The effects of hormone replacement and tamoxifen on spatial learning and active avoidance learning in ovariectimized rats

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Abstract

Background/Aims. *The aim of the present study is to investigate the effects of an estrogen receptor modulating agent tamoxifen, and different protocols of hormone replacement therapy that mimic clinical applications, on spatial learning and active avoidance learning in rats.* ***Methods.*** *We used 2 groups of normal rats for tamoxifen experiments (control group and drug group) and 4 groups for hormone replacement experiments: (1) ovariectomized rats with sesame oil injection, (2) ovariectomized rats with continuous estrogen injection, (3) ovariectomized rats with continuous combined estrogen and progesterone injection and (4) ovariectomized with continuous estrogen and intermittent (sequential) progesterone injection. Properly assigned control groups were used and cognitive processes were studied on animal models of surgical menopause using the Morris water maze and active avoidance learning paradigms.* ***Results.*** *In the Morris water maze no significant differences in spatial learning were observed between the hormone replacement and tamoxifen groups. Active avoidance learning was impaired by ovariectomy.* ***Conclusions.*** *In female ovariectomized rats, spatial learning is not influenced by circulating ovarian hormones and tamoxifen. On the other hand, active avoidance learning is impaired by the absence of gonadal hormones.*

Keywords: *tamoxifen, hormone replacement, Morris water maze, spatial learning*

Introduction

During the postmenopausal period, reduced or deficient estrogen levels affect many systems of the body. Cognitive impairments are among these problems and have a negative impact on quality of life. Clinical studies provide evidence that estrogen improves cognitive functions during the postmenopausal period[1-4].

The adult hippocampus shows structural and functional plasticity in response to changing levels of estradiol[5]. In female mammals, estrogen affects the neurochemistry, structure and function of brain regions important for learning and memory, including the hippocampus[6]. Gould et al.[7] demonstrated significant loss of dendritic spine density on CA1 hippocampal pyramidal cells following ovariectomy in adult rats. From a functional perspective, estradiol modulates N-Methyl-D-Aspartate (NMDA) receptor binding in the CA1 region[8], as well as cholinergic neurotransmission in hippocampal areas[9]. Sutherland et al.[10] demonstrated that the rat whose hippocampus was destroyed had impaired spatial learning in the Morris water maze (MWM).

Several natural or synthetic estrogenic molecules are commonly used in hormone replacement therapy during the postmenopausal period. Selective estrogen receptor modulators (SERMs) are agents that have variable agonistic and antagonistic effects depending on the tissue. The two widely used SERMs, tamoxifen and raloxifen act as estrogen agonists on bone and brain while their action on breast and uterus resembles estrogen antagonists[11]. Tamoxifen is used successfully in adjuvant treatment of breast cancer patients and there are clinical studies on cognitive effects of tamoxifen in postmenopausal women reporting memory impairment[12-14].

The objective of the present study was to investigate tamoxifen and hormone replacement therapy using different hormone regimens to determine their role in cognitive processes. We used two learning paradigms (active avoidance and spatial learning) to study cognitive processing involving different brain regions and mechanisms.

Materials and Methods

Experimental Animals. Adult, mature female Sprague-Dawley rats (190-250 g) were used. In the experiments designed to test the effects of hormone replacement, postmenopausal status was obtained by bilateral surgical ovariectomy. Intact rats were used for tamoxifen experiments. Following recovery from surgery animals were kept under standard conditions (five animals per cage, 20-22ºC, 12 hs light/dark cycle) with food and water ad libitum. After 21 days, rats were randomly allocated to study and control groups.

The animals were handled at all times under the regulations for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC) and all procedures were approved by the Institutional Animal Ethics Committee of Ege University Faculty of Medicine.

Surgical procedure. Rats were anaesthetized by sodium thiopental (40 mg/kg, i.p., Pentotal). Ovaries were pulled out through small bilateral flank incisions (1 cm each), the junction

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between the oviduct and the ovary were ligated, the side of the ovary was cut and the ovaries were removed. The horns were returned into the abdominal cavity through the openings and skin incisions were closed with sutures; rats were housed separately until they recovered from surgery.

