Molecular biology and genetics/Biologie et génétique moléculaires

# Gene expression profile of estrogen receptors alpha and beta in rat brain during aging and following high fat diet

Rosaria Scudiero, Mariailaria Verderame *

Department of Biology, University of Naples Federico II, via Mezzocannone 8, 80134 Napoli, Italy

A B S T R A C T

The "sex-hormone" estrogen-17β promotes several cognitive functions and is a master regulator of brain bioenergetics via the estrogen receptors α and β ($ER\alpha$ and $ER\beta$). In this work, by using Real-Time PCR analysis, we evaluated the effect of aging and high fat diet (HFD) on $ER\alpha$ and $ER\beta$ expression in rat hippocampus and cortex. In young rats, $ER\beta$ is abundant in cortex and $ER\alpha$ in hippocampus. During the aging, in the cortex, we observe a general decrease in $ER\alpha$ and $ER\beta$ expression; in hippocampus $ER\alpha$ increases and $ER\beta$ decreases. $ER$ expression patterns in rat brain are also affected by the administration of an HFD. In cortex, after 4 weeks of HFD, $ER\beta$ transcripts are down-regulated, whereas $ER\alpha$ levels remain unchanged; after 12 weeks, both ER$\alpha$ and $ER\beta$ expression is up-regulated. In the hippocampus, the level of $ER\beta$ transcripts does not change following HDF, whereas $ER\alpha$ expression is affected by HDF, in a time-dependent manner: it increases after the 4-week treatment and decreases after 12 weeks. Possible involvements of these receptors in the control of cortex and hippocampus functions during aging and in the modulation of energetic metabolism and feeding behaviour are discussed.

© 2017 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

## 1. Introduction

Estrogens are sex hormones involved in the development and maintenance of normal reproductive functions [1–3]. These hormones exert their biological effects through the interaction of two primary estrogen receptors, members of the nuclear steroid receptors superfamily: estrogen receptor-alpha (ERα), and estrogen receptor-beta (ERβ).

17β-Estradiol (E2) is the most potent and predominant form of estrogen; besides its recognized role in reproduction, it shows a number of effects on cognition and brain function [4–7]. In different brain regions, aromatase activity catalyzes E2 synthesis from androgen precursors and/or cholesterol. E2 is neurotrophic and neuroprotective and some recent results, although controversial, suggest that E2 synthesized within hippocampus and prefrontal cortex may contribute to the normal processes of memory consolidation [8–11]. The effects of E2 on several brain processes decline with age, including the ability to preserve the blood–brain barrier and to protect from stroke and neuroinflammation [12,13].

Accordingly with E2 functions, both $ER\alpha$ and $ER\beta$ subtypes are widely distributed in the brain regions, in particular in the hippocampus, the frontal cortex, and the amygdala [4,14]. The alteration in the normal distribution of ERs has been observed in hippocampal neurons of Alzheimer's disease (AD) patients [15].

It has been demonstrated that the two ER subtypes play different roles in E2-regulated gene expression and neuronal physiology [16]; the differential activation of $ER\alpha$ or $ER\beta$ is able to influence the expression of neuroprotective genes [17,18].

Data on the differential expression of $ER$ genes in the central nervous system (CNS), mostly achieved in females

* Corresponding author.
  E-mail address: ilaria.verderame@unina.it (M. Verderame).

and collected in the rat, the mouse or the human, are often contradictory [19–21]. In the rat hippocampus, $ER\alpha$ is transcriptionally more active than $ER\beta$ [7], and a significant decrease in the number of neurons positive to estrogen receptors during aging is observed [22]. In addition, $ER$s seem to have a gender specificity; indeed, $ER\beta$ expression in the female hippocampus is greater than in the male one [23]. In the cerebral cortex of the same animals, the limited data available show $ER\beta$ as the predominant receptor subtype immunolocalized [24].

In mouse, $ER\alpha$ is the predominant subtype in the hippocampus and in most of hypothalamus, whereas in the cerebral cortex $ER\beta$ is the main receptor subtype observed [25]. Conversely, in human and primates $ER\beta$ is the primary receptor reported in hippocampus [26,27]. In humans, it has been found that following the neurological diseases influencing memory, such as AD, the expression of $ER\alpha$ decreases while $ER\beta$ increases [28–31].

The relationship between E2 and ERs in the brain involves the regulation of food intake. In women, the daily food intake changes in relation to the E2 secretion during the menstrual cycle: during the follicular phase, the daily food intake is lower in respect to the luteal phase [32,33]. Also in rat, the increase in plasma E2 concentration during the preovulatory phase is associated with a transient decrease in food intake [34]. In the CNS, both $ER\alpha$ and $ER\beta$ are expressed in areas associated with satiety and feeding, such as hypothalamus and pituitary [35]; it has been also demonstrated that ER activation is involved in the metabolic effect of insulin signaling in the brain [36]. Finally, E2 has been proposed to act directly and indirectly to decrease orexigenic or anorexigenic peptides and food intake: for instance, $ER\alpha$ null mice are obese, insulin resistant, and show decreased energy expenditure [35,37].

