

Modulation of NR1 subunit of N-methyl-D-aspartate receptor by ovariectomy and passive avoidance learning

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Mahnaz Taherianfard¹, Maryam Sharifi², Mina Tadjali³, Mahboubeh Kohkiloezadeh⁴

¹ Associate Professor, Department of Physiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

² Department of Physiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

³ Department of Histology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

⁴ Department of Surgery, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Keywords

Ovariectomy, NR1 subunit of N-methyl-D-aspartate Receptor, Hippocampus, Cerebellum, Rat

Abstract

Background: Learning and memory are the most intensively studied subjects in neuroscience. Two sites of mammalian brain which are important in learning and memory are CA1 region of hippocampus and Purkinje cell layer of cerebellum. So, the aim of present investigation was to study of the effect of ovariectomy and passive avoidance learning on NR1 subunit of N-methyl-D-aspartate (NMDA) receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum.

Methods: Twenty four Sprague-Dawley rats were used in 4 groups: control-1 (intact without learning), control-2 (intact with learning), ovariectomy without learning, and ovariectomy with learning. Immunohistochemical procedure was used for determination of NR1 subunit of NMDA receptor. A shuttle box apparatus used for passive avoidance learning procedure. The determination of color intensity was done by Photoshop software.

Results: Immunohistological findings indicated that ovariectomy has a negative effect on density of NR1 subunit of NMDA receptors in two brain regions. Passive avoidance learning significantly increased density of NR1

subunit of NMDA receptors in two brain regions.

Conclusion: The results indicated that the sex hormone can modulate function and expression of the NR1 subunit of NMDA receptor in CA1 region of hippocampus and Purkinje cell layer of cerebellum.

Introduction

The significance of estrogen in cognitive processes has also been highlighted by evidence that estrogen may act as neuroprotective agent and may have beneficial effects on cognitive function in Alzheimer's disease.¹⁻³ In mammals, sex steroid hormones are known to influence spatial learning and memory abilities.⁴ The hippocampus, which is involved essentially in learning and memory processes, is known to be a target for the neuromodulatory actions of the steroid hormones. Extensive studies have been performed on the role of steroids in modulating hippocampus plasticity and functions.^{5,6}

In fully developed brain, estrogen can exert effects by up-regulating the gene expression of excitatory N-methyl-D-aspartate (NMDA) receptor subunit.^{7,8} Another study has shown that sex hormones may alter glutamatergic transmission in the brain by regulating expression of glutamate receptors.⁹ Immunohistochemical and in situ hybridization studies in the rat brain demonstrated ubiquitous

expression of NR1 while the NR2 subunits had unique expression pattern in the brain.¹⁰ The NMDA receptor plays a major role in the hippocampus where it is believed to be involved in mechanisms underlying memory acquisition and epilepsy.¹¹ Extensive evidence indicates that NMDA glutamate receptors in the hippocampus and the amygdala are involved in the formation of aversive memory.^{12,13}

There are few studies on the NR1 subunit of NMDA receptor distribution after ovariectomy (OVX). Therefore, the purpose of present study was to investigate the effect of OVX on NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum and also the effect of OVX and learning on NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum.

Materials and Methods

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of the Shiraz University, Iran.

Animals

Twenty four female Sprague Dawley rats weighing 180-200 g were used. Food and water were made available ad libitum. The rats were housed under a 12-hour light/dark (light on at 6 a.m.) and controlled temperature (20 ± 4 °C) condition. The rats were randomly divided (random assignment) into 4 equal groups (n = 6 each): 1) Control-1 group (intact without learning); 2) control-2 group (intact with learning); 3) OVX without learning; and 4) OVX with learning. For OVX, all rats were anesthetized (by 120 mg/kg thiopental sodium) and ovariectomized through a midline laparotomy under sterile conditions.

Learning and memory Procedure

A two-way shuttle-box (made by Aryoazma Co.) with acrylic walls and steel floor bars were used for learning procedure.

