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Research Article

THE EFFECT OF 17B-ESTRADIOL ON CA1 HYPPOCAMPAL NEUROGENESIS DURING THE AGING PROCESS

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ABSTRACT
The aging process affects all body tissues including the brain and different hormones reduced with age in mammals. This research was performed to study the effects of hormonal changes during the aging process on neurogenesis in the hippocampus. The female Wistar rats were divided into five groups including pre-pubertal, pubertal in metestrus phase, pubertal treated with 17β-estradiol, post-pubertal in metestrus phase and post-pubertal treated with 17β-estradiol. The groups received 50 µg/kg 17β-estradiol subcutaneously daily injections for 7 days. Groups in metestrus phase were detected in based on the observed cells in the vaginal smear. The results revealed that cell proliferation along with 17beta-estradiol injection in the CA1 region of hippocampus was increased and cell proliferation in the adult group was significantly higher than the after puberty group.

Keywords: *Neurugenesis, Hyppocampus, 17β-estradiol, CA1, Metestrus*

INTRODUCTION
Estrogens have well known effects on reproductive behaviors and associated brain regions; however, estrogens also influence non-reproductive behaviors such as cognition and associated brain regions such as the hippocampus. Past research in this area has shown a strong but complex relationship between estrogens and cognition, with many profound alterations in neuroplasticity in the hippocampus coinciding with estrogens' influence on cognition. The hippocampus is a limbic structure that is critical for spatial, contextual, and relational memory formation (Eichenbaum, 2004) and is involved in and responsive to stress (McEwen *et al.*, 2007). Interestingly, the hippocampus shows a remarkable degree of plasticity in response to steroid hormones such as estrogens and glucocorticoids (Galea *et al.*, 2006; Galea, 2008; Galea *et al.*, 2008).

Hormones are changed somewhat during the aging process**.** Sexual hormones are decreased with age in mammals and the changes in the human appear with having disorders in the nerve cells regeneration, depression and other psychological disturbances (Garcia-Segura, 2009). The wide variety of the neurological disorders will necessitate more research on treatment and since the majority of neurons are involved in neurological disorders, more studies are required regarding the proliferation and persistence issues. Furthermore, Neurogenesis process decreases with age (Kuhn *et al.*, 1996; Kempermann *et al.*, 1997; Nilsson *et al.*, 1999). Estrogen is a sexual hormone involved in the reproductive system, and the nervous system is also affected. Thus, estrogen acts more than a sexual hormone; steroids perform their actions by binding to two intracellular receptors, ERα and ERβ (Sherwin, 2006; Shughrue *et al.*, 2000). Aging affects all body tissues, including the brain and different hormones, including growth hormone, IGF-1 and finally sexual hormones are reduced with aging in mammals. This suggests that changes in hormone levels due to aging may have negative effects on brain function (Garcia-Segura, 2009). Several studies indicate that the older brains in comparison with younger brains respond in different ways to estrogen therapy. Less spines, including the alpha estrogen receptors, are on CA1 pyramidal cells in aged female rats compared to young females (Adams *et al.*, 2002). Levels of sexual hormones are related to sexual cycle in females. Because of the short reproductive cycle of rodents named estrus cycle, they are used as ideal animal models for research on the reproductive cycle changes. Estrous cycle happens every

four or five days and includes four stages: proestrus, estrus, metestraus and diestrus. Proestrus stage has the highest hormonal level and the metestrus stage has the lowest level in most periods of time (Caligioni, 2009). Estradiol affects cell proliferation and cell survival in adult female rodents. However, these effects vary with the hormonal status of the individual, the duration of exposure to estrogen and estradiol dose (Tanapat *et al.*, 2005; Tanapat *et al.*, 1999; Barker and Galea, 2008; Barha *et al.*, 2009).

