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Short Report

Effect of Ovariectomy on Reference Memory Version of Morris Water Maze in Young Adult Rats

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ABSTRACT

Background: The effect of ovariectomy and accompanying sudden loss of circulating gonad hormones on spatial learning performance in the young adult rats was examined. We hypothesized that spatial learning and memory in a considerable number of women who undergo a surgical menopause and estrogen deprivation before their natural menopause be impaired. **Methods:** In this study, we used 26 Wistar rats (approximately five months of age) and divided them into two groups: intact and ovariectomized (OVX). They were tested for spatial reference memory in Morris water maze 6 weeks after OVX. **Results:** The results showed that the performance of OVX group in the water maze was significantly lower than the control group. Although, mean path length decreased across blocks in both groups, OVX rats had significantly longer path length than controls across blocks 2-6 ($P<0.05$). OVX rats had lower percent of total time spent in target quarter than controls in probe trials ($P<0.05$). Body weight gain was significant only in OVX group during the experiment ($P<0.05$). Plasma estrogen significantly decreased after OVX ($P<0.05$). **Conclusion**: This finding provides further evidence for the role of estrogen, a gonadal steroid hormone, in the manipulation of functions related to learning and memory. It is suggested that estrogen loss following OVX impaired spatial reference memory in young adult rats. Our results suggest that it is necessary to protect women who undergo a surgical menopause before their natural menopause from cognition impairments. *Iran. Biomed. J. 12 (2): 123-128, 2008*

Keywords: Ovariectomy, Spatial memory, Morris water maze, Estrogen

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INTRODUCTION

Menopause marks the start of a new phase in a woman's life that is associated with a decrease in circulating estrogen levels. The average age at menopause has remained essentially constant at 50. Thus, 50-year-old women now spend nearly a third of their lives in an estrogen deficient state [1]. This normal aging process in women is associated with increasing health problems such as osteoporosis, cardiovascular disease, cancer and neurodegenerative disease. Although, estrogen deficiency has been linked to changes in several physiological processes, the extent to which estrogen loss is associated with cognitive changes noted by postmenopausal women has been more difficult to determine for a variety of reasons [2].

Furthermore, the specific neural mechanisms by which estrogen may affect cognitive function in women have not identified yet. Like humans, rodents exhibit an age-related cognitive decline, and thus provide good models for testing. The role of female sex hormones may play in cognition during the aging process [3]. During the past 25 years, findings from basic neuroscience have provided us with a great deal of information concerning the mechanisms of action of estrogen on brain structure

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and function. Estrogen has very marked effects on hippocampal synaptic function. Estrogen increases hippocampal dendritic spine density and increases the number of varicosities that can form multiple synapses with different cells [4].

In addition, estrogen can influence on other synaptic signaling processes including the balance of protein phosphatase and kinase activity [5]. While estrogen enhances performance on some tasks of learning, it impairs or has no effects on others. Results of numerous studies indicate that estrogen exerts positive effects on tasks that primarily require the use of working memory, defined as memory for information that relevant to a single trial [6, 7]. For example, chronic estrogen replacement in ovariectomized (OVX) rats increased the number of visits to correct arm choices during acquisition of working memory tasks in radial-arm maze and increased the number of reinforced alternations made in a T-maze [8]. Estrogen replacement in OVX rats had no effect on arm-choice accuracy in reference memory versions of a radial-arm maze task [9, 10]. Estrogen replacement also improved acquisition procedure [11] and on delayed matching-to-position spatial memory tasks [12, 13].

In contrast to the positive effects of estrogen on working memory, many studies report that endogenous and exogenous estrogen impair or have no effect on task dependent reference memory primarily, defined as memory for information consistent across trials [14-16]. For example, gonadally intact female rats and mice [17] exhibited longer escape latencies to locate a hidden platform than OVX controls in a reference memory version of the Morris water maze. On the other hand, estrogen replacement therapy reduced the number of reference memory errors in radial arm maze in aged sham-operated and OVX mice, but unlike young mice, it had no effect on working memory errors [18].

The memory impairment induced by an early age OVX attenuates as the mice get close to their estropausal age [19]. Some evidences suggest that estrogen given to young OVX rodents can improve both spatial and non-spatial learning and memory. One study indicates that the cyclic estrogen replacement regimen does not influence spatial memory function in young or middle aged animal in the hippocampal-dependent appetitive radial maze task [19].