In this study, by using Real-Time PCR analysis, we investigated the expression pattern of $ER\alpha$ and $ER\beta$ genes during aging and after a high fat diet (HFD) in rat brain. In particular, we evaluated the $ER\alpha$ and $ER\beta$ expression profiles in the cerebral cortex and hippocampus of pubertal young (2-months), adult (4- and 8-month), and middle-aged (16-month) rats. Our results demonstrate that $ER\alpha$ and $ER\beta$ subtypes have specific tissue- and age-related expression patterns that are unbalanced by an HFD that leads to the obese phenotype.

## 2. Materials and methods

### 2.1. Animal and experimental design

Male Wistar rats (Charles River, Calco, Como, Italy) of 2 months of age (adolescent), with the same starting body weight ($160 \pm 10$ g), were individually caged in a temperature-controlled room ($23 \pm 1$ °C) with a 12 h light/12-h dark cycle. Animals were housed in the Animal Care Facility at the Department of Biology, with *ad libitum* access to water and to a standard diet (SD, 10.6% fat J/J, 60,4% carbohydrate, 15.47 KJ/g) (Mucedola 4RF21; Settimo Milanese, Milan, Italy) up to 4 (social maturity, group 1), 8 (adulthood, group 2), or 16 (middle-aged, group 3) months. Six animals for each group

were anesthetized with chloral hydrate (40 mg/100 g body wt) and killed by decapitation. Group 4 was made of eight adolescent rats sacrificed four days after their arrival in the animal facility.

In parallel, another group of rats (referred to as HFD, $n = 12$) received a high-fat diet rich in lard (40% fat J/J, 31% carbohydrate, 19.23 KJ/g). The HFD was formulated to differ from the standard diet in the fat and carbohydrate contribution to the energy value but to be identical in terms of proteins, vitamins, minerals and fibers [38]. Six animals of both SD and HFD groups were sacrificed after 4 weeks and other six individuals after 12 weeks. Throughout the experimental period, body weights and food intakes were monitored daily to calculate the body-weight gain. Spilled food was collected and compensated in readjusting the calculation of food intake.

At the end of the experimental period, animals of each group were anesthetized by chloral hydrate (40 mg/100 g body wt) and killed by decapitation. The brains were quickly removed and the cerebral cortex and hippocampus were dissected on ice. Samples of each brain region were snap frozen in liquid nitrogen immediately and stored at $-80$ °C for subsequent RNA isolation. The study was performed in strict accordance with the criteria established by the National Institutes of Health. The protocol for animal care and use was approved by the Committee on the Ethics of Animal Experiments of the University of Naples Federico II.

### 2.2. RNA purification and cDNA synthesis

Total RNA was extracted according to the TRI-Reagent (Sigma Aldrich) protocol. The quality of each total RNA was checked by electrophoresis on 2% agarose gel stained with ethidium bromide and measuring the optical density at 260/280 nm. A ratio of 1.8–2.0 was accepted for further reverse transcription. The QuantiTect Reverse Transcription Kit (Qiagen) was used for the removal of genomic DNA contamination and for the subsequent cDNA synthesis. Approximately 1 μg of total RNA was used, according to the kit's protocol.

### 2.3. Quantitative Real-Time PCR analysis

The Real-Time PCR reactions were carried out in triplicate for each sample in an Applied Biosystems 7500 Real-Time System by using the Power SYBR Green Master Mix PCR (Applied Biosystems) following the procedures recommended by the manufacturer [39]. Each SYBR Green reaction (20-μl total volume) contained 12 μL of Real-Time PCR Master Mix, 1 μL of each of the forward and reverse primer (10 μM), 2 μL of cDNA diluted 1:1 and 4 μL of nuclease free water. For internal standard control, the expression of the β-actin gene was quantified [40]. A single pair of specific primers for both $ER\alpha$ and $ER\beta$ isoforms was designed on the nucleotide sequences of *Rattus norvegicus* $ER\alpha$ (NM012689.1) and $ER\beta$ (AF042058.1); β-actin primers were designed on the *R. norvegicus* template (NM031144.2). Primer sequences are reported in Table 1. The lengths of the obtained cDNA fragments were: $ER\alpha$, 216 bp; $ER\beta$, 262 bp; β-actin, 128 bp.

**Table 1**
Specific primers used for Real-Time PCR analyses.

| Name | Nucleotiode sequence (5'–3') | Length (mer) |
| --- | --- | --- |
| ERα_F | GCACATTCCTTCCTTCCGTC | 20 |
| ERα_R | CTCGTTCCCTTGGATCTGGT | 20 |
| ERβ_F | ACAGTCCTGCTGTGATGAAC | 20 |
| ERβ_R | ACTAGTAACAGGGCTGGCAC | 20 |
| β-act_F | ACCCGCCACCAGTTCGCCAT | 20 |
| β-act_R | CGGCCCACGATGGAGGGGAA | 20 |

The PCR was performed under the following conditions: holding stage of 95 °C per 10 min; cycling stage (45 cycles): 95 °C × 10 s–60 °C × 10 s–72 °C × 10 s; melting stage: 95 °C × 5 s–65 °C × 1 min–95 °C × 30 s–40 °C × 30 s. The melting curve analysis of PCR products was performed in order to ensure gene-specific amplification. Changes in the gene expression relative to the different samples were calculated according to the standard $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen [41].