MATERIALS AND METHODS

In this study, subjects were female Wistar rats, aged 3 weeks (pre-pubertal), aged 8-6 weeks (pubertal) and aged 48-46 weeks (post-pubertal). Rats were purchased from Pasteur Institute of Iran and 15-30 days were allocated for their fully adaptation to the new environment. Animals were kept under a 12-hour-light-dark cycle at an ambient temperature of 23 ± 2 ° C and relative humidity of 60-40. The animals had free access to food and water at standard conditions. The animals were divided into five groups (n= 7 per group):

1- Pre-pubertal rats (3-week-old, body weight 60-80 g).

2- Adult rats (6-8-week-old, body weight 180-220 g) in metestrus phase.

3- Adult rats (6-8-week-old, body weight 180-220 g) + 17β-estradiol subcutaneous injections.

4- Post-pubertal rats (one-year-old, body weight 230-250 g) in the metestrus phase.

5- Post-pubertal rats (one-year-old, body weight 230-250 g) +17β-estradiol subcutaneous injection.

To assess estrous cycling, vaginal smears were taken and the cells that desquamate from the epithelium were gently smeared on a slide, and the metestrus stage of the estrous cycle was determined (Montes and Luque, 1988). Tip of the micropipette was filled with approximately 10 ml PBS or saline and was slowly discharged into the vagina. The pipettage of the vagina was conducted three to five times. For vaginal cytological study, the final volume of 10 ml of saline solution containing sufficient material was collected. The final solution containing vaginal secretions was spread on a dried slide that had been degreased with detergent. After smear preparation, slides were dried at ambient temperature and fixed with methanol for 5 minutes. For staining, slides were inserted onto the jar and their surface was covered with methylene blue 2%. After a minute, extra color on the slides was removed with distilled water. After that, drying the slides was observed under light microscopy with 10X and 40X magnificent. Metestrus cycle was identified based on the characteristics of the cells of vaginal smears. Groups 3 and 5 were treated once daily by subcutaneous injection of 50 µg/kg 17β-estradiol hormone with sesame oil solvent for 7 days at a specific time of the day. The animals were deeply anesthetized by intraperitoneal injection of ketamine (100 mg/kg of body weight) and xylazine (10 mg/kg of body weight) before removing the brain from the skull. After opening the chest, needle was entered through the left ventricle into the aorta and the right atrium was cut with scissors to drain blood. Perfusion was done by intra-cardiac injection of phosphate buffer solution (PBS), followed by 4% cold paraformaldehyde. Then brain was removed and fixed in 10% formalin. Histological studies and cell counting in CA1 region of hippocampus was performed by staining with Cresyl Violet and Hematoxylin-Eozin.

To evaluate the changes in the cell number of the CA1 region of hippocampus, 10-15 slides were prepared from each brain with 5 microns thickness. Cells in all sections were counted using graticule in objective lens at 400 µm2 area. Finally, data was analyzed using SPSS 16 and one way-ANOVA and LSD test.

RESULTS AND DISCUSSION

According to cell counting of CA1 region of hippocampus, there are significant differences among experimental groups ($p \leq 0.05$). Results are shown in Figures 1, 2 and 3. The results of counting neural cells stained by Cresyl Violet indicated that cell density in CA1 region of hippocampus in the adult rats treated by hormone (*) was significantly more than post-pubertal, adult in metestrus stage (**), post-pubertal rats in metestrus stage (***) and the post-pubertal rats (***) in metestrus stage which had a significant difference with other groups ($p \leq 0.05$).