Thus, the discrepancies among these studies could be partly due to different ages of OVX and different effects on tasks of learning and memory. In the present study, we examined the effects of ovariectomy-induced estrogen reduction (or deprivation) on the reference memory version of the Morris water maze in the young adult rats.

MATERIALS AND METHODS

Subjects. Female Wistar rats (n = 26), approximately five months of age, were purchased from Ahvaz Jondishapour University of Medical Sciences (AJUMS) animal house (Ahvaz, Iran). The rats were housed individually in a temperature-controlled vivarum under a 12-h light/dark cycle (light on at 7:00 AM). The animals were allowed free access to water and food. After one week, animals were ovariectomized under anesthesia induced by injection of ketamine hydrochloride (90 mg/kg, i.p., Rotex Medica, Trittau, Germany) and Xylazine (10 mg/kg, i.p., Miles Laboratories, Shawnee, Kansas, USA). All efforts were made to minimize the number of animals used.

Groups. Subjects were divided into two groups. The first one (control) was gonadally intact, while the second one was OVX.

Morris water maze. The water maze was a black circular pool (140 cm in diameter, 70 cm in height) located in a well lit room and filled with water (50 cm height, 27°C). The maze performance was recorded by a video camera suspended above the maze and interfaced with a video (Teevanich Instruments Tracking System, Tehran, Iran). Extra maze cues surrounding the maze were fixed at specific locations and were visible to the rats. A platform (12 cm in diameter), was located in the center of north-east quadrant of the pool that allowed rats to escape the water. The escape platform was positioned 2 cm below the water surface.

Acquisition trials. Six weeks following OVX surgery, the water maze training began. In this task, the rats were trained to find a submerged platform using extra maze cues. Prior to water maze testing, all rats were habituated to swimming in water using a three-trial shaping procedure. This procedure habituated the rat to the water and taught that to escape from the water by climbing onto a platform. Subjects were trained across one day. Each rat received 18 trials over a period of 3 to 4 h. There was a 20-min break between each 3 trials (6 blocks, each block consists of 3 trials). The location of submerged platform did not change throughout the experiment. For each trial, the subject was placed in water facing the edge of the tank from random start points. On each trial, the subject was allowed 60 seconds to escape to the submerged platform; rats that failed to escape were led to the platform by experimenter and were allowed to remain on it for 15 seconds before being removed from the maze and dried off [20].

Probe trial. Following the one-day acquisition period, a probe trial was ordered. The probe trial was identical to the acquisition trials with one exception. During the probe trial, the submerged platform was removed. Multiple measures of water maze performance were recorded. Swim distance (cm), quadrant time (percent time that each subject spent in the quadrant containing the platform), and swim speed (cm/s) were recorded during 18 trials and one probe trial.

Body weight and plasma estrogen. In order to confirm that the ovariectomy was effective, a record of the body weight of each animal was kept at the beginning of the study and six weeks later and also plasma estrogen was measured by ELIZA test 15 days after ovariectomy.

Statistical analysis. Independent sample student's *t*-test was run to determine whether group differences existed in terms of percent time spent in the target quadrant and path length during acquisition and probe trials. Two-way ANOVA was run to determine if differences between groups for each block existed in path length during acquisition. A paired student's *t*-test analysis was used to determine whether significant differences existed in the OVX group weight at the baseline and one month after ovariectomy. Student's *t*-test was run to determine whether group differences existed in weight. All *post hoc* comparisons were computed using the least significant difference method. The criterion for significance was $P<0.05$ in all statistical evaluations.

RESULTS

Acquisition trials-path length. The results indicate that mean path length decreased across blocks in both groups. On the other hand, OVX rats had significantly longer path length than that of controls across blocks 2-6 (at least $P<0.05$, $F_{1, 154} = 30.89$, Fig. 1A). In addition the OVX rats had significantly longer path lengths than controls for total acquisition trials ($P<0.05$, Fig. 1B). Ovariectomy had no significant effect on swim speed in the water maze.

Probe trials- time. As in acquisition trial the OVX animals had significantly lower percent of total time spent in target quarter than controls in probe trials ($P<0.05$, student *t*-test, Fig. 2). There were no significant differences between swim speeds in both groups during probe trials.