### 2.4. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) from four separate experiments of each sample. Statistical analyses were carried out by GraphPad Prism software. The differences between the mean values were analyzed by one-way analysis of variance (ANOVA), followed by Fisher's LSD test. The differences were considered significant when $p < 0.05$.

## 3. Results

### 3.1. Expression of ERα and ERβ in cortical and hippocampal areas of 2 month-old rats

First, we investigated the relative abundance of ERα and ERβ in the cerebral cortex and hippocampus of young rats. We found that ERβ was more abundant in the cerebral cortex, whereas in the hippocampus ERα was dominant compared to the cortex (Fig. 1). In general, however, low
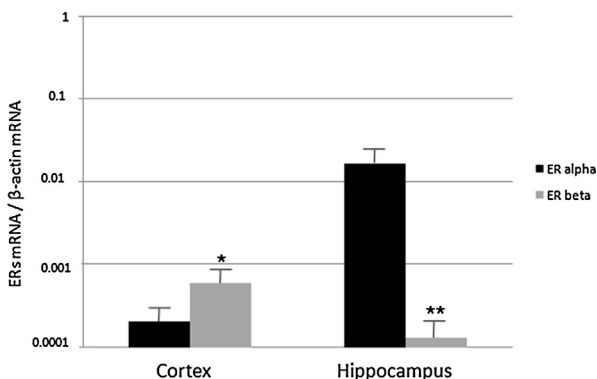


**Fig. 1.** Real-Time PCR analysis of ERα and ERβ expression in cerebral cortex and hippocampus of 2 month-old rats. ERα and ERβ mRNA levels are shown relative to the β-actin mRNA. The data represent the mean ± s.e.m.

but significant amounts of both ERα and ERβ were found in these brain regions.

Real-Time PCR analysis carried out in the cerebral cortex revealed a general decrease in ERα and ERβ expression during aging (Fig. 2). In particular, ERα transcripts decreased by half in 4 month-old rats, then the amount of transcripts remained constant; the ERβ transcript amount rapidly decreased to about one-tenth in 4 month-old rats, before rising in middle-aged rats at a fifth of the value measured in young rats (Fig. 2).

In the hippocampus, the expression of ERα greatly increased with aging, whereas ERβ expression generally decreased (Fig. 2). Compared to 2 month-old rats, the level of ERα transcript was almost unchanged in 4-month-old rats and sharply increased by about 5 and 25 times in adult (8-month-old) and middle-aged (16 month-old) rats, respectively (Fig. 2). As regarding ERβ, a significant decrease in expression was observed in 4- and 8 month-old rats, with mRNA levels 1/5 lower than in 2 month rats; in middle-aged rats, the ERβ expression level slightly increased, although remaining below the value observed in young rats (Fig. 2).

### 3.2. HFD-related changes of ERα and ERβ expression in rat brain

As no data are available on the effect of a HFD on the brain expression of ERs, we evaluated ERα and ERβ mRNA levels in cortex and hippocampus of rats fed a HFD for 4 or 12 weeks.

First of all, we determined the weight gain of HFD-fed rats. Both control and HFD rats showed a significantly weight gain during housing (Table 2). The increase in body weight was affected by the duration of the housing and diet administration. In control animals, the weight gain was of 18% after 4-week and 35% after 12-week housing, respectively. In HFD rats, the weight gain was 47% after 4 weeks and about 80% after 12 weeks, with an increase by 25% between the two 4-week groups and of 48% in the 12-week group.

ER expression, as detected by Real-Time PCR, was significantly affected by the diet. In the cortex of HFD rats receiving the diet for 4 weeks, the level of ERα transcripts did not change, whereas the level of ERβ transcripts was lower than in control animals (about 1/5, Fig. 3). Interestingly, after 12 weeks of HF diet, both ERα and ERβ transcription was dramatically up-regulated (54-fold and 141-fold, respectively) (Fig. 3).

In the hippocampus of rats receiving the HF diet for 4 weeks, the level of ERα transcripts increased about two-fold with respect to control rats, but decreased below the value determined in the control rats, when the treatment was extended for 12 weeks (Fig. 3). As regarding ERβ, the expression level was not affected by the HFD, no matter how long it was (Fig. 3).

