Int J Endocrinol Metab. 2010;8(2):82-89
International Journal of
Endocrinology & Metabolism
Journal home page: www.IJEM.org

# The effect of pre-training infusions of estrogen receptor ligands in the CA1 region of hippocampus on passive avoidance task

Zohreh Sharifkhodaei [1,2], Nasser Naghdi [1*], Shahrbano Oryan [2,3]

[1] Department of Physiology and Pharmacology, Pasteur Institute of Iran, Tehran, IR Iran
[2] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, IR Iran
[3] Department of Biology, College of Science, Tarbiat Moalem University, Tehran, IR Iran

ARTICLE INFO

*Article Type:*
Original Article

*Article history:*
Received: 7 May 2010
Revised: 10 Jul 2010
Accepted: 4 Sep 2010

*Keywords:*
Estrogen receptor beta
Estrogen valerat
Cyclofenil

ABSTRACT

*Background:* Neurohormones like testosterone and estrogen play important roles in learning and memory. Estrogen receptors, densely expressed in the CA1 region of rat hippocampus, mediate the effects of estrogen on learning and memory. Estrogen receptors belong to a family of transcription factors, the nuclear receptor super family, and have two subtypes, estrogen receptor α and estrogen receptor β.
*Objectives:* Study the effect of pre-training infusions of estrogen receptor ligands in the CA1 region of hippocampus on passive avoidance task
*Materials and Methods:* The current research has been conducted to assess the effects of estradiol valerat, estrogen receptor β selective agonist, diarylpropionitrile, non-steroidal selective estrogen receptor β modulator, and Cyclofenil on passive avoidance task on adult male rats. Male adult rats were bilaterally cannulated into the CA1 area of hippocampus, and then administered vehicle dimethyl sulfoxide or estradiol valerat (15 μg/0.5μl/side), diarylprop-ionitrile (0.2, 0.5, 1 μg/0.5μl/side), Cyclofenil (5, 7.5,10 μg/0.5μl/side), 30 min before training.
*Results:* Results showed that pre-training intra CA1 injections of EV (15 μg/0.5μl/side), diarylpropionitrile (0.5, 1 μg/0.5μl/side), and Cyclofenil (10 μg/0.5μl/side), significantly decreased step-through latencies and increased time spent in the dark chamber in inhibitory avoidance learning.
*Conclusions:* Our data suggest that estrogen receptor β plays has an important role in learning and memory acquisition in the inhibitory avoidance task.


*Please cite this paper as:*
Sharifkhodaei Z, Naghdi N, Oryan S. The effect of pre-training infusions of estrogen receptor ligands in the CA1 region of hippocampus on passive avoidance task. *Int J Endocrinol Metab*. 2010;**8**(2):82-89.

## Background

Substantial evidence suggests that estrogen can enhance or impair learning and memory depending on the qualities of the task and schedule of estrogen replacement used (1). Performance on place learning tasks, requiring the use of spatial location is enhanced by estrogen treatment to ovariectomized rats (2, 3). Estradiol binds with a high affinity to E2 receptor (ER) isoforms, estrogen receptor α (ERα) and estrogen receptor β (ERβ). Although there is differential distribution of ERα and ERβ throughout the central nervous system, both ERα and ERβ are expressed in the hippocampus and cortex (4) and may influence cognitive processes that rely on hippocampal and cortical function. Estrogen receptors belong to a family of transcription factors, the nuclear receptor superfamily (5), and have two subtypes, ERα and ERβ (6). Several studies have shown that ERβ is an important modulator of cell proliferation and learning and memory (7, 8). Localized predominantly in the limbic system, like amygdala, septum, and also in the hippocampus, and hypothalamus, ERs are involved in emotional processing and cognition (9,10). Estradiol me-

* *Corresponding author at:* Nasser Naghdi, Department of Physiology and Pharmacology, Pasteur Institute of Iran, Pasteur Ave., 13164, Tehran, IR Iran. Fax: +98-2166968854.
*E-mail:* naghdi@pasteur.ac.ir

diates structural changes at synapses of the hippocampus, an area in the brain important for learning and memory (11). In addition, estradiol has a variety of effects in the brain, including the modulation of cholinergic, serotonergic, and catecholaminergic neurons in different brain areas (12).

Several concepts have been proposed to account for the mixed effects of estrogen on cognition, including the possibility that the efficacy of estrogen treatment varies with the motivating factors of the task (13), stress state of the animals (14), type of memory (15) and duration and type of hormone treatment (3). ER$\beta$ knock out (KO) mice, which lack functional ER$\beta$, show impaired acquisition in the Morris Water Maze task compared to their wild type counterparts (7), and show increased anxiety in the open Weld and elevated plus maze tasks (16). In addition, administrations of selective ER modulators that target ER$\beta$, but not ER$\alpha$, have been shown to enhance cognitive performance, and antianxiety and anti-depressive behavior in female rats (17, 18). Given these different patterns of effects with SERMs, it is important to further investigate the role of ER$\beta$ for the effects of E2 on performance in hippocampally mediated tasks.

## Objectives

In this study, we used the selective agonist ER$\beta$, diarylpropionotril (DPN), and Cyclofenil that is a non-steroidal selective ER$\beta$ modulator (SERM). Cyclofenil display a 4.9-fold affinity for ER$\beta$ versus ER$\alpha$(19, 20), to describe the effect of ER$\beta$ in the CA1 region of adult male rat hippocampus on learning and memory in the passive avoidance (PA) apparatus.