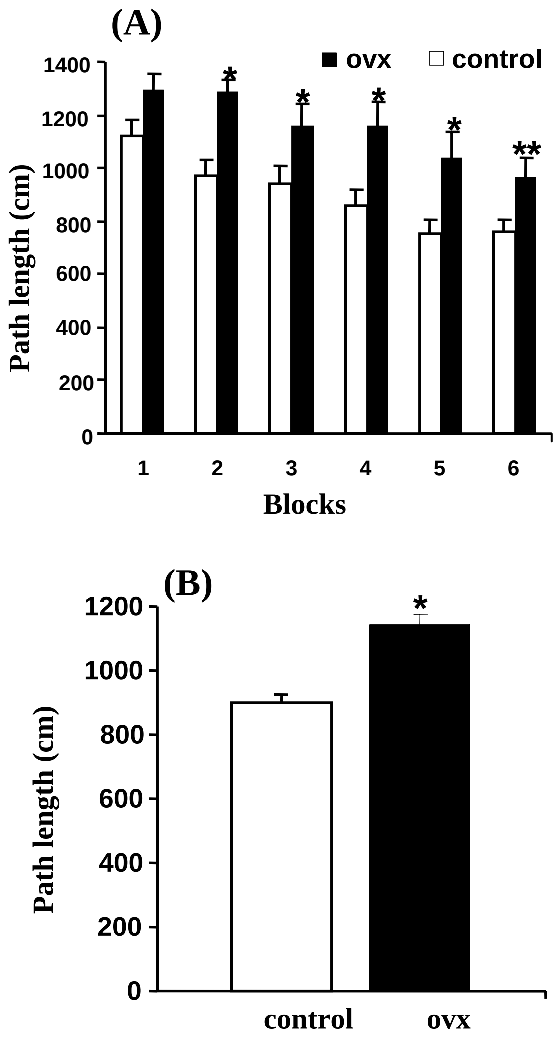

Fig. 1. Path length (Mean ± SEM, n = 13) to locate the escape platform for each block **(A)** and for total acquisition trials **(B)**. (*$P<0.05$, **$P< 0.005$, OVX vs. control, 2-ways ANOVA followed by least significant difference test, $F_{1, 154} = 30.89$, for **(A)** and student's *t*-test for **(B)**.

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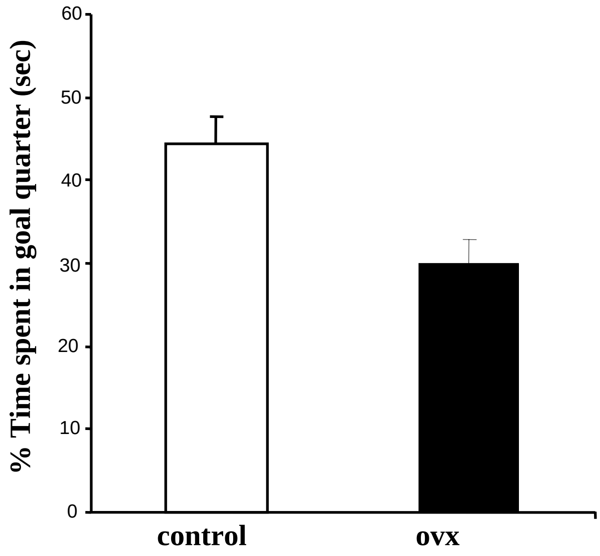

Fig. 2. Percent of total time (Mean ± SEM, n = 13) spent in target quarter for probe trial (*P<0.05, student's *t*-test).

Efficacy of OVX. Data analysis indicated that Mean ± SEM of weight gain was significant only in OVX group during the experiment. The body weight changes at the beginning of the study (260.17 ± 5.43 g and 267.17 ± 8.23 g for control and OVX groups, respectively) and six weeks later (282.21 ± 10.5 g and 96.71 ± 9.94 g for control and OVX groups, respectively, n = 13, *P<0.05, paired student's *t*-test).

Plasma estrogen. Plasma estrogen significantly decreased after ovariectomy. (410.4 ± 27.5 in OVX vs. 1758.4 ± 109.8 pg/ml in control, n = 13, *P<0.05).