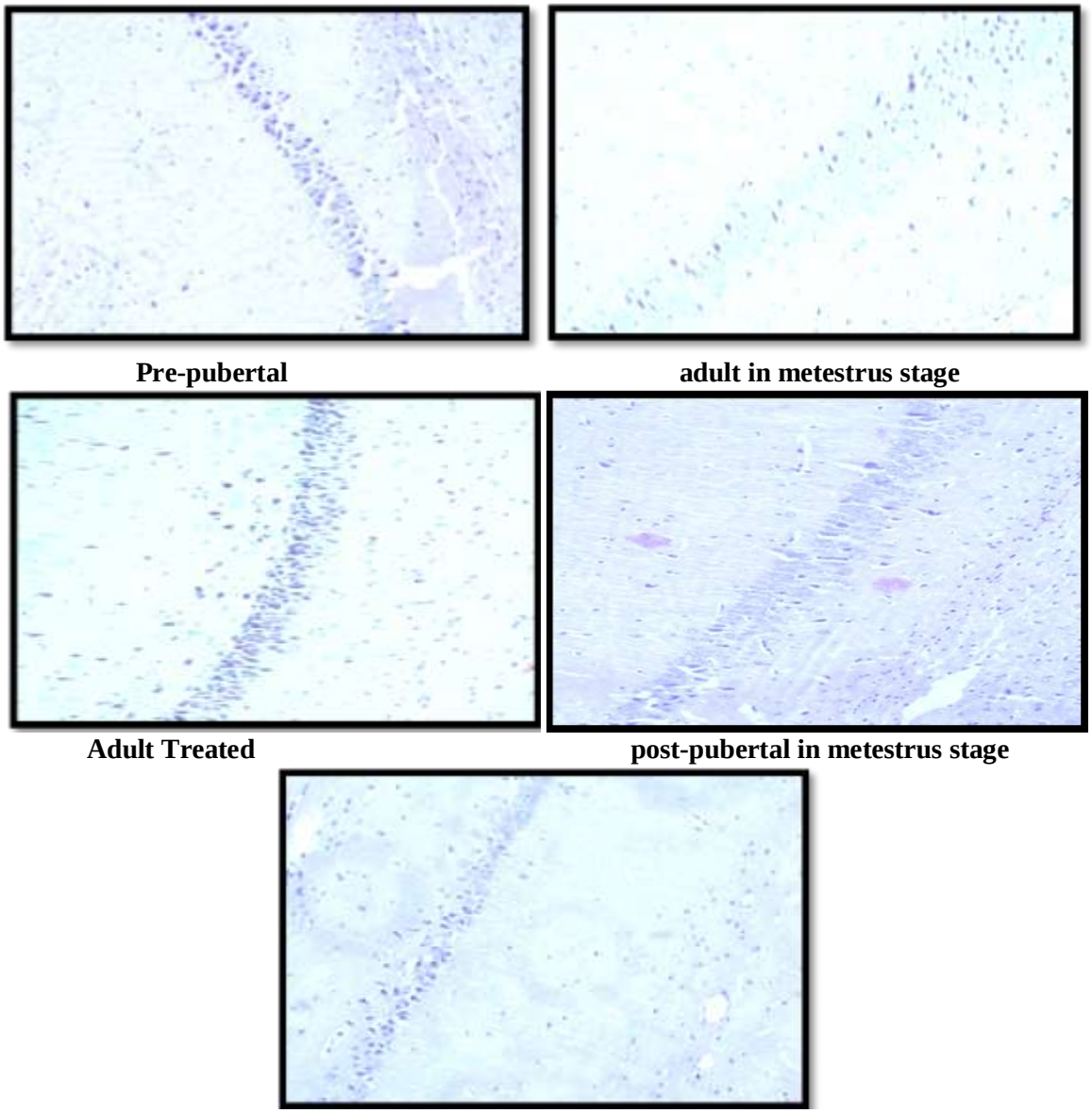
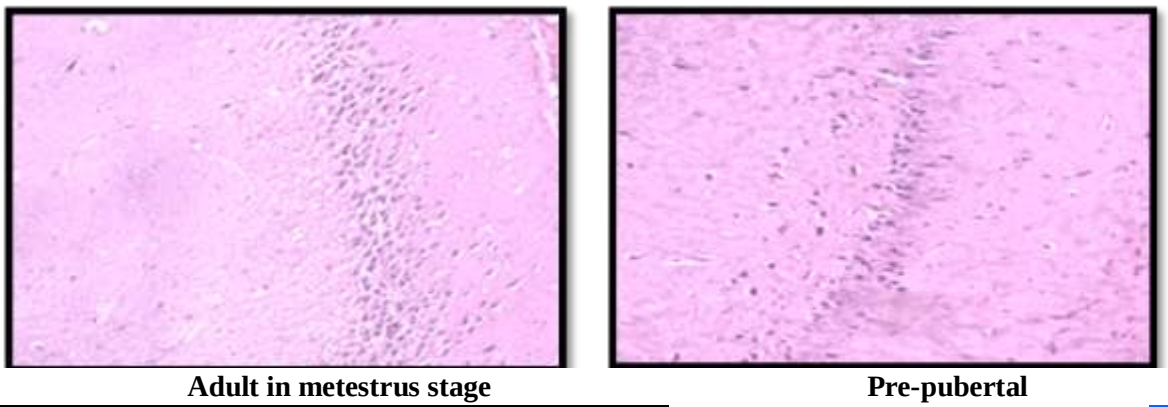