Hormones/Drugs. All hormone/drug treatments were applied for 12 days i.p. each day. Tamoxifen (5 mg/kg, Sigma) was dissolved in 10% dimethyl sulphoxide (DMSO). The vehicle (10% DMSO) was used for control injections. Medroxyprogesterone (2.5 mg/kg) and 17-β estradiol (50 μg/kg) were dissolved in sesame oil. Progesterone, tamoxifen and vehicle (sesame oil or DMSO) injections were applied between 08:30-09:00 a.m. Estrogen injections were applied between 4:00-5:00 p.m. The rat menstrual cycle is generally 4 days. Therefore, for the sequential hormone replacement regime progesterone injections were intermittently applied with 2-day injections and 2 day intervals. Drug treatment was continued for an additional 12 days in the MWM groups (total 24 days). Drug/hormone injections were also continued during the active learning experiments for an additional 5 days (total 17 days).

Experimental groups

- Tamoxifen groups: Normal animals were used.

1. Control (10% DMSO) (n=20)
2. Tamoxifen (5 mg/kg) (n=20)

- Hormone replacement groups: Ovariectomized animals were used.

1. Ovariectomized rats with sesame oil injection (n=20)
2. Ovariectomized rats with continuous estrogen injection (n=20)
3. Ovariectomized rats with continuous combined estrogen and progesterone injection (n=20)
4. Ovariectomized rats with continuous estrogen and intermittent (sequential) progesterone injection (n=20)

MWM for spatial learning and memory testing. MWM was a circular pool (130 cm diameter and 75 cm height), filled to a depth of 45 cm with a water temperature of 22ºC, made opaque and dark yellow by a non-toxic, water soluble dye. The hidden platform was square shaped (12 x 12 cm), painted yellow, and submerged 1.5 cm below water level. The same platform was made visible on the first day by elevating it so that it protruded 2.5 cm above the surface of the water. The maze was located in a 4 x 3 meter room and extramaze (spatial) cues included posters, racks, lights and two experimenters blind to the treatment. The experiments were recorded using a camera which was displayed on a video monitor. The output of the camera was captured by the tracker (HVS Image, UK) and analyzed with a computer using the HVS-Water software. The parameters analyzed include escape latency to the platform, path length to reach the platform and swim speed; on day 13 (probe trial) time spent (actual and percentage of total time) and path length in the quadrant, where the platform had been during acquisition, was recorded.

Rats received 4 consecutive training trials on each of 11 consecutive days with an interval of approximately 30 minutes for each rat. On the first day, the platform was elevated above the water surface. The rats were put into the pool from one of four different starting points (each in a different quadrant) to find the platform. Each rat was given 30 seconds to find the platform. If the rat could not find the platform within 30 seconds, the experimenter led the rat gently to the platform. After finding the platform, rats were allowed to stay 15 seconds on the platform and then they were returned to their cage.

On the last test day (day 13) the platform was removed and MWM testing was performed for 60 seconds The time spent and path length in the quadrant where the platform had been during acquisition, were recorded.

Active avoidance learning. Using an instrumental training procedure, rats were trained to jump onto a pole after hearing a tone to prevent getting a foot shock. Active avoidance learning trials were carried out daily and consisted of 15 trials conducted on each of the 5 days. The rats were placed in a plexiglas active avoidance chamber. An auditory stimulus (78 decibels, 380 Hz) was presented for 3 seconds, followed by a 50-V(AC) foot shock 5 seconds after the auditory stimulus. The rat had to jump on to a pole to avoid or escape the shock; if the rat did not jump the shock was terminated at 3 seconds. The interval between the trials was 15 seconds. If the rat jumped onto the pole, the next trial was started 15 seconds after the rat climbed down from the pole and stepped on the grid floor[15,16].

The responses were defined as follows:

- avoidance response: the rat jumped onto the pole after the onset of the auditory stimulus and avoided the shock;
- escape response: the rat jumped onto the pole during the shock;
- response failure: the rat did not jump onto the pole even after receiving the shock.