## 4. Discussion

Over the past 25 years, evidence shows that estradiol modulates cognitive function in mammals, including humans. The E2 action begins in utero when estrogens
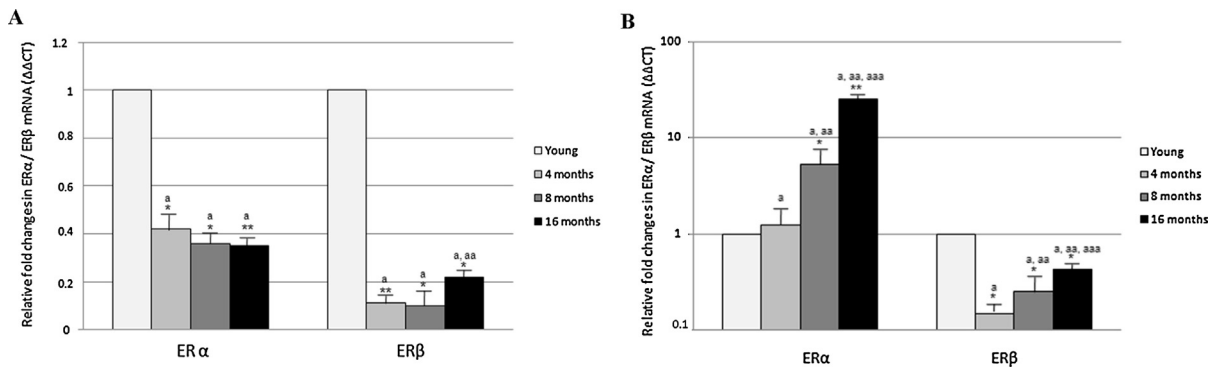
**A**



**B**

Fig. 2. Effect of aging on *ERα* and *ERβ* gene expression in rat cerebral cortex (A) and hippocampus (B). The *ERα* and *ERβ* mRNA expression was normalized to that of β-actin mRNA and converted in fold change, compared with the 2 month-old rats. The data represent the mean ± s.e.m. Significance of differences is shown. $^*p < 0.05$, $^{**}p < 0.001$. a: significance *vs.* 2 months; aa: significance *vs.* 4 and 8 months; aaa: significance *vs.* 8 month.
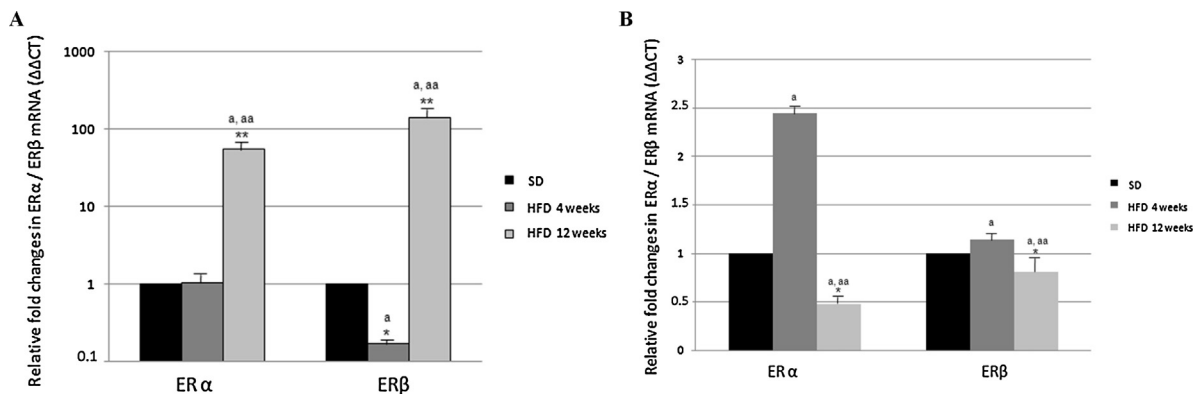
**A**



**B**

Fig. 3. Effect of high fat diet on *ERα* and *ERβ* gene expression in rat cerebral cortex (A) and hippocampus (B). The *ERα* and *ERβ* mRNA expression was normalized to that of β-actin mRNA and converted in fold change, compared with rats fed with a standard diet. The data represent the mean ± s.e.m. Significance of differences is shown. $^*p < 0.05$, $^{**}p < 0.001$. a: significance *vs.* standard diet; aa: significance *vs.* high fat diet for 4 weeks.

**Table 2**
Average body weight in control and HFD rats.

| | Control | | | High fat diet | |
|---|---|---|---|---|---|
| | 0 weeks | 4 weeks | 12 weeks | 4 weeks | 12 weeks |
| Body weight (g) | 311.47 ± 14.3 | 369.22 ± 15.0 | 422.69 ± 18.9 | 460.17 ± 32.1 | 615.4 ± 41.2 |
| Weight gain (%) | | 18.5 | 35.7 | 47.7 | 97.5 |

direct sexual differentiation of the brain regions controlling reproduction and some cognitive functions linked to it. They reach the highest levels during puberty; with aging, levels of circulating estrogens collapse, contributing to the age-related declines in learning and memory function.

The aim of this study was to assess whether changes in *ER*s occur with aging in *rattus* cerebral cortex and hippocampus. Investigations were performed in males to avoid the influence of the fluctuations of steroids, mainly of E2, on the expression and localization of these receptors in brain [42–44].

