

**THE COMPERATION BETWEEN CA3 NEURONAL DENSITY IN  
OVARIECTOMIZED AND NORMAL RATS**

**Maryam Tehranipour\* and Lila Yosofi Sangani**

**Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.**

**Summary**

Ovarian hormones regulate a wide variety of non-reproductive functions in the central nervous system by interacting with several molecular and cellular processes. The aim of this study is the evaluation of CA3 neuronal density in ovariectomized rats in compare with normal rats. Eighteen female Wistar Rats weight (300-350 g) were completely divided into two experimental groups and one control group. Animals were anesthetized under interaperitoneal injection of a mixture (1:2) of 10% ketamin and 2% xylazine then the gonads were removed. After one month and two, Animals were decapitated and their brain dissected, fixed in 10% formalin, sectioned in 7 $\mu$ m thickness and stained by H.E. By applying stereological techniques and systematic random sampling scheme the neuronal density of CA3 were estimated. Statistical analyses showed significant decrease ( $p<0/01$ ) in the CA3 neuronal density in experimental groups. Then reduce the steroidal hormones induced neuronal degeneration in CA3 in way that reduced the neuronal density of CA3.

**Key words:** CA3, stereo logy, ovarectomy

**Running title:** CA3 neuronal density in ovariectomized rats

**Corresponding Author:** Tehranipour Maryam, Department of Biology, Faculty of Science, Islamic Azad University, Mashhad Branch, Mashhad, Iran. Tel: +98511835050 Fax: +985118424020E mail: maryam\_tehranipour@mshdiau.ac.ir

**Introduction**

Accumulating evidence indicates that ovarian hormones regulate a wide variety of non-reproductive functions in the central nervous system by interacting with several molecular and cellular processes (1). Estrogen loss and memory loss have been shown to be related in those suffering AD. Estrogen receptors have been found in several places throughout the brain including the hypothalamus, preoptic area, anterior pituitary, and most importantly, the hippocampus (2).

The hippocampus is an important structure for memory processing. It is a particularly vulnerable and sensitive region of the brain that is also very important for declarative and spatial learning and memory (3). Hippocampus neurons are vulnerable to seizures, strokes, and head trauma, as well as responding to stressful experiences. At the same time they show remarkable plasticity, involving long-term synaptic potentiation and depression, dendrite remodeling (synaptic turnover, and neurogenesis in the case of the dentate gyrus (4).The hippocampus has been implicated in certain short-term memory. Indeed hippocampus lesions often produce short-term memory deficits (5).The hippocampus is preferentially susceptible to a wide variety of toxic insults and disease processes, including hypoxia-ischemia and hypoglycemia (6).Metabolic diseases such as diabetes and obesity have been associated with increased vulnerability to stress (7) and cognitive dysfunction (8).

In animal models, ovarian hormones have been shown to influence memory via actions on neurons, particularly in the hippocampus. Gonadal hormones affect the nervous system in ways that extend beyond their essential actions of regulating gonadotropin and PRL secretion and modulating sexual behavior(9). Confronting the efficacy of a regenerative therapeutic is the degenerative environment that is characterized by neuronal loss, physical plague and glial scar barriers and inflammation(10,11), But perhaps more fundaments from a regenerative prospective, are changes in the biochemical milieu of steroid and peptide growth factors, cytokines and neurotransmitter systems(11,12,13). Data from multiple researches indicate that gonadal steroid hormones and their metabolites can promote neural health whereas their decline or absence is associated with reduction in neural health and increased risk of neurodegenerative diseases including Alzheimer's (14,15). In ovariectomized mice with or without estrogen replacement, regeneration of the sciatic nerve after crush injury was studied. Functional recovery, quantified with sciatic functional index was significantly accelerated in estrogen – treated mice throughout the regeneration (10). Sexosteroid hormones are potential neuroprotective candidates following CNS injury (16) and can play an important role in promoting and enhancing repair after traumatic brain injury and stroke. Although many of its specific actions on neuroplasticity remain to be

discovered. There is growing evidence that these hormones may be a safe and effective treatment for traumatic brain injury and other neural disorders in humans (17). In this research, the neuroprotective actions of testosterone on populations of injured rat spinal motoneurons will be discussed. The aim of present experimental design was to evaluation of CA3 neuronal density in ovariectomized rats in compare with normal rats.