The number of correct responses (i.e. avoidance responses) was taken as the measure of learning performance. The experimenter did not interfere with the performance of the rat, i.e. placing the rat on the pole to prevent confounding effects of handling. Some rats did not acquire pole jump active avoidance learning and therefore rats with a score of 7% or lower (one or no avoidance responses) on the 4th day were excluded from the study. Therefore, while each group contained 10 animals in the beginning of the experiments, the behavioral data analyzed contains 6 animals in the vehicle group, 7 in the estrogen group, 8 in the combined estrogen-progesteron group, 8 in the sequential estrogen-progesteron group and 9 in the tamoxifen group and its control.

Statistics. The data were analyzed using SPSS version 8.0 to perform ANOVAs. Differences between groups were evaluated by the post hoc Duncan test. Effects with degrees of freedom (df) values exceeding unity in the numerator were set to unity in a conservative Geisser-Greenhouse correction procedure. The level of rejecting the null hypothesis was 0.05. Mean escape latencies; path length, swim speed and correct response of active avoidance were initially analyzed in separate repeated measures ANOVA, with days of testing and treatment (hormone replacement) as the factor. On the probe day of the water maze time spent in the target quadrant path length and swim speed were analyzed in separate one-way ANOVA.

Results

MWM performance. During the acquisition phase, in all of the groups, latency to find the platform decreased across days, a main effect of days ($F_{(6,54)}$ = 64,165, P<0.001) was observed (Figure 1).

Similarly, the path length taken to locate the platform was significantly shorter across days ($F_{(6,54)}$ = 45,0, P<0.001). A significant increase in swimming speed was also noted ($F_{(6,54)}$ = 8,463, P<0.001); rats swam faster across the 11 days of the acquisition phase. On the 13th day of the MWM experiment, the hidden platform was removed from the pool to test memory performance. One-way ANOVA revealed that the groups were not different from each other regarding the percent of time spent and path length in the quadrant where the platform used to be during acquisition (indicating that the rats remembered the platform position and were searching for it). However, ovariectomized rats without hormone replacement had the lowest memory performance (Figure 2).

Active avoidance learning. The number of correct responses (climbing onto the pole after the tone and avoiding the electric shock) increased significantly across days [$F_{(5,42)}$ = 323,523, P<0.001]. The groups were significantly different from one another in the number of correct responses [$F_{(5,42)}$= 3,243, P<0.01]. Post hoc tests (Bonferoni Test) showed that the performance of the normal rats (tamoxifen and controls) was highest compared with the ovariectomized rats without hormone replacement (P=0.18) (Figure 3). When the data were analyzed for each day separately, ANOVA revealed significant differences between the groups on day 3 (P<0.05), day 4 (P<0.01) and day 5 (P<0.001).

Discussion

Neuroendocrine systems show important changes when gonadal hormones are not present and the impairment of the cholinergic, adrenergic, opioid and serotonergic systems are the most striking[17]. SERMs activate estrogen receptors with tissue-specific effects. They have a mixed estrogenic and antiestrogenic activity and act on the two estrogen receptors (α and β). In a recent study we investigated the effects of raloxifen on cognition and depression in a rat model of surgical menopause[18]. In the MWM no significant differences were depicted between the groups regarding swim speed or acquisition of place learning. In the memory test, raloxifen provided an advantage over controls (P<0.05) and a positive impact on memory despite the negligible effects on spatial learning[18].

Some studies have investigated the effects of estrogen on an animal's ability to learn and remember. While research has produced somewhat conflicting results, in general hippocampal-dependent memory showed improved performance with estrogen, while striatal or amygdala dependent memory showed deficits[6]. Elevated levels of estradiol were associated with enhanced performance on spatial maze tasks that primarily require the use of working memory, defined as memory for information that is relevant for a single session or behavioral episode. By contrast, elevated levels of estrogen impaired or had no effect on tasks dependent primarily on reference memory, defined as memory for information consistent across trials. Several studies[19-24] reported an estradiol-associated impairment in performance during place training, a reference memory version of the MWM task, which requires the rodents to swim to a hidden escape platform located in a fixed position across trials.