On the first day, all animals were individually subjected to 2 minutes of adaptation to the shuttle box,

in which the rat could explore the light compartment and move about freely. At this stage, since the rat likes dark compartment, if the rat did not move to dark compartment after 120 seconds it was removed from study. This adaptation was repeated 30 minutes later. On the second day, the rats were placed in the light compartment box and one second following entering to the dark compartment received a 0.6 mA foot shock for one second. On the third day, the procedure was similar to the second day; the third day considered as learning. On the fourth day, as memory consolidation, the procedure was like the learning days without foot shock. On the fifth day, as memory retention, the procedure was similar to the fourth day. The rats were considered as completely learned, if they did not move to dark compartment after 120 second during third, fourth and fifth session of experiments.

Tissue preparation

In all groups the rats were anesthetized by Natriopental (120 mg/kg) and after heart perfusion of 10% formaldehyde the brains were removed and washed by normal saline and fixed for 72 hours in 10% formaldehyde in 0.1 M phosphate buffer (PB, pH: 7.4), then the brains were post-fixed in 4% formaldehyde in 0.1 M phosphate buffer (PB, pH: 7.4).

Immunohistochemical study was done for labeling of NR1 subunit of NMDA receptor. Negative control sections were incubated with phosphate-buffered saline (PBS) in the absence of primary antibody and no immunoreactivity was detected. The figure 1 shows the negative control. In this photograph, there was no brown color due to reaction of primary antibody. After preparing of digital image from slides, distribution of NR1 subunit of NMDA receptor of glutamate were analyzed by Adobe Photoshop (version 8).¹⁴ This program determined the distribution of receptors according to three character, hue, saturation and intensity; and then showed the number that has reverse relation with the receptor distribution; meaning that higher receptor distribution represented by low number in the program.

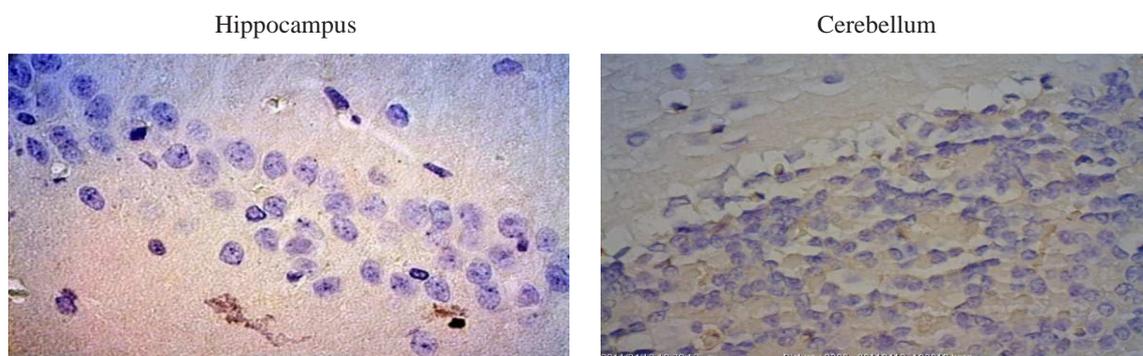


Figure 1. Photograph of negative control in CA1 region of hippocampus and Purkinje cell layer of cerebellum

Statistical analysis

Statistical analyses were performed using SPSS (version 18; SPSS Inc., Chicago, IL.). Student's t-test was used to determine the difference between groups. Data reported as mean \pm standard error of mean and the level of significant was considered as $P < 0.05$.

Results

After passive avoidance learning, NR1 subunit of

NMDA receptor distribution significantly ($P < 0.05$) increased in CA1 region of hippocampus and Purkinje cell layer of cerebellum in comparison between control-1 and control-2 groups (Figures 2, 3, and 4).

After OVX, NR1 subunit of NMDA receptor distribution significantly ($P < 0.05$) decreased in CA1 region of hippocampus and Purkinje cell layer of cerebellum in comparison between OVX without learning and control-1 groups (Figures 2, 3, and 5).