### **Materials and methods**

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2011-2011).

#### **Animal subjects:**

Eighteen female, Wistar rats weighting between 300-350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights on at 6.00h. Ambient temperature in the animal facility was kept at  $22\pm 2^{\circ}\text{C}$ . Food and water was given ad libitum.

#### **Groups:**

1. Controls (N=8)

2.,3. Ovariectomized groups (N=8)

(In group2, the time for brain sampling was 1 month after ovariectomy but in group 3 was 2 month.)

#### **Surgery:**

Animals were anesthetized under interaperitoneal injection of an initial dose of  $60\text{mg kg}^{-1}$  ketamine and  $6\text{ mg kg}^{-1}$  xylazine (ip). Then gonads were removed. In group 2 after one month and in group 3 after 2 month, Animals were anesthetized with sodium pentobarbital( $64\text{mg/kg}$ ) and decapitated. The whole brain was removed and fixed in 10% paraformaldehyde. NaCl was added to the fixative to make the tissue float in order to overcome deformities during the fixation period(18). Paraffin embedded tissue blocks were sectioned at  $7\mu\text{m}$  thickness coronally and stained. A uniform random sampling scheme was employed so that about 10 sections from each block were sampled. With each section thus selected its immediately preceding neighbor was also collected. Sections were stained with toluidine blue staining method with special buffer of acetic acid, sodium acetate and distilled water (PH=4.65). Neuronal density in CA3 was determined, using stereological counting technique; physical dissector (19).

The dissector principle was used to determine the numbers of neurons in each section. From each section and its adjacent neighbor two photos were taken, one from each section with a final magnification of 100. A two-dimensional unbiased counting frame was overlaid in a uniform, random manner on to regions of any two photos taken of both sections. Those cell nuclei selected by the frame on the reference plane but not appearing on the adjacent look-up frame section were deemed to have their tops in the volume described by the product of the area of the counting frame and the distance between sections. These nuclei were counted (Q) to provide the numerical density of cells (NV) in the ventral horns of 100-spinal cord according to the equation:

$$NV = \frac{\sum a}{\sum frame \times V_{di\ sec\ tor}}$$

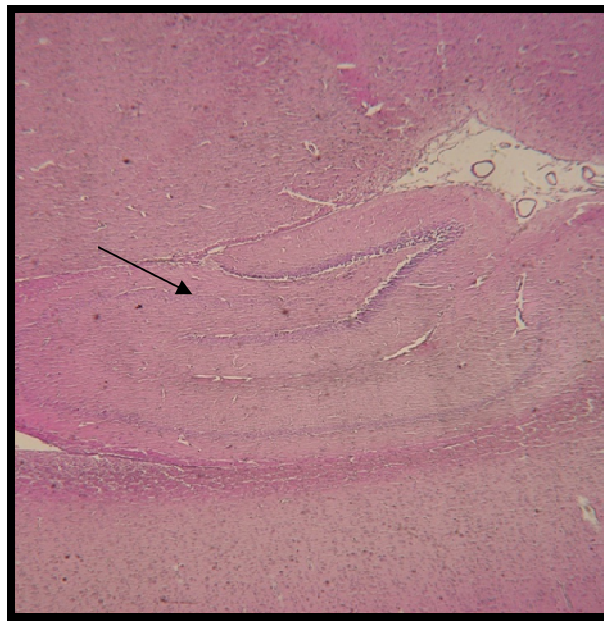
Where  $\sum a$  is the sum of counted neurons, h is the depth of the dissector equal to the section thickness (7 micron) and a (frame) is the scaled area of the dissector frame (20).

### **Statistical analyze**

The ratio of numerical density of neurons in samples of brain was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test. Student's t test was used for comparison when only 2 groups were analyzed. Statistical significance was chosen as  $p < 0.05$ . All results are reported as mean  $\pm$  SEM.