First, our results demonstrate that the two *ER* subtypes are differentially expressed in the two brain regions examined. Indeed, regardless of the rats' age, in cerebral cortex the *ERβ* subtype is more abundant with respect to the hippocampus; conversely, in the latter, *ERα* is more abundant. However, our results show also a very low transcription rate of both *ERα* and *ERβ* in rat brain. From these data, we can speculate that in male rats, *ERβ* is involved in the control of the complex mental functions of the cerebral cortex, whereas *ERα* may have a role in the hippocampus activities, such as learning and long-term memory. This hypothesis is corroborated by the findings showing that the learning performance in *ERβ*-KO mice was higher to that observed in *ERα*-KO, indicating that removal of *ERβ* preserves the learning abilities [44].

Many studies reported age-related changes in the transcript level of several genes in the mammalian nervous system [45,46]. We found that the relative abundance of *ERα* and *ERβ* transcripts changes during aging in both

cortex and hippocampus. In the cortex, we observed a general and significant decrease in *ER* content from 2- to 16-month-old rats, and these findings are in agreement with that found in the cerebral cortex of mice, where *ERα* and *ERβ* transcription rate decreases in males with increasing age [42]. The *ER* expression trend in the hippocampus is different, as with aging *ERα* is up-regulated, while *ERβ* is down-regulated. In particular, in the male 4-month-old rats, the level of ERs does not change significantly compared to 2 month-old rats, but in adult (8-month) and middle-aged (16-month) rats, the *ERα* expression considerably increases by about 5 and 25 times, and the *ERβ* decreases by about 1/5 and 1/2, respectively.

On the basis of the current knowledge, the *ER* down-regulation observed in the cortical area could be linked to a neuroprotective effect [47]. It is known that Folstein test scores in women in AD terminal stage show an increase in *ERα* levels in the frontal cortex [48], and that estrogen and ER agonists are capable of altering the transcription of genes involved in neuroinflammatory responses in astroglia, interneurons and microglia of the frontal cortex [49–51].

Also in the hippocampus, the observed *ERα* up-regulation could have a neuroprotective role against the onset of neurodegenerative diseases, contributing to the effectiveness of the hormonal therapy ameliorating the memory loss that often accompanies advanced age [52]. In transgenic mice and in humans, it has been demonstrated that cytoplasmatic *ERα* inhibits the development of AD pathology [53,54], whereas *ERβ* increased in the hippocampal cells of AD patients [28]. Moreover, the expression of *ERα* seems to restore the protective action of estrogen against amyloid-β peptide, supporting the key role of *ERα* in estrogen-mediated neuroprotection [55].

Because *ERβ* may act as a negative regulator of *ERα*-mediated transcription [7,22], a general low level of *ERβ* transcripts during aging in brain is not surprising.

Another objective of this work was to evaluate the expression of *ERα* and *ERβ* in brain of rats fed with an HFD for 4 and 12 weeks, a condition that is able to induce systemic and peripheral inflammation, insulin resistance, hepatic steatosis, higher serum glucose and leptin levels [56], as well as inflammation and decrease in the haptoglobin level in the brain [38]. Once again, from our data emerges a different response in the two brain regions. In cortex, the 4-week HFD treatment affects only *ERβ* expression, which significantly decreased; conversely, the 12 weeks of HFD induced a huge increase (by about 100-fold) in both *ERα* and *ERβ* expression. In the hippocampus, the *ERβ* transcripts level did not change following HFD, whereas *ERα* transcript level showed a two-fold increase after 4 weeks of treatment and a halving after 12 weeks.

Evidence exists that circulating estrogen levels are able to induce the expression of leptin receptors, probably through estrogen responsive elements present in the gene promoter region [57]. A crosstalk between ER and leptin receptors has also been evidenced [58–60]. For this reason, the weight gain observed in postmenopausal women is often associated with the decrease in estrogen levels occurring in this period. It has also been demonstrated that estrogen is involved in the regulation of ghrelin expression, so the *ER* down-regulation may jointly cause a reduced response to leptin and an increased susceptibility to ghrelin.

Further, it is interesting to note that obesity increases the risk of age-related cognitive decline and is accompanied by peripheral inflammation [61–63]. In particular, dietary obesity impaired hippocampus-dependent memory and reduced long-term potentiation [63].

In this scenario, it is conceivable that the huge increase in *ER* expression observed in the cortex of rats fed for 12 weeks with a hypercaloric diet may represent a defense mechanism to counterbalancing the excessive energy intake, increasing sensitivity to leptin and reducing hunger. Conversely, the lack of *ER* responsiveness to HFD in hippocampus might be one of the motives for which the dietary composition negatively affects hippocampal functions, making this brain area, crucial for learning and memory, one of the most vulnerable sites in inflammation, in early AD and in other neurodegenerative diseases development.

Taken together, our data add a new slice to identify factors that significantly change in the brain following aging and HFD administration and unveil another possible molecular mechanism involved in the brain consequences of dietary fats.