## Materials and Methods

### *Animals*

Adult male Wistar rats (200–250g) were obtained from the breeding colony of the Pasteur Institute of Iran. Rats were housed, three per cage in a temperature (23 ± 1 °C) controlled room, maintained on a 12:12h reversed light cycle (light on at 07:00 a.m.). Rats had unrestricted access to food and water in their home cage. These animal experiments were carried out in accordance with the recommendations of the Helsinki declaration and the internationally accepted principles for the use of experimental animals.

### *Surgical procedures*

Approximately 7–8 days prior to initiation of the behavioral experiments, the rats were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (25 mg/kg, i.p.) and were bilaterally implanted with cannulae (23-gauge) aimed at a site immediately above the CA1 (AP: −3.8mm from Bregma, ML: ± 2.2 mm from midline, and DV: −2.7 mmfrom the skull surface) according to the atlas of Paxinos and Watson(21). Two screws were inserted into the skull and cannulae fixed to these with dental cement.

### *Microinjection procedure*

Intra-hippocampal injections were given via guided cannulae with injection needles (30-gauge) that were connected by polyethylene tubing to a 10-µl Hamilton micro-syringe. The injections (0.5µl total volume) were delivered over two minutes with a syringe pump, and the injection needles (extending 0.5mm from the end of the guide cannulae) were left in place an additional minute before they were slowly withdrawn.

### *Behavioral testing*

### *Inhibitory avoidance*

### *Apparatus*

The step-through PA apparatus consisted of a lighted chamber (30 cm × 20 cm × 20 cm) made of transparent plastic and of a dark chamber (30 cm × 20 cm × 20 cm), the walls and ceiling of which were made of dark opaque plastic. A rectangular opening (8 cm × 8 cm) was located between the two chambers and could be closed by an opaque guillotine door. The floor of both chambers was made of stainless steel rods (2mm diameter), spaced 1 cm apart. The floor of the dark compartment could be electrified. The apparatus was placed in an acoustically insulated room, kept under standard conditions.

### *Procedure*

The stepthrough type of passive avoidance task was used to examine the long-term memory based on the negative reinforcement. A day before initiation of tests, animals were familiarizedand habituated to the testing room, for which on day one, 30 min before training, rats were placed in the lighted chamber and were allowed to explore for 30 s, and then guillotine door was raised. After entering of the rats to the dark chamber, the guillotine door was lowered and the rats remained there for 30s. Following the habituation of all animals, the first rat was again placed into the lighted chamber for 10 s, the door was lifted, and the crossover latency was recorded; the door was closed behind it and a shock was delivered (1 mA, 5-s duration). The retention test was performed 24 h after training (day 2). The rats were placed in the lighted chamber, 10 s later the door was opened, and the step-through latency (STL) and the time spent in dark compartment during the retrieval test was recorded, up to 600 s, during which time electric shocks were not applied to the grid floor(22). All experiments were done between 9 and 11 o'clock.

### *Experimental protocol*

### *Experiment 1*

The aim of this experiment was to assess the effect of

pre-training injections of EV into the CA1 region of hippocampus on passive avoidance task. Eight rats in one group (No. = 8) received effective dose of EV (15μg) dissolved in 0.5μl dimethyl sulfoxide (DMSO), 30 min before the training in passive avoidance. STL during the training session, STL and the time spent in the dark compartment during the retrieval test were recorded.

*Experiment 2*

The aim of this experiment was to assess the effect of pre-training injections of DPN into the CA1 region of hippocampus on passive avoidance task. Twenty-four rats were divided into three groups (No. = 8 each) that received different doses of DPN (0.2, 0.5, 1 μg dissolved in 0.5 μl DMSO), 30 min before the training in passive avoidance test. STL during the training session, and STL and the time spent in dark compartment during the retrieval test were recorded.

*Experiment 3*

The aim of this experiment was to assess the effect pre-training injections of Cyclofenil into the CA1 region of hippocampus on passive avoidance task. Twenty four rats were divided into three groups (n=8) that received different doses of Cyclofenil (5, 7.5, 10 μg dissolved in 0.5 μl DMSO), 30 min before the retrieval of passive avoidance test. STL during the training session and STL and the time spent in dark compartment during the retrieval test were recorded.

*Experiment 4*

The aim of this experiment was to assess the effect pre-training injections of Cyclofenil plus DPN into the CA1 region of hippocampus on passive avoidance task. Eight rats in one group (No.=8) according to the dose levels of10 μg + 0.5 μg for Cyclofenil and DPN respectively, dissolved in 0.5 μl DMSO, 30 min before the retrieval of passive avoidance test. STL during the training session, and STL and the time spent in dark compartment during the retrieval test were recorded.

*Histology*

Following behavioral testing, animals were sacrificed by decapitation and the brains were removed and fixed in formalin. For histological examination of cannulae and injection placement in CA1 area, 100-μm thick sections were taken and cannulae and injection tracks were examined for each side with light microscopy. Only data obtained from animals, whose cannulae and injections were inserted precisely in the CA1 region, were used for analysis.

*Statistical analysis*

Results of STL during the training session, and STL and time spent in the dark chamber during the retrieval for groups are expressed as one way ANOVA followed by Tukey or LSD test. Unpaired T test was performed for comparison between the two groups. In all comparisons, values of P<0.05 were considered significant.