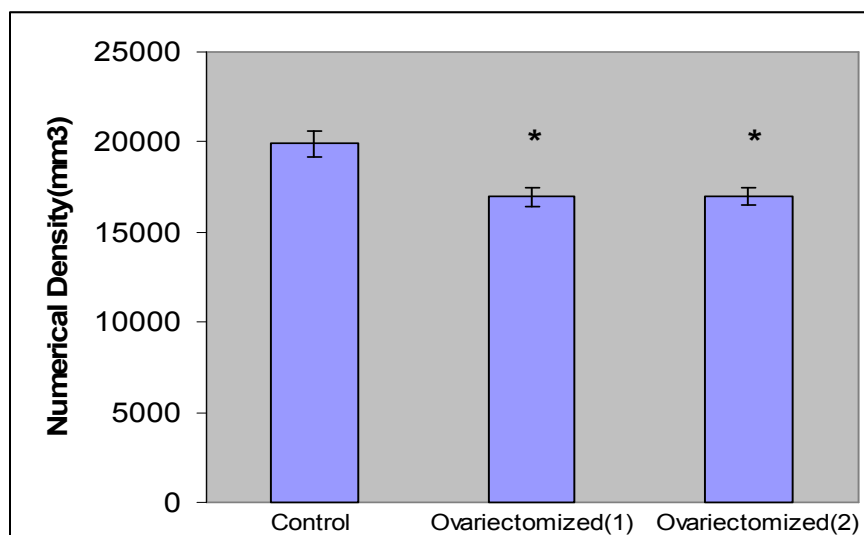
### **Results**

The effects of gonad hormone on the numbers of intact neurons in the CA3 region of hippocampus after ovariectomy in rats are shown in (Fig.1,2,3).



**Fig.1.**Photomicrograph illustrates neurons of the CA3 region of hippocampus at magnification of (20×).  
spiks show the CA3.

1-The control group revealed healthy neuronal cells amounted by  $(19919 \times 10^{-9} \pm 731)$  intact neurons. The neuronal cells in ovariectomized (1) were  $(16957 \times 10^{-9} \pm 516)$  and ovariectomized (2) group  $(16975 \times 10^{-9} \pm 479)$ . The numerical density in control group has significant increased ( $P < 0.05$ ) in comparison to ovariectomized groups (Fig.2).

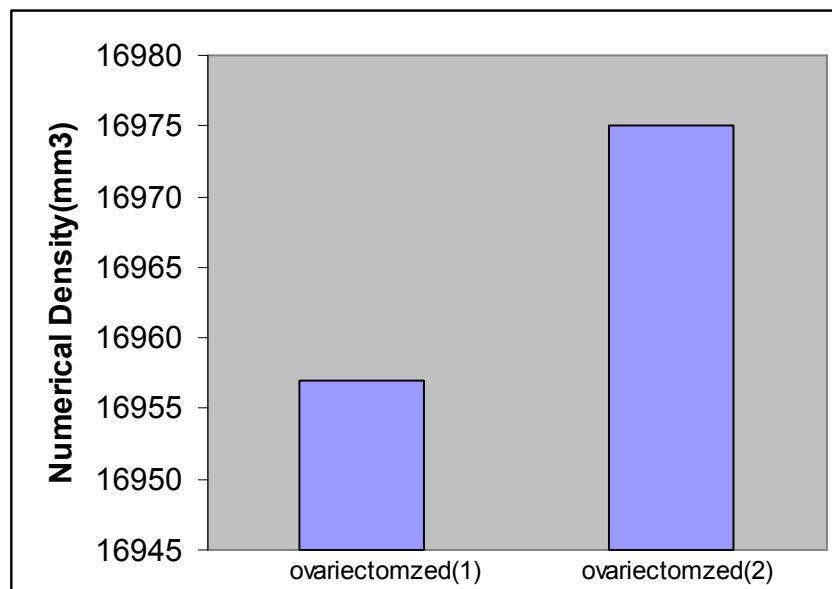


**Fig.2:** Effects of gonad hormone on the intact neurons numerical density of hippocampus CA3 region in rats. Results are expressed as Mean± SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test.

2- As has shown in fig.2, ovariectomy resulted in massive neuronal damage manifested as a significant ( $P < 0.05$ ) 25% decrease in the number of normal appearing neurons after one month.

3- After two month this reduction was not clear more. There wasnot a remarkable decrease in this group in compare with ovariectomized (1) ( $P > 0.05$ ).

4- When compare ovariectomzed (1) with (2), there was not any significant changes in numericl density (Fig.3).