## Acknowledgments

## References

[1] P. Dostalova, E. Zatecka, K. Dvorakova-Hortova, Of oestrogens and sperm: a review of the roles of oestrogens and oestrogen receptors in male reproduction, Int. J. Mol. Sci. 18 (5) (2017), pii: E904.
[2] S. Farzaneh, A. Zarghi, Estrogen receptor ligands: a review (2013-2015), Sci. Pharm. 84 (3) (2016) 409–427.
[3] M. Verderame, F. Angelini, E. Limatola, Expression of the estrogen receptor alpha switches off the secretory activity in the epididymal channel of the lizard *Podarcis sicula*, Mol. Reprod. Dev. 79 (2) (2012) 107–117.
[4] P.J. Shughrue, I. Merchenthaler, Estrogen is more than just a "sex hormone": novel sites for estrogen action in the hippocampus and cerebral cortex, Front. Neuroendocrinol. 21 (2000) 95–101.
[5] B.S. McEwen, Estrogen actions throughout the brain, Recent Prog. Horm. Res. 57 (2002) 357–384.
[6] T.C. Foster, Interaction of rapid signal transduction cascades and gene expression in mediating estrogen effects on memory over the life span, Front. Neuroendocrinol. 26 (2005) 51–64.
[7] T.C. Foster, Role of estrogen receptor and expression and signaling on cognitive function during aging, Hippocampus 22 (2012) 656–669.
[8] J. Nilsen, R. Diaz Brinton, Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression, Proc. Natl. Acad. Sci. U S A 100 (2003) 2842–2847.
[9] K.B. Jelks, R. Wylie, C.L. Floyd, A.K. McAllister, P. Wise, Estradiol targets synaptic proteins to induce glutamatergic synapse formation in cultured hippocampal neurons: critical role of estrogen receptor-α, J. Neurosci. 27 (2007) 6903–6913.
[10] K.K. Aenlle, T.C. Foster, Aging alters the expression of genes for neuroprotection and synaptic function following acute estradiol treatment, Hippocampus 20 (2010) 1047–1060.
[11] V. Luine, Recognition memory tasks in neuroendocrine research, Behav. Brain. Res. 15 (2015) 158–164.
[12] S. Suzuki, C.M. Brown, P.M. Wise, Neuroprotective effects of estrogens following ischemic stroke, Front. Neuroendocrinol. 30 (2009) 201–211.

[13] V. Benedusi, C. Meda, S. Della Torre, G. Monteleone, A. Vegeto, A. Maggi, A lack of ovarian function increases neuroinflammation in aged mice, Endocrinology 153 (2012) 2777–2788.

[14] S.A. Hart, J.D. Patton, C.S. Woolley, Quantitative analysis of ERα and GAD colocalization in the hippocampus of the adult female rat, J. Comp. Neurol. 440 (2001) 144–155.

[15] X.A. Liu, L.Q. Zhu, Q. Zhang, H.R. Shi, S.H. Wang, Q. Wang, J.Z. Wang, Estradiol attenuates tau hyperphosphorylation induced by upregulation of protein kinase-A, Neurochem. Res. 33 (2008) 1811–1820.

[16] J.F. Couse, K.S. Korach, Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20 (1999) 358–417.

[17] J.C. Carroll, C.J. Pike, Selective estrogen receptor modulators differentially regulate Alzheimer-like changes in female 3xTg-AD mice, Endocrinology 149 (2008) 2607–2611.

[18] S. Gingerich, G.L. Kim, J.A. Chalmers, M.M. Koletar, X. Wang, Y. Wang, D.D. Belsham, Estrogen receptor alpha and G-protein coupled receptor 30 mediate the neuroprotective effects of 17β-estradiol in novel murine hippocampal cell models, Neuroscience 170 (2010) 54–66.

[19] A.H. Lauber, C.V. Mobbs, M. Muramatsu, D.W. Pfaff, Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose, Endocrinology 129 (1991) 3180–3186.

[20] P.J. Shughrue, I. Merchenthaler, Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system, J. Comp. Neurol. 436 (2001) 64–81.

[21] C. Orikasa, Y. Kondo, S. Hayashi, B.S. McEwen, Y. Sakuma, Sexually dimorphic expression of estrogen receptor β in the anteroventral periventricular nucleus of the rat preoptic area: implication in luteinizing hormone surge, Proc. Natl. Acad. Sci. U S A 99 (2002) 3306–3311.

[22] L.A. Bean, L. Ianov, T.C. Foster, Estrogen receptors, the hippocampus, and memory, Neuroscientist 20 (2014) 534–545.

[23] J.Q. Zhang, W.Q. Cai, D.S. Zhou, B.Y. Su, Distribution and differences of estrogen receptor beta immunoreactivity in the brain of adult male and female rats, Brain Res. 935 (2002) 73–80.

[24] A. Mitra, C. Lenglos, E. Timofeeva, Inhibition in the lateral septum increases sucrose intake and decreases anorectic effects of stress, Eur. J. Neurosci. 41 (2015) 420–433.