## Results

*Experiment 1: Effect of EV on acquisition of passive avoidance task*

The results showed that pre-training injections of

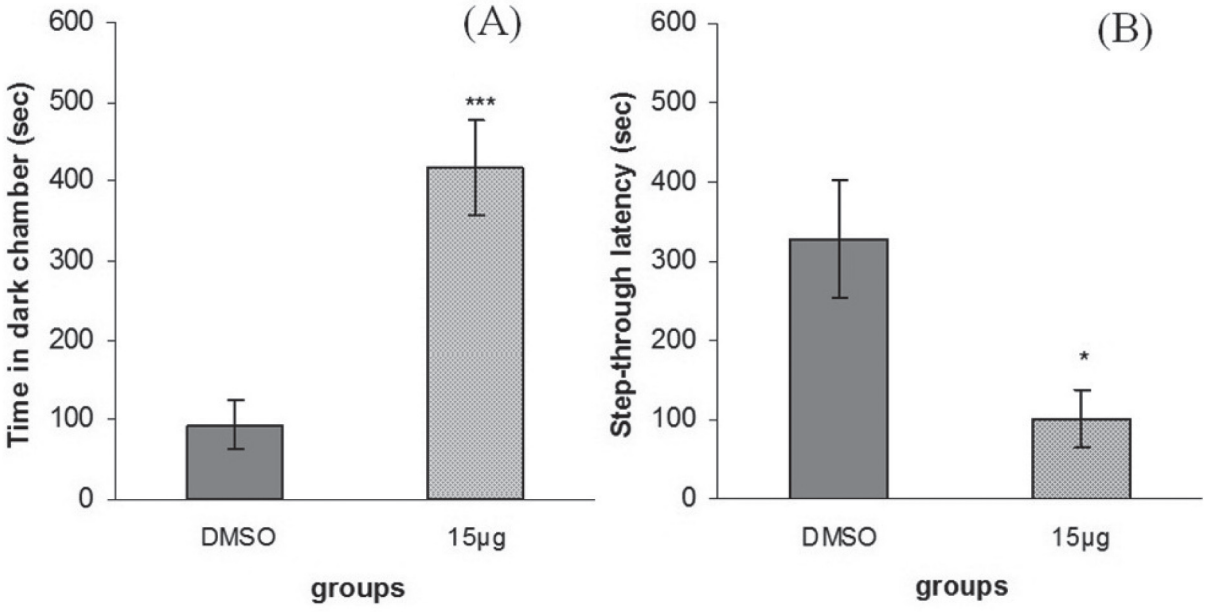


**Figure1.** The effects of EV on acquisition of inhibitory avoidance learning (A) step-through latency and (B) time spent in dark chamber. Mean ± SE. (*P<0.05 and ***P<0.001, indicate significant difference vs. control)

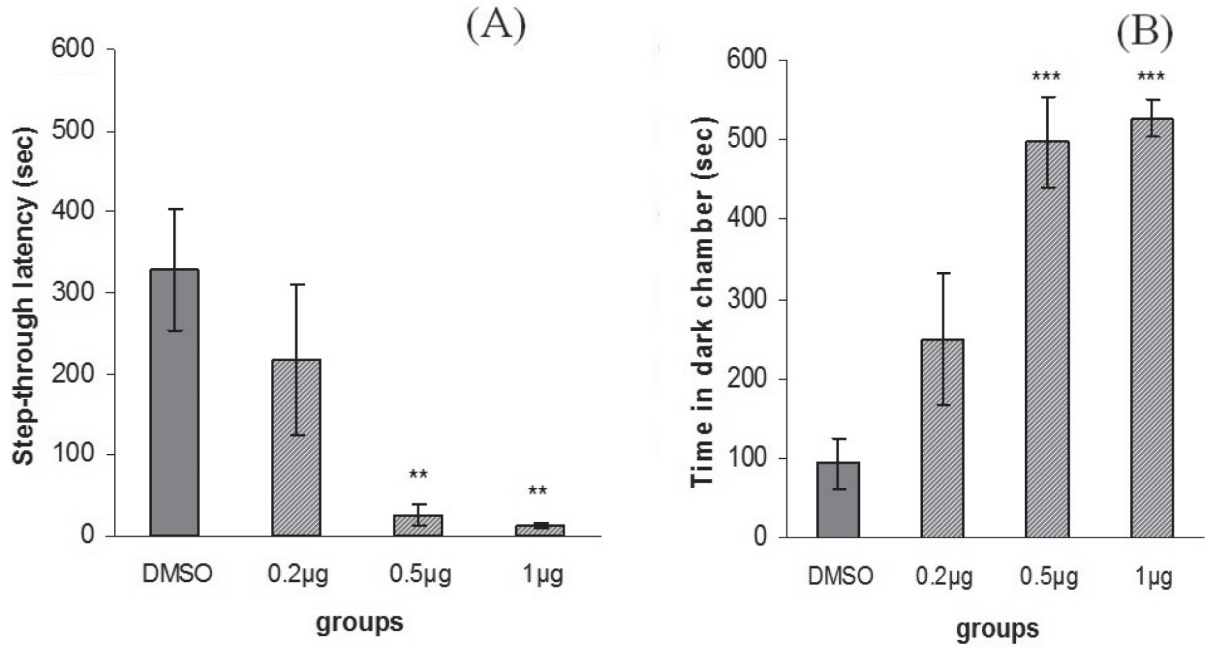


**Figure 2.** The effects of DPN on acquisition of inhibitory avoidance learning. (A) step-through latency and (B) time spent in dark chamber. Mean ± SE. (*P<0.05 and ***P<0.001, indicate significant difference vs. control)