DISCUSSION

The results of the present study indicate that ovariectomy in the young adult rats affects the strategy used to locate a hidden escape platform in the Morris water maze. Mean path length to locate the escape platform decreased across blocks in both groups but OVX rats' path lengths were significantly longer than controls. Percent of total time spent in target quarter increased across blocks in both groups but OVX rats had significantly lower percent of total times than controls during acquisition and probe trials.

The Morris water maze tasks require the engagement of multiple neural areas that are not involved in memory. However, the lack of any group differences in swim speed during probe trials suggests that different performance between groups is not due to sensorimotor differences between groups.

Our results revealed that both groups of rats are able to demonstrate learning across blocks, but OVX rats learned given task in a lower level than control intact rats. This finding of decreased spatial reference memory in OVX rats is similar to the previous reports [2-4, 6-9]. EL-Bakri *et al.* [21] reported that ovariectomy severely impaired spatial reference memory. Estrogen induced regulation of spatial memory and N-methyl-D-aspartic acid (NMDA) receptors are likely to be mediated by the two-nuclear estrogen receptors, estrogen receptor α (ER α) and β (ER β). Both receptors are expressed in the hippocampus and neocortex. There is also the possibility of indirect effect through estrogen interaction with other neurotransmitters such as cholinergic system which in turn affect the glutamate system. It has earlier been shown that the ability of estrogen to alter NMDA receptor binding to CA1 is related to its ability to alter cholinergic system. Previous studies show that estradiol plays a dual effect on NMDA receptors. It enhances the cognitive function and at the same time exerts a neuroprotective effect. Thus, estrogen is thought to be responsible for memory fluctuation during the menstrual cycle [21].

Heikkinen T. *et al.* [19] reported that ovariectomy impaired the performance of aged mice in T-maze. In addition, Struse H. [22] found that the OVX rats evidenced superior performance on the maze task, as measured by latency to reach goal (running time) and error scores. Yamada *et al.* [23] reported that neither long-term (3 month) nor short-term (1 month) deprivation of estrogen by ovariectomy caused a significant impairment in spatial learning and memory in water maze and spontaneous alteration behavior in a Y-maze. Wilson *et al.* [17] suggested that short-term estrogen deprivation has no effect upon spatial-reference memory, while it impairs spatial working memory. Numerous reasons could be offered to explain these discrepancies in research findings such as differences in type of memory that is studied [11, 17, 19, 21] or the age of OVX animals [18, 19, 24].

Based on some results [3, 4, 7, 8], it is proposed that estrogen biases an animal towards using the hippocampus whether or not it is advantageous to do so. The hypothesis that estrogen may influence

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cognitive strategy selection may provide a framework to explain why estrogen has positive effects on some tasks of learning and memory and impairing or no effects on others. If a task is best solved using a hippocampally based strategy, estrogen may enhance performance. However, if a task is best solved using a non-hippocampally based strategy, estrogen may impair performance. Finally, if multiple strategies are equally effective in solving a task, estrogen may have no effect [11]. Furthermore, the effect of estrogen on learning and memory is dependent on age of animal. It is proposed that many brain regions influenced by estrogen, most notably the hippocampus, are sites of age-related neurodegenerative changes in both sexes, which may render the aged brain less responsive to estrogen. Thus, it seems that long-term ovariectomy lose some of its effects as the female rat reaches the post-estropausal age [19].

The precise mechanism(s) by which ovariectomy influences learning and memory are not clear. It is possible that the chronic loss of estrogen (and progesterone) may lead to subtle decreases in NMDA receptor binding and/or calcium signaling pathways in hippocampal CA1 dendrites [25]. Carrer *et al.* [26] reported that the slow after hyper-polarization (sIAHP) was significantly larger in cells from OVX rats than in cells from control rats. Furthermore, they reported that the excitability of neurons taken from ovariectomized rats was considerably reduced when compared to the control rats and this effect was reversed by estrogen treatment. Ovariectomy can therefore influence post-synaptic calcium ion signals that in turn may influence the balance between kinase and phosphatase pathways and thus influence the dynamic range of CA1 response to synaptic input [27].

In summary, the present study has shown that estrogen loss following ovariectomy impaired spatial reference memory in young adult rats. Our results suggest that it is necessary to protect women who undergo a surgical menopause before their natural menopause from cognition impairments.

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