[25] S.W. Mitra, E. Hoskin, J. Yudkovitz, L. Pear, H.A. Wilkinson, S. Hayashi, D.W. Pfaff, S. Ogawa, S.P. Rohrer, J.M. Schaeffer, B.S. McEwen, S.E. Alves, Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha, Endocrinology 144 (2003) 2055–2067.

[26] M.K. Osterlund, J.A. Gustafsson, E. Keller, Y.L. Hurd, Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERalpha mRNA, J. Clin. Endocrinol. Metab. 85 (2000) 3840–3846.

[27] M. Gonzalez, A. Cabrera-Socorro, C.G. Perez-Garcia, J.D. Fraser, F.J. Lopez, R. Alonso, G. Meyer, Distribution patterns of estrogen receptor alpha and beta in the human cortex and hippocampus during development and adulthood, J. Comp. Neurol. 503 (2007) 790–802.

[28] E. Savaskan, G. Olivieri, F. Meier, R. Ravid, F. Muller-Spahn, Hippocampal estrogen beta-receptor immunoreactivity is increased in Alzheimer's disease, Brain Res. 908 (2001) 113–119.

[29] W.R. Perlman, E. Tomaskovic-Crook, D.M. Montague, M.J. Webster, D.R. Rubinow, J.E. Kleinman, C.S. Weickert, Alteration in estrogen receptor alpha mRNA levels in frontal cortex and hippocampus of patients with major mental illness, Biol. Psychiatry 58 (2005) 812–824.

[30] T.A. Ishunina, D.F. Fischer, D.F. Swaab, Estrogen receptor alpha and its splice variants in the hippocampus in aging and Alzheimer's disease, Neurobiol. Aging 28 (2007) 1670–1681.

[31] Y.L. Lan, J. Zhao, S. Li, Update on the neuroprotective effect of estrogen receptor alpha against Alzheimer's disease, J. Alzheimers Dis. 43 (2015) 1137–1148.

[32] P.M. Lyons, A.S. Truswell, M. Mira, J. Vizzard, S.F. Abraham, Reduction of food intake in the ovulatory phase of the menstrual cycle, Am. J. Clin. Nutr. 49 (1989) 1164–1168.

[33] L. Dye, J.E. Blundell, Menstrual cycle and appetite control: implications for weight regulation, Hum. Reprod. 12 (1997) 1142–1151.

[34] L.A. Eckel, T.A. Houpt, N. Geary, Spontaneous meal patterns in female rats with and without access to running wheels, Physiol. Behav. 70 (2000) 397–405.

[35] P.A. Heine, J.A. Taylor, G.A. Iwamoto, D.B. Lubahn, P.S. Cooke, Increased adipose tissue in male and female estrogen receptor-alpha knockout mice, Proc. Natl. Acad. Sci. U S A 97 (2000) 12729–12734.

[36] W. Pratchayasakul, N. Chattipakorn, S.C. Chattipakorn, Effects of estrogen in preventing neuronal insulin resistance in hippocampus of obese rats are different between genders, Life Sci. 89 (2011) 702–707.

[37] N. Geary, L. Asarian, K.S. Korach, D.W. Pfaff, S. Ogawa, Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-alpha null mice, Endocrinology 142 (2011) 4751–4757.

[38] M.S. Spagnuolo, M.P. Mollica, B. Maresca, G. Cavaliere, C. Cefaliello, G. Trinchese, R. Scudiero, M. Crispino, L. Cigliano, High fat diet and inflammation: modulation of haptoglobin level in rat brain, Front. Cell. Neurosci. 15 (2015) 1–13.

[39] M. Agnese, S. Valiante, L. Rosati, P. Andreuccetti, M. Prisco, Pituitary adenylate cyclase-activating peptide (PACAP) and PAC1 receptor in the testis of cartilaginous fish *Torpedo marmorata*: a molecular and phylogenetic study, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 191 (2016) 26–35.

[40] G.S. Julian, R.W. de Oliveira, J.C. Perry, S. Tufik, J.R. Chagas, Validation of housekeeping genes in the brains of rats submitted to chronic intermittent hypoxia, a sleep apnea model, Plos One 9 (10) (2014) e109902.

[41] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)), Methods 25 (2001) 402–408.

[42] M.K. Thakur, P.K. Sharma, Transcription of estrogen receptor and in mouse cerebral cortex: effect of age, sex, 17-estradiol and testosterone, Neurochem. Int. 50 (2007) 314–321.

[43] T.C. Foster, A. Rani, A. Kumar, L. Cui, S.L. Semple-Rowland, Viral vector-mediated delivery of estrogen receptor- to the hippocampus improves spatial learning in estrogen receptor- knock-out mice, Mol. Ther. 16 (2008) 1587–1593.

[44] X. Han, K.K. Aenlle, L.A. Bean, A. Rani, S.L. Semple-Rowland, A. Kumar, T.C. Foster, Role of estrogen receptor alpha and beta in preserving hippocampal function during aging, J. Neurosci. 33 (2013) 2671–2683.