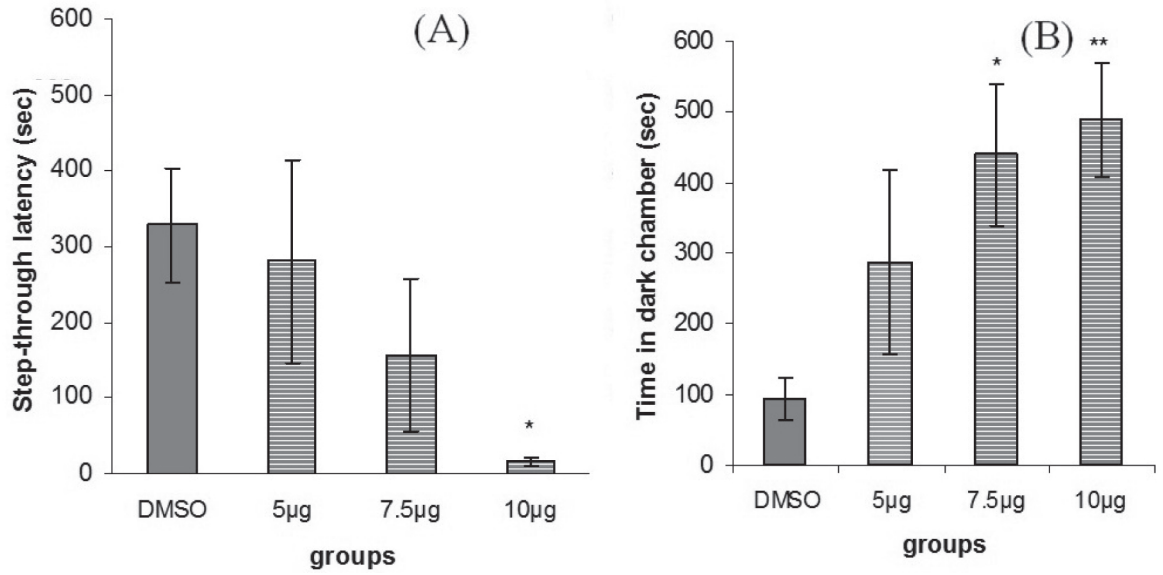


**Figure 3.** (A) The effects of Cyclofenil on acquisition of inhibitory avoidance learning.(A) step-through latency and (B) time spent in dark chamber. (Mean ± SE) (*P<0.05 and **P<0.01, indicate significant difference vs. control)

EV15µg did not have any significant effects on STL during the training session as compared to the DMSO group. *Figure 1A* shows a significant decrease in STL in the EV15µg as compared to the DMSO group ($F(1, 14) = 2.041$, $P < 0.05$). *Figure 1.* B shows the effect of pre-training injections of EV15µg on time-spent in dark chamber. Unpaired T test shows that EV15µg significantly increased time spent in dark chamber, as compared to the control group ($F(1, 14) = 8.225$, $P < 0.001$).

### *Experiment 2: Effect of DPN on acquisition of passive avoidance task*

The results showed that pre-training injections of DPN 0.2, 0.5, 1µg had no significant effects on STL during the training session as compared to the DMSO group.The results revealed significant differences between the groups ($F(2, 21) = 6.496$, $P = 0.002$). *Figure 2A* shows that STLs were significantly decreased at doses 0.5µg and 1µg DPN in comparison to those of the control group (P<0.01). *Figure 2B* shows the effect of pre-training injections of DPN on time-spent in the dark chamber. Data analyses from this experiment also showed the increase of time-spent in the dark chamber at doses 0.5µg and 1µg significantly different to those of the control group ($F(2, 21) = 14.833$, $P<0.0001$).