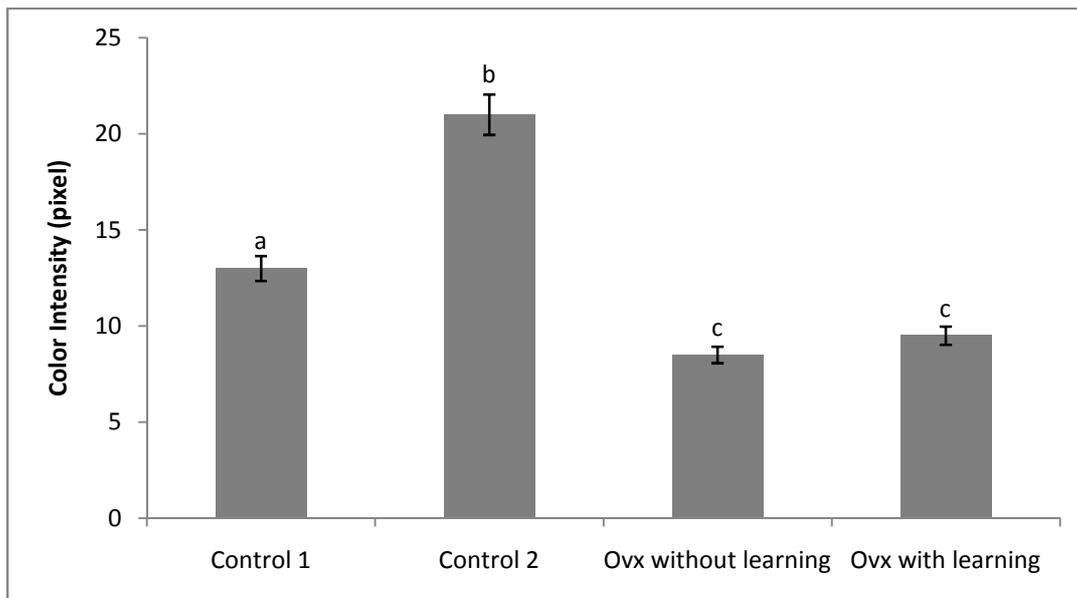


Figure 2. Effect of ovariectomy on NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus

Characters (a, b, and c) shows statistically significant difference between groups ($P < 0.05$).

OVX: Ovariectomy

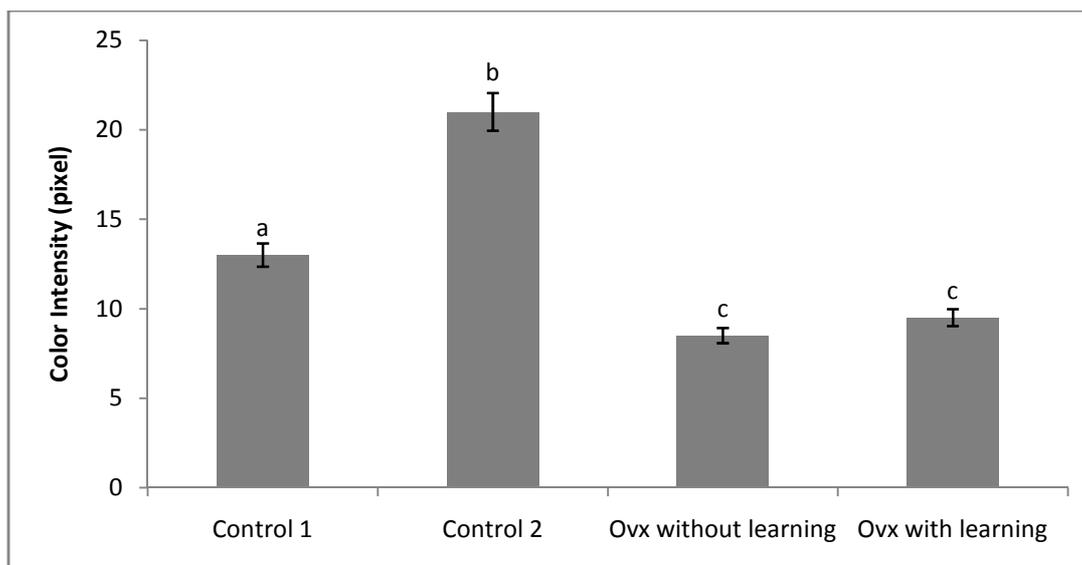


Figure 3. Effect of ovariectomy on NR1 subunit of NMDA receptor distribution in Purkinje cell layer of cerebellum

Characters (a, b, and c) shows statistically significant difference between groups ($P < 0.05$).