Figure 1: Coronal sections of hippocampus staining by Cresyl Violet. Microscopic view of the CA1 region in the different experimental groups with × 400 magnificent



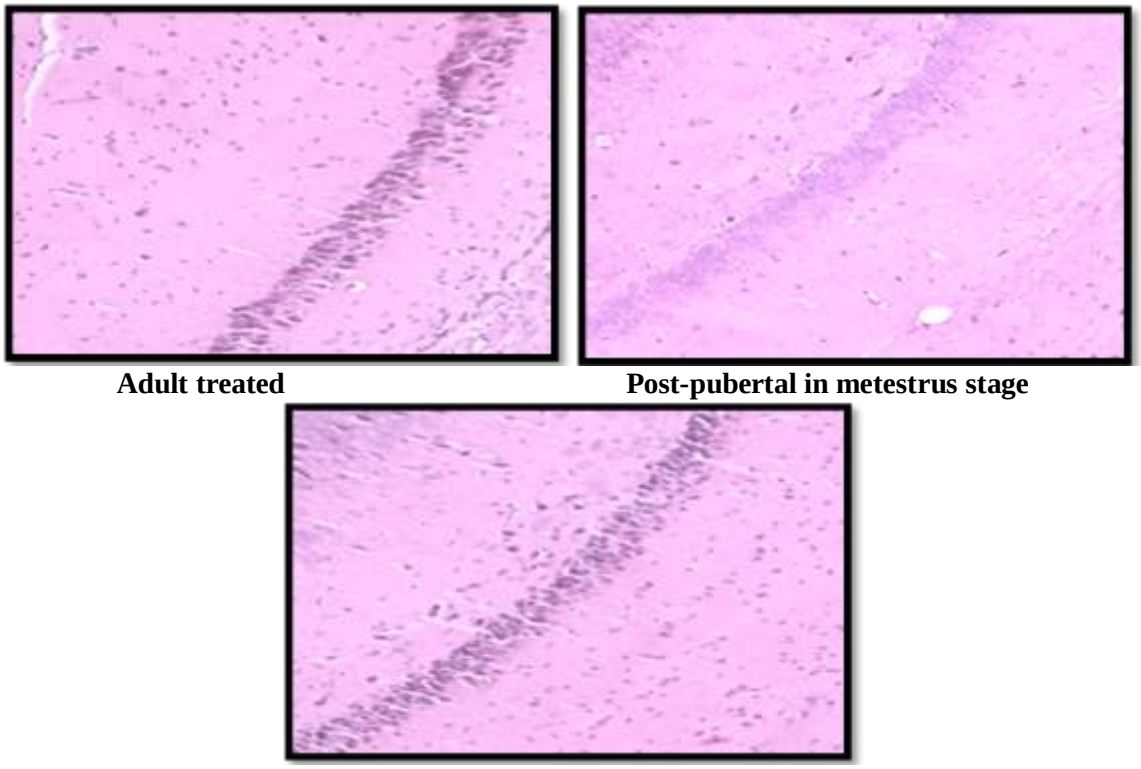


Figure 2: Coronal sections of hippocampus stained by Hematoxylin-Eozin. Microscopic view of the CA1 region in the different experimental groups with × 400 magnificent