[45] O.Y. Naumova, M. Lee, S.Y. Rychkov, N.V. Vlasova, E.L. Grigorenko, Gen expression in the human brain: the current state of the study of specificity and spatiotemporal dynamics, Child Dev. 84 (2013) 76–88.

[46] R. Scudiero, L. Cigliano, M. Verderame, Age-related changes of metallothionein 1/2 and metallothionein 3 expression in rat brain, C. R. Biol. 340 (2017) 13–17.

[47] M. Chakrabarti, A. Haque, N.L. Banik, P. Nagarkatti, M. Nagarkatti, S.K. Ray, Estrogen receptor agonists for attenuation of neuroinflammation and neurodegeneration, Brain Res. Bull. 109 (2014) 22–31.

[48] C. Kawas, S. Resnick, A. Morrison, R. Brookmeyer, M. Corrada, A. Zonderman, C. Bacal, D.D. Lingle, E. Metter, A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging, Neurology 48 (1997) 1517–1521.

[49] A. Sierra, A. Gottfried-Blackmore, T.A. Milner, B.S. McEwen, K. Bulloch, Steroid hormone receptor expression and function in microglia, Glia 56 (2008) 659–674.

[50] G. Barreto, M. Santos-Galindo, Y. Diz-Chavez, O. Pernia, P. Carrero, I. Azcoitia, L.M. Garcia-Segura, Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones, Endocrinology 150 (2009) 5010–5015.

[51] Z. Madak-Erdogan, T.H. Charn, Y. Jiang, E.T. Liu, J.A. Katzenellenbogen, B.S. Katzenellenbogen, Integrative genomics of gene and metabolic regulation by estrogen receptors α and β, and their coregulators, Mol. Syst. Biol. 9 (2013) 676.

[52] K.K. Aenlle, A. Kumar, L. Cui, T.C. Jackson, T.C. Foster, Estrogen effects on cognition and hippocampal transcription in middle-aged mice, Neurobiol. Aging 30 (2009) 932–945.

[53] A.F. Jorm, A.E. Korten, A.S. Henderson, The prevalence of dementia: a quantitative integration of the literature, Acta Psychiatr. Scand. 76 (1987) 465–479.

[54] V.W. Henderson, A. Paganini-Hill, C.K. Emanuel, M.E. Dunn, J.G. Buckwalter, Estrogen replacement therapy in older women. Comparisons between Alzheimer's disease cases and nondemented control subjects, Arch. Neurol. 51 (1994) 896–900.

[55] H. Kim, O.Y. Bang, M.W. Jung, S.D. Ha, H.S. Hong, K. Huh, S.U. Kim, I. Mook-Jung, Neuroprotective effects of estrogen against beta-amyloid toxicity are mediated by estrogen receptors in cultured neuronal cells, Neurosci. Lett. 302 (2001) 58–62.

[56] L. Lionetti, M.P. Mollica, R. Sica, I. Donizzetti, G. Gifuni, A. Pignalosa, G. Cavaliere, R. Putti, Differential effects of high-fish oil and high-lard diets on cells and cytokines involved in the inflammatory process in rat insulin-sensitive tissues, Int. J. Mol. Sci. 15 (2014) 3040–3063.

[57] P.A. Bennett, K. Lindell, C. Wilson, L.M. Carlsson, B. Carlsson, I.C. Robinson, Cyclical variations in the abundance of leptin receptors, but not in circulating leptin, correlate with NPY expression during the oestrous cycle, Neuroendocrinology 69 (1999) 417–423.

[58] L.M. Brown, L. Gent, K. Davis, D.J. Clegg, Metabolic impact of sex hormones on obesity, Brain Res. 1350 (2010) 77–85.

[59] R. Fusco, M. Galgani, C. Procaccini, R. Franco, G. Pirozzi, L. Fucci, P. Laccetti, G. Matarese, Cellular and molecular crosstalk between leptin receptor and estrogen receptor-{alpha} in breast cancer: molecular basis for a novel therapeutic setting, Endocr. Relat. Cancer 17 (2010) 373–382.

[60] Z. He, S.S. Zhang, Q. Meng, S. Li, H.H. Zhu, M.A. Raquil, N. Alderson, H. Zhang, J. Wu, L. Rui, D. Cai, G.S. Feng, Shp2 controls female body weight and energy balance by integrating leptin and estrogen signals, Mol. Cell Biol. 32 (2012) 1867–1878.

[61] A.M. Stranahan, E.D. Norman, K. Lee, R.G. Cutler, R.S. Telljohann, J.M. Egan, M.P. Mattson, Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats, Hippocampus. 18 (2008) 1085–1088.

[62] E.C. McNay, C.T. Ong, R.J. McCrimmon, J. Cresswell, J.S. Bogan, R.S. Sherwin, Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance, Neurobiol Learn. Mem. 93 (2010) 546–553.

[63] S. Haoa, A. Dey, X. Yu, A.M. Stranahan, Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity, Brain Behav. Immun. 51 (2016) 230–239.