.1. Animals

Subjects were C57BL/6J female mice (8 weeks of age at the beginning of the experiments) purchased from the Animal Facility of the Universidade Federal de Minas Gerais (Brazil). The animals were maintained in a climate-controlled animal housing unit (Alesco, Brazil): 12 h/12 h light/dark cycle, room temperature at 22 ± 2 °C and relative humidity at 55 ± 10%. Food and water were available *ad libitum*. Experimental procedures were conducted during daytime. Experiments are licensed under the Protocol 035/2009 of the Institutional Animal Care and Ethics Committee of the Universidade Federal de Minas Gerais.

2.2. Ovariectomy

Eight weeks old female mice were anesthetized using a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). An abdominal incision was made just lateral to the midline at the pelvic level. In the OVX group, the ovary, oviduct, and top of the fallopian tubes were clamped and removed. The surgical procedure for the control group, named sham-operated mice, was the same except that the ovaries were not removed. The abdominal wall and the skin were sutured (Capettini et al., 2011) and animals allowed recovering for at least 1 week. After recovery from anesthesia, mice received a unique injection of analgesic, anti-inflammatory and anti-thermal mixture (0.3 mg/kg, Banamine, Shering-Plough, Brazil) and returned to their home cages for at least 1 week.

2.3. Estrogen replacement therapy (ERT)

After 12 weeks of ovariectomy, mice were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg). A 1 cm incision was made in the animal's nape and a silastic capsule (0.04 in./0.085 in., inner/outer diameter; volume, 4 μL; Down Corning, Midland, MI) containing either corn oil (vehicle, OVX_{oil} group) or 17-β estradiol (E2; Sigma: 0.18 mg/4 μL, OVX_{E2} group) was implanted subcutaneously. After recovering from anesthesia, mice received a unique injection of analgesic, anti-inflammatory and anti-thermal mixture (0.3 mg/kg, Banamine, Shering-Plough, Brazil) and returned to their home cages for at least 5 weeks. This protocol produces levels of E2 in serum around 50–100 pg/ml, which is slightly higher than the serum estradiol levels of 35–75 pg/ml reported in mice during proestrus (Grasso & Reichert, 1996; Heikkinen et al., 2004; Nelson, Felicio, Osterburg, & Finch, 1992). We used the uterus weight to evaluate the ERT effectiveness. At the end of the experiment, animals were euthanized by decapitation and the uterus was removed. During the procedure of weighting, we lost the data of three animals from OVX_{oil} group.

2.4. Novel Object Recognition (NOR)

All animals were given two 20 min habituation session, one per day, in a plastic cage (50 cm × 40 cm × 20 cm) with no objects, which was equally illuminated. Twenty-four hours after the last habituation session, animals were allowed to explore two identical objects, each one always presented at the same location inside the box, for a total of 10 min (training session: TR). Memory retention was evaluated during the test session (TT) carried out 24 h after the TR. During TT, with duration of 10 min, the familiar and the novel objects were presented at the same location the objects were presented during the training phase. However, during the test phase, to avoid a natural preference of the animals for one location or another, we pseudo-randomly interchanged the location of the new object. All objects (available in duplicate) presented similar material and size, but distinct shape. The objects had been selected from a large pool of objects on the criterion that mice would spend approximately equal amounts of time exploring each of them (data not shown). Between each change of animals, box and objects were cleaned with 70% alcohol and air-dried. Exploration time was defined as sniffing or touching the object with the nose and was quantified by the software Debut Video Captura®. Data are expressed as recognition index, calculated according to the following formulae: time exploring the new object/(time exploring the familiar object + time exploring the new object) (Capettini et al., 2011; Gusmao et al., 2012; Lazaroni et al., 2012).

2.5. Immunohistochemistry

Animals were exposed to the context (box groups) or to the context containing two identical objects (object groups). After 10 min, the animals return to their home cage and 1.5 h after they were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and underwent transcardial perfusion with 0.01 M phosphate buffer saline (PBS), followed by 4% paraformaldehyde (PFA) in PBS. The brains were removed and placed overnight in the same fixing solution and afterwards in 30% sucrose in PBS at 4 °C for 3 days. Brains were frozen in 99% isopentane and maintained at −45 °C for 1 min. Shortly after freezing, 40 μm thick coronal brain cryo-sections (from Bregma −1.06 mm to −2.80 mm, Paxinos & Franklin, 2001) were obtained (cryostat model 300) and stored at −20 °C in PBSAF [PBS, 20% sucrose, 15% ethylene glycol, 0.05% NaN_3]. The sections were washed three times in 0.01 M Trizma base saline buffer (TBS) before being placed in hydrogen peroxide (3% H_2O_2/TBS) for 10 min. Next, the sections were washed three times for 6 min in TBS/0.3% Triton X-100, followed by 2 h of incubation in a blocking solution [3% normal goat serum (NGS) in TBS/0.3% Triton X-100]. Then, the primary antibody against c-Fos (Santa Cruz, sc-52), diluted 1:1000, was added and incubated overnight at room temperature. Next day, the sections were washed three times for 6 min in TBS with 0.3% Triton X-100 and incubated with the secondary antibody (1:1000, biotinylated anti-IgG antibody goat anti-rabbit; Vector Laboratories) for 2 h at room temperature. After, the sections were washed three times for 6 min in TBS with 0.3% Triton X-100 and incubated with avidin–biotin horseradish peroxidase complex (1:500 in TBS; Vector Laboratories) during 1 h at room temperature. Subsequently, the sections were washed three times for 6 min in TBS and three times for 6 min in 175 mM acetate buffer. The sections were developed with a solution containing 0.2 mg/ml diaminobenzidine (DAB), 25 mg/ml nickel sulfate and 0.0025%H_2O_2 in acetate buffer for 15 min. Finally, the sections were mounted on gelatin-coated slides, air-dried, dehydrated in xylene, and embedded in entellan.