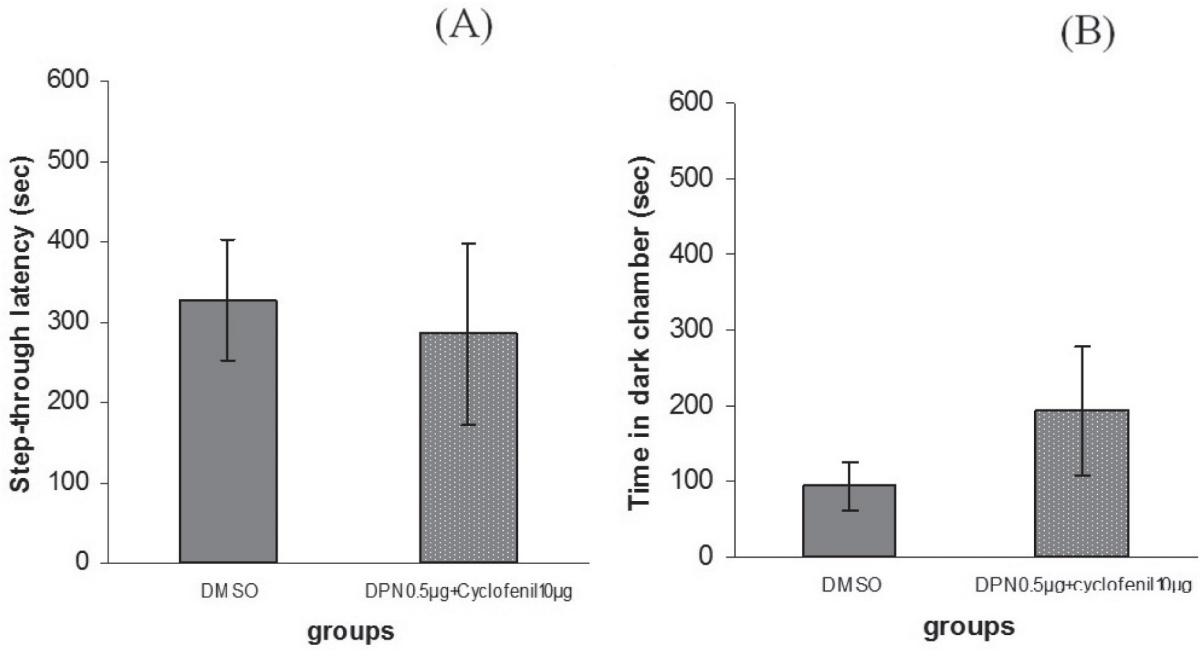


**Figure 4**. The effects of DPN0.5µg + Cyclofenil 10µg on acquisition of inhibitory avoidance learning. (A) step-through latency and (B) time spent in dark chamber (Mean ± SE). P > 0.05, indicate not significant difference vs. control.

*Experiment 3: Effect of Cyclofenil on acquisition of passive avoidance task*

The results showed that pre-training injections of Cyclofenil 5, 7.5, 10µg had no significant effects on STL during the training session as compared to the DMSO group. Data analyses from this experiment revealed significant differences between groups (F (2, 21) = 3.062, P = 0.049). *Figure 3A* shows that STLs were significantly (P = 0.043) decreased at dose 10µg of Cyclofenil in comparison to those of the control group. *Figure 3B* shows the effect of pre-training injections of Cyclofenil on time-spent in the dark chamber. Data analyses from this experiment also showed the increases in time-spent in the dark chamber at doses 7.5µg (P<0.05) and 10µg (P<0.01) that are significantly different to those of the control group (F (2, 21) = 5.293, P = 0.007).

*Experiment 4: Effect of Cyclofenil plus DPN on acquisition of passive avoidance task*

The results showed that pre-training injections of Cyclofenil plus DPN had no significant effects on STL during the training session as compared to the DMSO group. *Figure 4A* shows no significant difference between the DPN 0.5µg + Cyclofenil 10µg and the DMSO groups. *Figure 4B* reveals no significant difference in time spent in the dark chamber, between the DPN 0.5µg + Cyclofenil 10µg and the DMSO groups.

## Discussion

Our results showed that EV (15µg/0.5µl), DPN (0.5 and 1 µg/0.5µl) and Cyclofenil (10µg/0.5µl) significantly decreased STL, while it increased time-spent in the dark chamber in comparison to the control group, findings suggesting that EV, DPN and Cyclofenil could impair acquisition in pas-sive avoidance task. Our results also showed that Cyclofenil (10µg/0.5µl) could eliminate impairment caused by DPN (0.5µg/0.5µl). Estradiol valerat as a general ER agonist, could impair acquisition in the passive avoidance task, as compared to vehicle-treated rats, a finding consistent with previous studies showing that estradiol valerat (15µg/0.5µl) could impair acquisition in the Morris water maze (3) and a high level of estradiol is associated with impaired initial performance in the MWM in both laboratory rats and meadow voles (4, 15). It is well established that many of the actions of steroid hormones occur by means of activation of intracellular hormones (23), which diffuse into the cell, bind to their individual receptors and transformation and activation of the receptors occur; activation is dissociation of the receptor-heat shock protein complex, formed with unbound receptors in order to stabilize, keep inactive and protect the receptor. The hormone-receptor complex dimerizes, i.e. two activated receptors bind to each other; the dimer binds to specific DNA sites, hormone response elements, in the promoter region of target genes; this initiates transcription, subsequently leading to translation, and synthesis of new proteins (24). ERs consist of different domains: N-terminal domain, DNA-binding domain, hinge region, large ligand binding domain, and the C-terminal domain (25). Aside from the well-known classical ER (now termed ER**α**), a novel nuclear receptor, ER**β**, has recently been cloned. Both receptor sub forms are coded by separate genes located on chromosomes 6 (ER**α**) and 14 (ER**β**). These nuclear estrogen receptors

exhibit an identical exon structure and share high homology in the coding regions for the ligand- and DNA-binding domains (23); with respect to their binding and transactivation properties, **α** and **β** receptors show distinct differences. Saturation binding analysis revealed a higher affinity of 17**β**-estradiol for the **α**-receptor than for the **β**-receptor, and the activated **α**-receptor complex was found to act as a transcriptional activator from the AP1 site, whereas, the **β**-receptor complex inhibited transcription (26). Both receptors are widely expressed in the brain with a great overlap but also with quantitative regional and sex-specific differences in expression patterns (24). In this study we have suggested that distinct differences in binding and transactivation properties of **α** and **β** receptors may be the molecular basis for estrogen-dependent impairing in learning and memory. Another hypothesis that may explain our results is that estradiol increases glutamate spillover; in this hypothesis, the enhanced concentration of diffusing glutamate contributes to the larger, late NMDAR-mediated EPSP in CA1 by activation of NMDARs, located extrasyn-aptically but adjacent to the same synapse from which glutamate was released and/or by activating NMDARs at neighboring synapses (27). It has been suggested that structural changes in the hippocampus may be the neural basis for estrogen-dependent impairing in learning and memory.