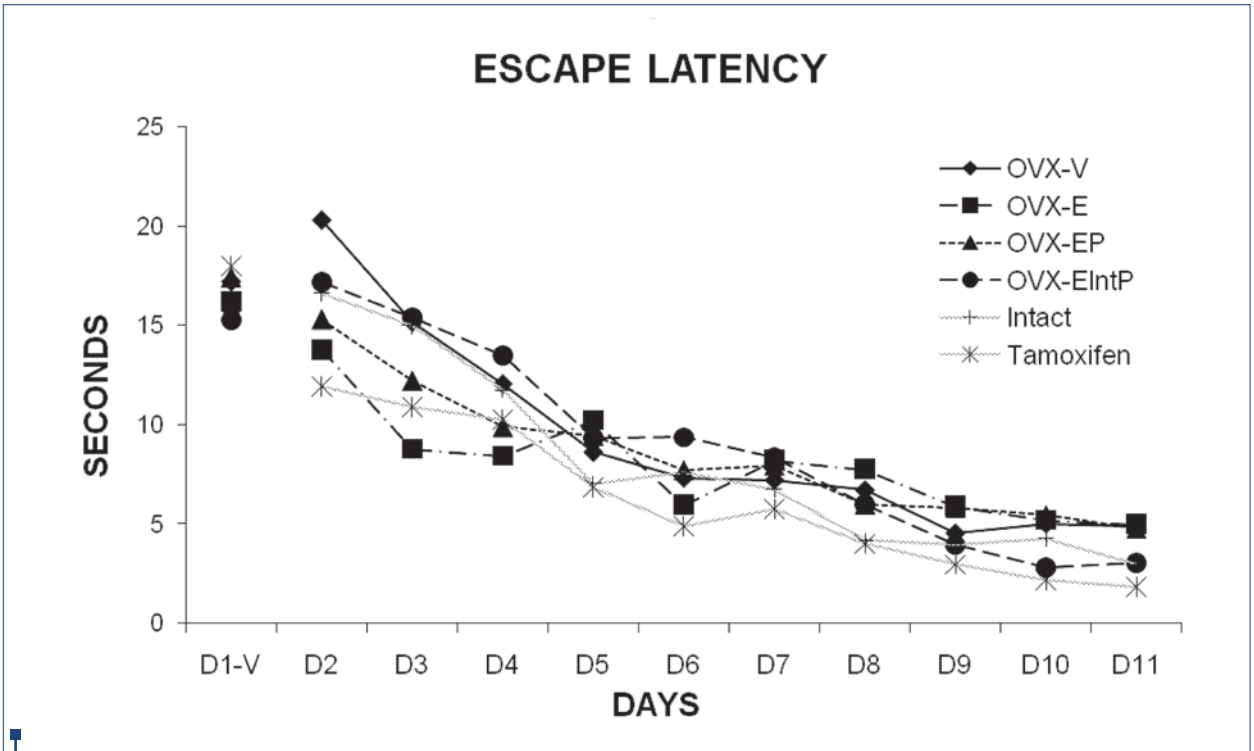

Figure 1. Escape latencies in the MWM. OVX-V: Ovariectomized rats with vehicle, OVX-E: Ovariectomized rats with estrogen, OVX-EP: Ovariectomized rats with combined estrogen and progesterone, OVX-EintP: Ovariectomized rats with combined estrogen and intermittent (sequential) progesterone, Intact: normal control rats, D1-V: first day with visible platform.