OVX: Ovariectomy

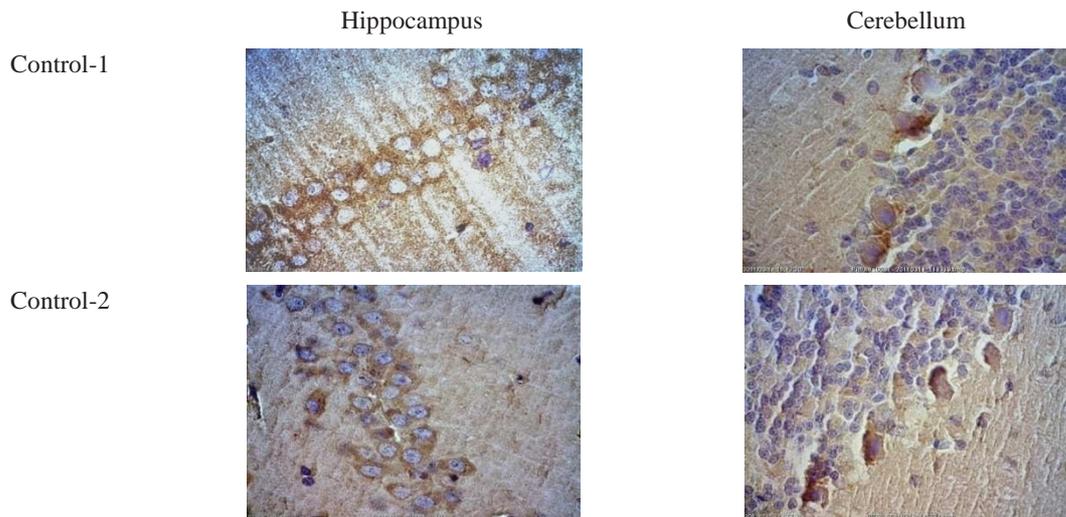


Figure 4. Photograph of NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum in control-1 and control-2 groups ($\times 640$)

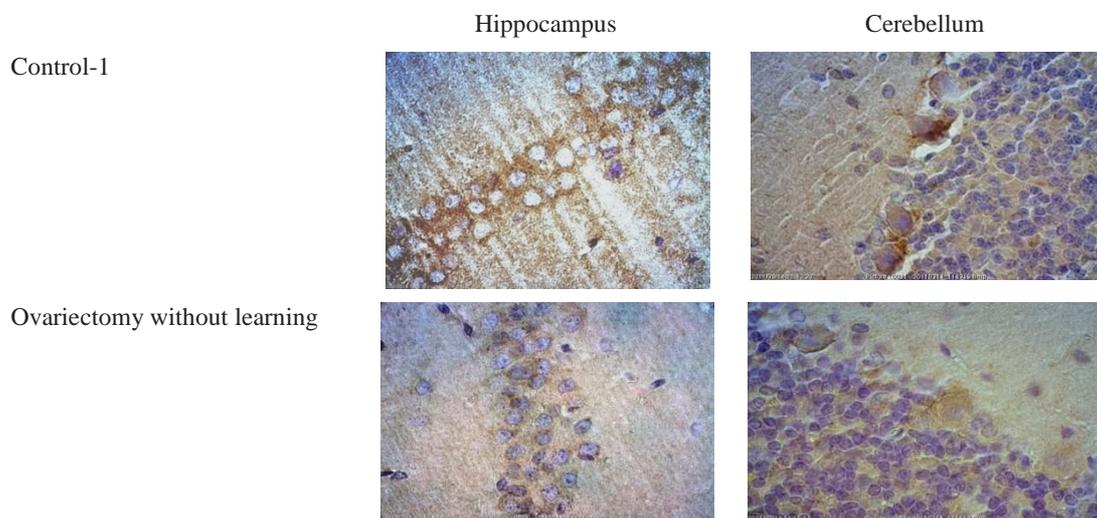


Figure 5. Photograph of NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum in control-1 and ovariectomy without learning group ($\times 640$)