As regards estradiol acting via receptors in the cell, our use of DPN and Cyclofenil to describe the role of ER**β** on learning and memory, showed that DPN (0.5 and 1 μg/0.5μl) could impair acquisition in the passive avoidance task, indicating the possibility that estrogen caused its impai-rment via ER**β**. It has become evident in recent years that there are important reciprocal relationships between brain steroid hormone systems and neurotransmitter systems such as the cholinergic (23, 28), dopaminergic (29, 30), GABAergic, serotonergic and the glotaminergic (23, 31, 32). There are two possible explanations for this; first, the most abundant cortical nuclear estrogen receptor, ER**β**, is present in GABAergic neurons; data show that ER-beta-bearing inhibitory neurons project onto other GABAergic neurons that lack nuclear estrogen receptors (33). The GABA system is the major inhibitory system in the brain and GABAA receptor active substances, like benzodiazepine, can inhibit learning and memory in human and animals (34); in addition, GABAA receptor activation with propofol can inhibit LTP induction (35); Thus, ER-beta exhibits extensive co localization with a subclass of inhibitory neurons, suggesting a potential mechanism whereby estrogen can regulate neuronal excitability in diverse and broad brain regions by modulating inhibitory tone (33). Second, serotonin neurons also express the nuclear estrogen receptors beta (ER**β**) which are transcription factors. Neurotran-smitters, like serotonin are also involved in memory function; 5HT1A and 5HT2C receptor knockout/mutant mice also show impaired spatial learning. Estradiol decreases 5HT1A and 5HT2A mRNA expression in the hippocampus, and also decreases the 5HT2C receptor gene expression in the ventral hippocampus (36). 5HT1B knockout mice exhibit facilitation in the acquisition of a hippocampal-dependent spatial reference memory task in the Morris water maze, but an impairment of delay-dependent working memory in the radial arm maze (37-40). Interestingly, stimulation of the 5HT1B receptor inhibits the release of acetylcholine in the hippocampus, but stimulates its release in the frontal cortex (39). Reduction in both cholinergic and serotonergic functions causes severe memory impairment in young as well as in aged rats (41). It is hence possible that DPN affects serotonin neurons by ERβ and serotonin via 5HT1B receptor impaired learning and memory.

In addition, despite our results showing Cyclofenil could impair acquisition in passive avoidance task, they also showed that injection of Cyclofenil (10μg/0.5μl) together with DPN (0.5μg/0.5μl) could eliminate impairment caused by use of each per se. It has been demonstrated that ERβ receptors play an important role in passive avoidance learning and memory.

Multiple ERβ isoforms exist as a result of alternative splicing of the last coding exon (exon 8), deletion of one or more coding exons, or alternative usage of untranslated exons in the 5' region (42); Among these, five full-length transcripts designated ERβ1-5, have been reported; the full-length mRNA translated from 8 exons, encoding 530 amino acids, is named ERβ1; the full-length ERβ2-5 transcripts share identical sequences with ERβ1 from exon 1 to exon 7, but have unique sequences in place of exon 8 (43); ERβ4 and ERβ5 isoforms were originally identified as truncated transcripts containing only part of the common exon 7 and different exon 8 sequences (43, 44). In vitro studies show that ERβ4 and β5 can heterodimerize with ERβ1 and enhance its transactivation in a ligand-dependent manner (44). The expression of ERβ3 appears to be restricted to the testis (43) and functional studies on this isoform have not been performed. ERβ2 lacks the AF-2 core region and has undetectable affinity for estradiol and other tested ligands. Interestingly, ERβ2 has been shown to inhibit ligand-induced ERα transcriptional activity on an ERE-reporter gene (45). Some studies report that ERβ1 is the only full-function isoform and that ERβ2, β4, and β5 do not have innate activities in their homodimeric forms but can heterodimerize with ERβ1 and enhance ERβ1-induced transactivation in a ligand-dependent manner. The ER-β isoforms would heterodimerize with ER- β1 and modulate its function. Although ER- β2, - β4, and - β5 do not form homodimers in Y2H, they readily heterodimerize with ER-β1 in the presence of physiological concent-rations of estradiol in a dose-dependent manner. The propensity to dimerize follows the descending order of β1– β4 ≥ β 1– β5 > β1– β1 > β1– β2 .On the basis of analyses, ER- β2, - β4 and, - β5 should have an AF-2 domain different from that of ER- β 1 and may not have a complete helix 12 (43, 46). The molecular weight of ER- β1, - β2, - β4, and - β5 was determined as 59, 56, 54 and 53 kDa, respectively. An in vitro estrogen receptor-binding assay was used to assess the binding affinities of the yeast recombinant proteins. ER- β 1 bound to estradiol