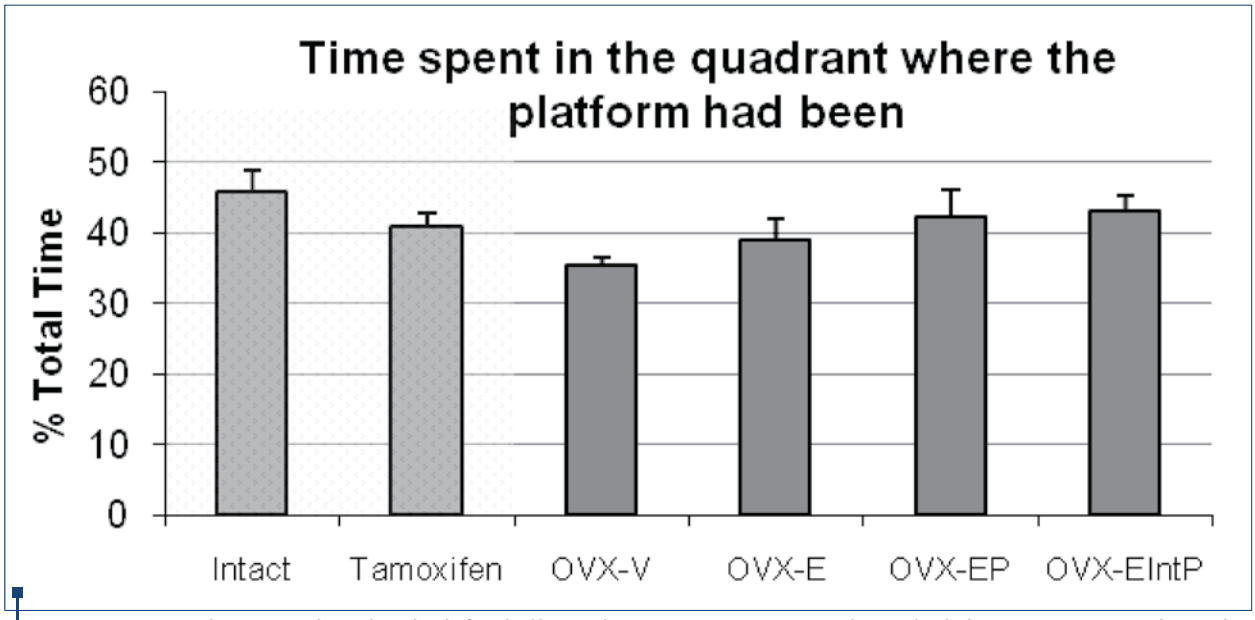

Figure 2. Time spent in the target quadrant where the platform had been in the MWM. OVX-V: Ovariectomized rats with vehicle, OVX-E: Ovariectomized rats with estrogen, OVX-EP: Ovariectomized rats with combined estrogen and progesterone, OVX-EintP: Ovariectomized rats with combined estrogen and intermitent (sequential) progesterone, Intact: normal control rats.