**Fig.3. Photomicrographs illustrate neurons of hippocampus CA3 region in rats at magnification of (20×), 30 and 60 days after injury.**

### **Discussion**

The hippocampus is especially important because it has been shown to be involved in certain aspects of learning and memory (21) the degeneration of which are primary effects of Alzheimer's. The reasons for this degeneration are many-fold. According to one study, estrogen is associated with an increase in the release of certain brain chemicals which transmit information across the space between two nerve cells, in the brain (2). Therefore, when estrogen presence decreases or disappears, neural chemicals which previously were available in high quantities are no longer largely present causing a decrease in

brain activity. A study of estrogen-treated rats tends to support this theory in that the treated rats had higher memory performances than did estrogen-deprived rats (2)

Our findings demonstrate that endogenous estrogen plays an important role in the maintenance and survival the cell. The data show that there is a remarkable change in the number of neurons in different groups. Endogenous estrogen in control animals resulted in a significant ( $P < 0.05$ ) increase in the number of intact neurons, respectively as compared to ovariectomized groups (Fig. 2). Result show that the rate of degeneration was increased in ovariectomized groups during the experiment. It means that in control group endogenous estrogen acts as a survival factor and suppresses the degeneration phenomena. It is also recommended that whenever in the animal gonad hormone decreased program cell death was happened. In ovariectomized groups even omitting the hormone was longer, the numerical density was reduced more (Fig.3).

Gonadal androgens (testosterone) can act as a regulator of the expression of receptors for trophic factors, proteins critical for the maintenance of normal structure and function. The cellular effects of gonadal hormone can be grouped into genomic and non genomic categories. Genomic effects are related to transcription and translation of genes, but non genomic effects occur very rapidly and involve in ion movements and/or initiation of signal transduction cascades (22). Testosterone might act directly through androgen pathways or indirectly via conversion to estrogen (8). There is a consensus that these hormones increase secretion of neuromediators, stimulate formation of new synapses and activation of certain genes, responsible for production of anti-apoptotic proteins and growth factors (23). Further more, accumulated evidence suggests that TP manifests its effect on neuronal regeneration in the emendate post operative or pre regenerative phase by altering the cellular stress response (24).

A report argues for a previously unrecognized antioxidant cycle for estrogen derived compounds (25). Other researchers reported that exogenous administration of testosterone immediately after nerve injury impacted positively on functional recovery through actions mediated by the androgen receptors (AR) (26). They have suggest that mechanism, by which steroidal enhancement of the regenerative properties of the injured motoneurons occurs, may involve pre-existing AR, modulation of the cellular stress response and heat shock proteins. It is interesting that steroid hormones regulate ribosomal gene expression and nuclear ultra structure in target tissues (8).

Furthermore, induction of hippocampal LTP is facilitated in ovariectomized rats treated with estrogen as compared to untreated ovariectomized rats (1). Generaly, the results of present study indicated that

endogenous estrogen may change the media for regenerating neurons or survival the cell. It is concluded that estrogen with the protective role is clinically beneficial in the cases of neuronal death. If estrogen provides neuroprotection as seen in rats, estrogen treatment would act to save a number of patients from CNS damage.

### **Acknowledgments**

Authors would like to thanks the Islamic Azad university of Mashhad for financial Supports.