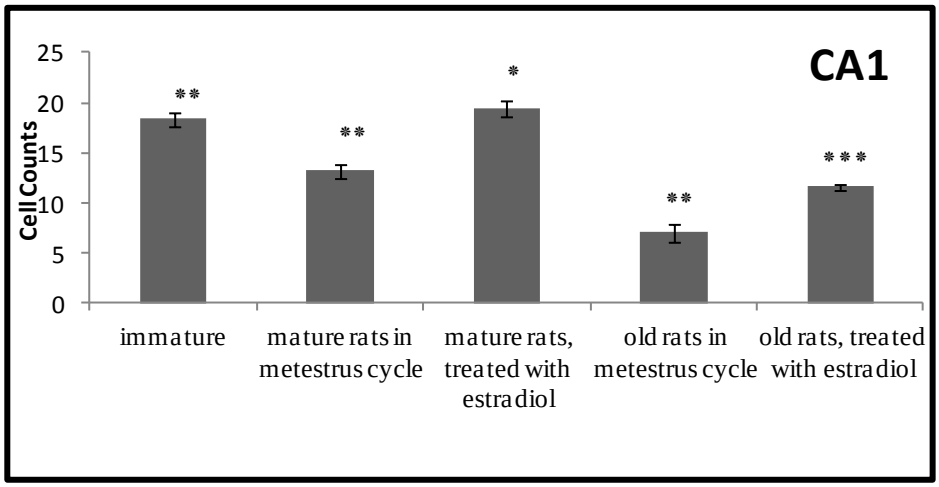


Figure 3: The cell counting of CA1 region of hippocampus stained by Cresyl Violet

DISCUSSION

Despite continuing neurogenesis of the hippocampus throughout its life, its rate decreases with age. This reduction in cell proliferation that occurs with aging can be attributed to many factors. For example, one possibility could be the duration increase of the cell cycle with age; or, gradual loss of granular cell precursors. Also, increasing glucocorticoid levels in old rats and age-related decreasing of neurogenesis occur simultaneously. In addition to all these factors, great reduction of sex hormone 17β-estradiol is known to be a significant factor in reduction of neurogenesis process during the maturation and adulthood

(Gustafsson, 1999). Differences in age-related neurogenesis process have forced researchers to recover variable factors associated with aging in modification the neurogenesis during adulthood and prevent the occurrence of abnormalities and neurological disorders in adulthood and aging. In this research, with 17β-estradiol treatment, the production of new nerve cells were assessed and compared in the process of aging. During adolescence, the environment is enriched in terms of levels of sex hormones and hence, neurotrophic factors are favorable. Clinical studies have shown that suffering certain neurological diseases such as memory loss and Alzheimer with aging occurs because circumstances have changed. Garcia-Segura *et al.*, showed that since the brain is a major center for fine control of endocrine, aging of brain can cause hormonal changes, which subsequently reduces neurogenesis (Garcia-Segura *et al.*, 2009). Several studies have proven that the old brain responds differently to treatment with estrogen compared to the young one. In older brains, ERα on CA1 pyramidal cells are reduced compared to young brains and estrogen therapy is not able to change the distribution of ERα in CA1 of old rats (Adams, 2001 & 2002). Based on the ideas of some researchers, since the brain will be under normal estrogen levels during long-term declines followed by aging, the loss of reactivity to estrogen and the estrogen sensitivity only can be maintained by replacement of the hormone thought aging. This idea is referred to as "healthy cell bias" or cell turns into estrogen health effect (Maki, 2006; Sherwin, 2006). However, the older groups in this study are 12 months olds have not entered the period of absolute aging and insensitivity to estrogen yet. According to the results in the CA1 region of the hormone 17β-estradiol-treated group, an increase in cell proliferation was observed compared to untreated hormone groups, which was in agreement with our claim in this study.

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