with high affinities (Kd=0.48 nM), comparable with values reported in previous studies (47, 48); ER- β 2 exhibited no binding, whereas ER- β 4 and - β 5 bound to estradiol with moderate affinities (9.87 and 23.45 nM, respectively) (48). These findings are in agreement with molecular modeling data, which demonstrated a relatively open configuration of the ligand-binding pocket in ER- β 4 and - β 5 and an apparent restriction of ligand access to the pocket of ER- β 2 because of its helix 12 positioning. Collectively, data indicates that the isoforms 2, 4, and 5 have no intrinsic transactivation activity for two plausible reasons: (1) They cannot form homodimers because of weak/no ligand binding, and (2) Their inability to recruit coregulators as a result of either the lack of helix 12 in ER- β4 and - β5 or the shrinkage of the coregulator binding cleft in ER- β 2. Yet another important conclusion drawn from these data is that ER- β1 is the only full-function ERβ, and it prefers to heterodimerize with ERβ isoforms, particularly ER- β4 and - β5, under the stimulation of estrogens, excluding phyto-estrogens. All heterodimers have higher transactivation activities than the ER-β1 homodimer. Some studies introduce a previously unrecognized concept for type I nuclear receptor signaling; ER- β 1 serves as the "obligatory partner" of a functional dimeric complex, whereas ER- β 2, - β 4, or - β 5 act as the "variable dimer partners" and serve as enhancers. This model differs from the original paradigm in which the two partners in a nuclear receptor dimer play identical roles in ligand binding and coacti-vator recruitment by way of helix 12. Hence, these data suggest that the ER- β heterodimer may recruit only one coactivator during transcriptional activation (46). To conclude, these findings suggest that estrogens induce the formation of different sets of heterodimers in a specific tissue/cell type, leading to widely varied biological responses, and it is possible that DPN and Cyclofenil affect on different heterodimers of ERβ isoforms, hence they could have same effect on passive avoidance learning and memory. Injection of Cyclofenil and DPN simultaneously could eliminate the impaired effect caused by each per se; so it is possible Cyclofenil which binds to ERβ isoforms, could inhibit the effect DPN has on the other isoforms.

## Financial support

None declared.

## Conflict of interest

None declared.

## References


1. Korol DL. Role of estrogen in balancing contributions from multiple memory systems. *Neurobiol Learn Mem.* 2004;**82**(3):309-23.
2. Holmes MM, Wide JK, Galea LA. Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behav Neurosci.* 2002;**116**(5):928-34.
3. Luine VN, Jacome LF, Maclusky NJ. Rapid enhancement of visual and place memory by estrogens in rats. *Endocrinology.* 2003;**144**(7):2836-44.
4. Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *J Comp Neurol.* 2001;**436**(1):64-81.
5. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A.* 1996;**93**(12):5925-30.
6. Bodo C, Rissman EF. New roles for estrogen receptor beta in behavior and neuroendocrinology. *Front Neuroendocrinol.* 2006;**27**(2):217-32.
7. Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA. Disruption of estrogen receptor beta gene impairs spatial learning in female mice. *Proc Natl Acad Sci U S A.* 2002;**99**(6):3996-4001.
8. Zhang JQ, Cai WQ, Zhou DS, Su BY. Distribution and differences of estrogen receptor beta immunoreactivity in the brain of adult male and female rats. *Brain Res.* 2002;**935**(1-2):73-80.
9. Osterlund MK, Hurd YL. Acute 17 beta-estradiol treatment downregulates serotonin 5HT1A receptor mRNA expression in the limbic system of female rats. *Brain Res Mol Brain Res.* 1998;**55**(1):169-72.
10. Osterlund MK, Keller E, Hurd YL. The human forebrain has discrete estrogen receptor alpha messenger RNA expression: high levels in the amygdaloid complex. *Neuroscience.* 2000;**95**(2):333-42.
11. Jelks KB, Wylie R, Floyd CL, McAllister AK, Wise P. Estradiol targets synaptic proteins to induce glutamatergic synapse formation in cultured hippocampal neurons: critical role of estrogen receptor-alpha. *J Neurosci.* 2007;**27**(26):6903-13.
12. Murakami G, Tsurugizawa T, Hatanaka Y, Komatsuzaki Y, Tanabe N, Mukai H, et al. Comparison between basal and apical dendritic spines in estrogen-induced rapid spinogenesis of CA1 principal neurons in the adult hippocampus. *Biochem Biophys Res Commun.* 2006;**351**(2):553-8.
13. Wilson IA, Puolivali J, Heikkinen T, Riekkinen P, Jr. Estrogen and NMDA receptor antagonism: effects upon reference and working memory. *Eur J Pharmacol.* 1999;**381**(2-3):93-9.
14. Wood GE, Shors TJ. Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. *Proc Natl Acad Sci U S A.* 1998;**95**(7):4066-71.
15. Galea LA, Wide JK, Paine TA, Holmes MM, Ormerod BK, Floresco SB. High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. *Behav Brain Res.* 2001;**126**(1-2):115-26.
16. Krezel W, Dupont S, Krust A, Chambon P, Chapman PF. Increased anxiety and synaptic plasticity in estrogen receptor beta -deficient mice. *Proc Natl Acad Sci U S A.* 2001;**98**(21):12278-82.
17. Rhodes ME, Frye CA. ERbeta-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. *Neurobiol Learn Mem.* 2006;**85**(2):183-91.
18. Walf AA, Rhodes ME, Frye CA. Antidepressant effects of ERbeta-selective estrogen receptor modulators in the forced swim test. *Pharmacol Biochem Behav.* 2004;**78**(3):523-9.
19. Mueller SO, Korach KS. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. *Curr Opin Pharmacol.* 2001;**1**(6):613-9.
20. Muthyala RS, Sheng S, Carlson KE, Katzenellenbogen BS, Katzenellenbogen JA. Bridged bicyclic cores containing a 1,1-diarylethylene motif are high-affinity subtype-selective ligands for the estrogen receptor. *J Med Chem.* 2003;**46**(9):1589-602.
21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition.* 6th ed. New York: Academic Press; 2007.
22. Harooni HE, Naghdi N, Sepehri H, Rohani AH. Intra hippocampal injection of testosterone impaired acquisition, consolidation and retrieval of inhibitory avoidance learning and memory in adult male rats. *Behav Brain Res.* 2008;**188**(1):71-7.
23. Beyer C. Estrogen and the developing mammalian brain. *Anat Embryol (Berl).* 1999;**199**(5):379-90.
24. Speroff L, Glass RH, Kase NG. Clinical gynecologic endocrinology and infertility. In: Mitchell C, editor. *Oncogene:* Scientific & Medical Division, Macmillan Press; 1999.
25. Vazquez-Pereyra F, Rivas-Arancibia S, Loaeza-Del Castillo A, Sch-