### **References**

- 1- Michael R. Foy, Michel Baudry, Roberta Diaz Brinton, and Richard F. Thompson. Estrogen and Hippocampal Plasticity in Rodent Models. *J Alzheimers Dis.* (2008);15(4): 589.
- 2- Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *Journal of Comparative Physiological Psychology.* (1979);93:74–104.
- 3-Artola A. Diabetes-, stress- and ageing-related changes in synaptic plasticity in hippocampus and neocortex the same metaplastic process? *Eur J Pharmacol.*(2008); 6;585(1):153-62.
- 4-McEwen BS. Stress and the aging hippocampus. *Front Neuroendocrinol.* (1999);20(1):49-70.
- 5-Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. Hippocampal damage and exploratory Preferences in rats: memory for objects, places, and contexts. *Learn Mem.*( 2002);9(2):49-57.
- 6-Piotrowski P, Wierzbicka K, Smiałek M. Neuronal death in the rat hippocampus in experimental diabetes and cerebral ischaemia treated with antioxidants. *Folia Neuropathol.*(2001); 39(3):147-54.
- 7-Damasceno DC, Volpato GT, de Mattos Paranhos Calderon I, Cunha Rudge MV. Oxidative stress and diabetes in pregnant rats. *Anim Reprod Sci.*(2002); 15;72(3-4):235-44.
- 8-Nazer J, Ramírez R. Congenital malformations in the offspring of diabetic mothers. *Rev Med Chil.*(2000); 128(9):1045-52.
- 9- Wojtal K, Trojnar M K and Czuczwar S J. Endogenous neuroprotective factors: Neurosteroids. *Pharmacological Reports* (2006); 58: 335–340.
- 10- Bramlett H M and Dietrich W D. Neuropathological protection after traumatic brain injury in intact female rats versus males or ovariectomized females. *Journal of Neurotrauma*(2001); 18: 891–900.
- 11- Islamov R R, Hendricks W A, Jones R J, Lyall G J, Spanier N S and Murashov A K. 17Beta-estradiol stimulates regeneration of sciatic nerve in female mice. *Brain Research* (2002);943: 283–286.
- 12- Fargo K N, Alexander T D, Tanzer L, Poletti A and Jones K J. Androgen regulates neuritin mRNA levels in an invivo model of steroid-enhanced peripheral nerve regeneration. *Journal of Neurotrauma*(2008); 25: 561–566.



- 13- Belle M D and Lea R W. Androgen receptor immunolocalization in brains of courting and brooding male and female ring doves (*Streptopelia risoria*). *General and Comparative Endocrinology*(2001);124: 173–187.
- 14- Kulkarni J, de Castella A, Fitzgerald P B, Gurvich C T, Bailey M, Bartholomeusz C and et al. Estrogen in severe mental illness: A potential new treatment approach. *Archives of General Psychiatry*(2008); 65: 955–960.
- 15- Wang J M, Irwin R W, Liu L, Chen S and Brinton R D. Regeneration in a degenerating brain: potential of allopregnanolone as a neuroregenerative agent. *Current Alzheimer Research*(2007); 4: 510–517.
- 16- Singh M, Sumien N, Kyser C and Simpkins JW. Estrogens and progesterone as neuroprotectants: What animal models teach us. *Frontiers in Bioscience* (2008); 13: 1083–1089.
- 17- Billeci A M, Paciaroni M, Caso V and Agnelli G. Hormone replacement therapy and stroke. *Current Vascular Pharmacology* (2008);6: 112–123.
- 18- Behnam-Rasouli M, Nikravesh M, Mahdavi N and Tehranipour M. Post-treatment time after sciatic nerve crush on the number of alpha motoneurons, using a stereological counting method (disector). *Iranian Biomedical Journal* (2000); 4(1): 45–49.
- 19- Tehranipour M, Khakzad M. Effect of maternal diabetes on hippocampus neuronal density in neonatal rats. *J. of biological Sciences*.(2008);8(6).1027-1032.
- 20- Gundersen HJG, Bendtsen TF and et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* (1988); 96(5):379-394.
21. Barnes CA, Rao G, Foster TC, McNaughton BL. Region-specific age effects on AMPA sensitivity: electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. *Hippocampus* 1992;2:457–468.
- 22- Falkenstein E, Tillman H C, Christ M, Feuring M and Wehling M. Multiple actions of steroid hormones a focus on rapid, nongenomic effects. *Pharmacological Reviews*(2000); 52: 513–555.
- 23- Rudziński W and Krejza J. Effects of estrogens on the brain and implications for neuro-protection. *Polish Journal of Neurology and Neurosurgery* (2002); 36: 143–156.
- 24- Tanzer L and Jones K J. Neurotherapeutic action of testosterone on hamster facial nerve regeneration: Temporal window of effects. *Hormones and Behavior* (2004); 45: 339–344.
- 25- Prokai-Tatrai K, Perjesi P, Rivera-Portalatin N M, Simpkins JW and Prokai L. Mechanistic investigations on the antioxidant action of a neuroprotective estrogen derivative. *Steroids*(2007);73: 280–288.
- 26- Jones K J, Brown T J and Damaser M. Neuroprotective effects of gonadal steroids on regenerating peripheral motoneurons. *Brain Research Reviews*(2001); 37: 372–382.