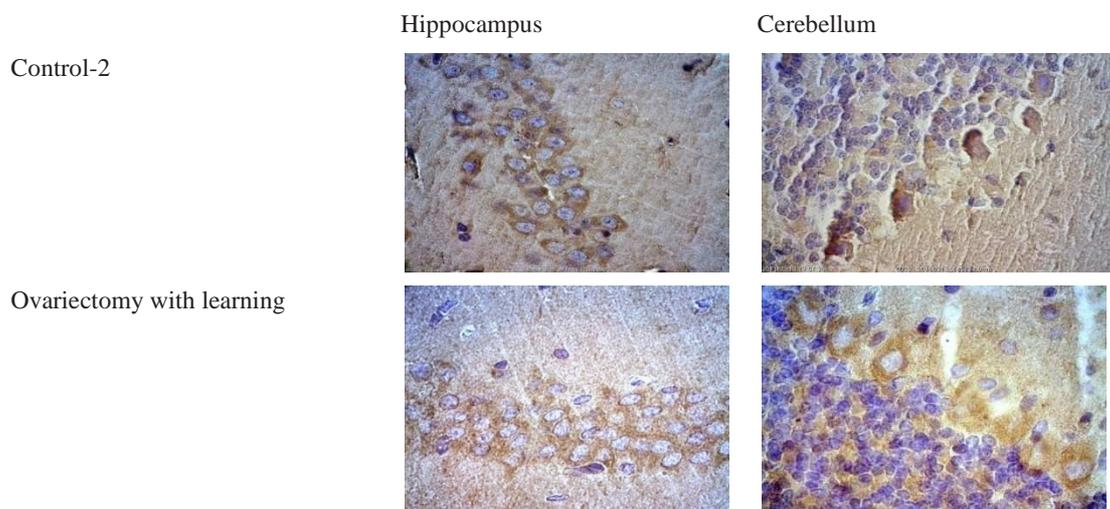


Figure 6. Photograph of NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum in control-2 and ovariectomy with learning ($\times 640$)