neider-Rivas S. Modulation of short term and long term memory by steroid sexual hormones. *Life Sci.* 1995;**56**(14):PL255-60.
26. Ruff M, Gangloff M, Wurtz JM, Moras D. Estrogen receptor transcription and transactivation: Structure-function relationship in DNA- and ligand-binding domains of estrogen receptors. *Breast Cancer Res.* 2000;**2**(5):353-9.
27. Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, et al. Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science.* 1997;**277**(5331):1508-10.
28. Jahr CE. Drooling and stuttering, or do synapses whisper? *Trends Neurosci.* 2003;**26**(1):7-9.
29. Packard MG, Kohlmaier JR, Alexander GM. Posttraining intrahippocampal estradiol injections enhance spatial memory in male rats: interaction with cholinergic systems. *Behav Neurosci.* 1996;**110**(3):626-32.
30. Warren SG, Juraska JM. Spatial and nonspatial learning across the rat estrous cycle. *Behav Neurosci.* 1997;**111**(2):259-66.
31. Handa RJ, Hejna GM, Lorens SA. Androgen inhibits neurotransmitter turnover in the medial prefrontal cortex of the rat following exposure to a novel environment. *Brain Res.* 1997;**751**(1):131-8.
32. Lephart ED. A review of brain aromatase cytochrome P450. *Brain Res Brain Res Rev.* 1996;**22**(1):1-26.
33. Maxwell JW, Croake JW, Biddle AP. Sex differences in the comprehension of spatial orientation. *J Psychol.* 1975;**91**(1st Half):127-31.
34. Blurton-Jones M, Tuszynski MH. Estrogen receptor-beta colocalizes extensively with parvalbumin-labeled inhibitory neurons in the cortex, amygdala, basal forebrain, and hippocampal formation of intact and ovariectomized adult rats. *J Comp Neurol.* 2002;**452**(3):276-87.
35. Cain DP. Prior non-spatial pretraining eliminates sensorimotor disturbances and impairments in water maze learning caused by diazepam. *Psychopharmacology (Berl).* 1997;**130**(4):313-9.
36. Wei H, Xiong W, Yang S, Zhou Q, Liang C, Zeng BX, et al. Propofol facilitates the development of long-term depression (LTD) and impairs the maintenance of long-term potentiation (LTP) in the CA1 region of the hippocampus of anesthetized rats. *Neuro sci Lett.* 2002;**324**(3):181-4.
37. Birzniece V, Backstrom T, Johansson IM, Lindblad C, Lundgren P, Lofgren M, et al. Neuroactive steroid effects on cognitive functions with a focus on the serotonin and GABA systems. *Brain Res Rev.* 2006;**51**(2):212-39.
38. Heisler LK, Tecott LH. Knockout Corner: Neurobehavioural consequences of a serotonin 5-HT(2C) receptor gene mutation. *Int J Neuropsychopharmacol.* 1999;**2**(1):67-9.
39. Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M. Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. *Proc Natl Acad Sci U S A.* 2000;**97**(26):14731-6.
40. Buhot MC, Wolff M, Benhassine N, Costet P, Hen R, Segu L. Spatial learning in the 5-HT1B receptor knockout mouse: selective facilitation/impairment depending on the cognitive demand. *Learn Mem.* 2003;**10**(6):466-77.
41. Wolff M, Savova M, Malleret G, Hen R, Segu L, Buhot MC. Serotonin 1B knockout mice exhibit a task-dependent selective learning facilitation. *Neurosci Lett.* 2003;**338**(1):1-4.
42. Richter-Levin G, Segal M. Age-related cognitive deficits in rats are associated with a combined loss of cholinergic and serotonergic functions. *Ann N Y Acad Sci.* 1993;**695**:254-7.
43. Lewandowski S, Kalita K, Kaczmarek L. Estrogen receptor beta. Potential functional significance of a variety of mRNA isoforms. *FEBS Lett.* 2002;**524**(1-3):1-5.
44. Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, et al. Cloning and characterization of human estrogen receptor beta isoforms. *Biochem Biophys Res Commun.* 1998;**247**(1):75-8.
45. Zhao C, Dahlman-Wright K, Gustafsson JA. Estrogen receptor beta: an overview and update. *Nucl Recept Signal.* 2008;**6**:e003.
46. Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y, et al. Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor ofestrogen action in human. *Nucleic Acids Res.* 1998;**26**(15):3505-12.
47. Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. *Proc Natl Acad Sci U S A.* 2006;**103**(35):13162-7.
48. Jisa E, Dornstauder E, Ogawa S, Inoue S, Muramatsu M, Jungbauer A. Transcriptional activities of estrogen receptor alpha and beta in yeast properties of raloxifene. *Biochem Pharmacol.* 2001;**62**(7):953-61.