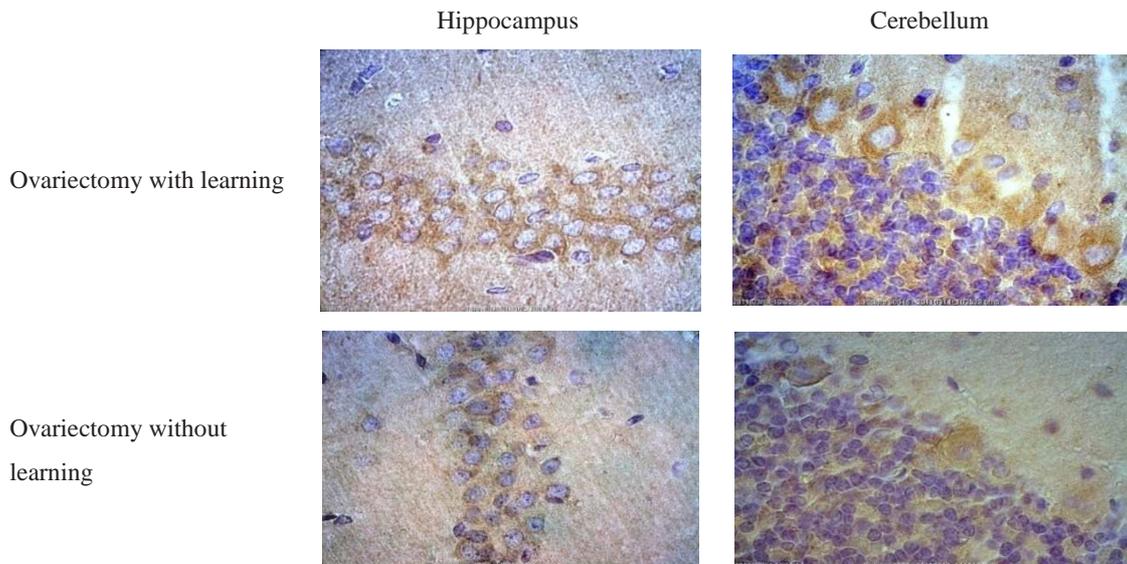


Figure 7. Photograph of NR1 subunit of NMDA receptor distribution in CA1 region of hippocampus and Purkinje cell layer of cerebellum in ovariectomy with learning and ovariectomy without learning groups ($\times 640$)

Comparing OVX with learning and control-2 groups, NR1 subunit of NMDA receptor distribution significantly ($P < 0.05$) decreased in OVX with learning group in CA1 region of hippocampus and Purkinje cell layer of cerebellum (Figures 2, 3, and 6).

In comparison between OVX with and without learning groups, there were no significant differences in CA1 region of hippocampus and Purkinje cell layer of cerebellum (Figures 2, 3, and 7).

Discussion

Neurosteroids positively and negatively modulate glutamate receptors, which underlie fastest inhibition and excitation in the central nervous system.¹⁵ In the present study, NR1 subunit of NMDA receptor distribution increased in response to learning. NMDA receptors are widely distributed in brain, but the highest concentrations of NMDA binding sites are found in area CA1 of hippocampus, with substantial concentrations also localized within the dentate gyrus. NMDA receptors in hippocampal CA1 area are very important in the regulation of synaptic plasticity and the processes of learning and memory, including short- and long-term memory.^{10,16}

In the present study, NR1 subunit of NMDA receptor decreased after OVX. Chronic ovarian hormone deficiency, which was provoked during development, reduced brain capability to propagate cortical spreading depression in adulthood.¹⁷ There is an interaction between estrogen and the NMDA receptor following middle cerebral artery occlusion that may contribute to estrogen's neuroprotective properties in the cortex.¹⁸

The effect of 17 β -estradiol in the hippocampus was confirmed; both estrogen receptor subtype α and β are found in the hippocampus although in low concentration.¹⁹ The estrogenic effect of other brain regions containing estrogen receptors, such as the septum mediates by NMDA receptors.²⁰

In the present study, NR1 subunit of NMDA receptor in ovariectomized rats with learning and without learning in hippocampal CA1 and Purkinje cell layer of cerebellum was the same. Ovarian hormone depletion without estradiol replacement reduced the sensitivity of the hippocampus to estradiol-induced increases in CA1 apical dendritic spine density. Although two acute injections of 17-B-estradiol (10 μ g each) increased CA1 apical spine density and spine heads in rats sacrificed after 10 weeks of ovarian hormone absence.²¹

Beltran-Campos et al. reported that after only one training session in water maze, the spine number in hippocampal CA1 increased by 38% relative to non-learning group; while OVX produced a clear spine pruning in the CA1 apical dendrite, with a 44% reduction from normal level.²² Heikkinen et al. showed that OVX impaired and estrogen treatment improved acquisition of the radial arm maze task in mice. The improvement induced by the estrogen treatment was even more pronounced in the mice with the longer, 40-day treatment than in the mice with the shorter, 7-day treatment.²³

Conclusion

According to our results:

- 1- Passive avoidance learning lead to increases in

NR1 subunit of NMDA receptor distribution in hippocampal CA1 and Purkinje cell layer of cerebellum in female rat.

2- OVX lead to decrease in NR1 subunit of NMDA receptor distribution in hippocampal CA1 and Purkinje cell layer of cerebellum.

3- NR1 subunit of NMDA receptor distribution in ovariectomized rats with and without learning was the same. It means that the absence of ovarian hormone prevents the up-regulation of NR1 subunit

of NMDA receptor following learning and memory in hippocampal CA1 and Purkinje cell layer of cerebellum.

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