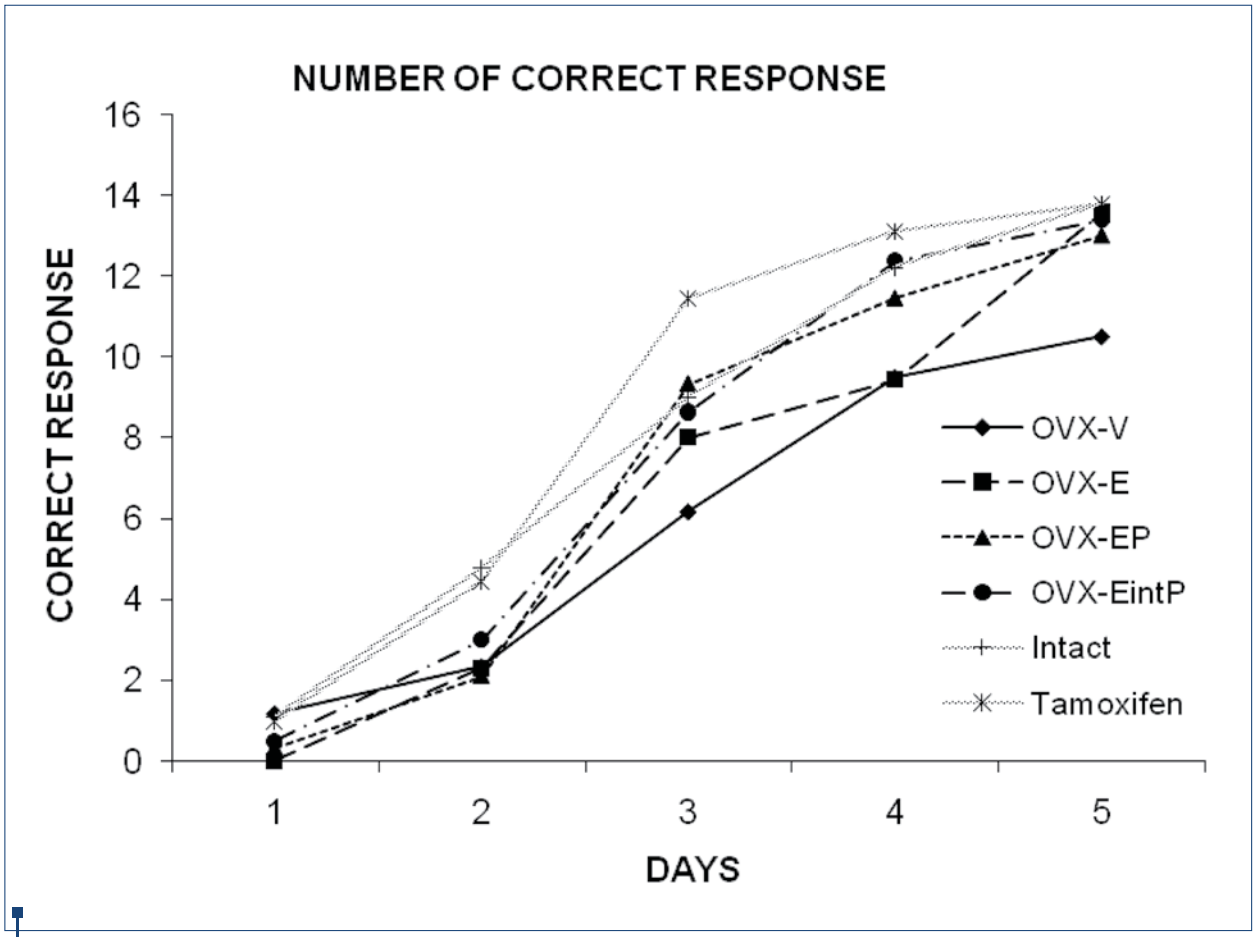

Figure 3. The number of correct responses during active avoidance learning experiments. OVX-V: Ovariectomized rats with vehicle, OVX-E: Ovariectomized rats with estrogen, OVX-EP: Ovariectomized rats with combined estrogen and progesterone, OVX-EintP: Ovariectomized rats with combined estrogen and intermitent (sequential) progesterone, Intact: normal control rats.

Moreover, estrogen levels seem to affect the strategy that an animal chooses to solve a problem which in turn impacts the learning ability[25]. Korol and colleages[26] demonstrated increased acquisition of a hippocampus-sensitive task and impaired acquisition of a striatum-sensitive task as a result of estradiol administration 48 and 24 hours before testing. They concluded that variations in the levels of estrogen may affect cognitive functioning by shifting the cognitive strategy to solve a task.

In our study, there was a main effect of days in the acquisition of place learning, with all groups swimming shorter periods over days to locate the platform using spatial cues. The path lengths to the platform were significantly shorter throughout the days and rats went directly to the platform instead of searching for the platform in different places. In the last days of the experiment, tamoxifen, control and sequential hormone replacement groups apparently performed better comparing with the other groups, but, again, the results were not significant. The significant progressive increase of swimming speed over days may reflect learning and the memory test of the probe trial when the platform was removed showed the disadvantage of the ovariectomized rats with sesame oil injection group. Using adult Sprague-Dawley rats, tamoxifen at the doses used did not have any pronounced effect on learning or memory. Chen et al.[27] demonstrated impaired retrieval of spatial information processing in mice with tamoxifen injection 30 minutes before the MWM experiment. The species used and the experimental protocol may underlie this observed discrepancy. Post-training estrogen is reported to enhance spatial and object memory consolidation in female mice[27]. Estrogen is reported to play a significant role in a water maze study where ovariectomized female mice could locate the hidden platform on the 2nd day only when they receive 0.2 mg/kg estradiol injection[28]. Estrogen and progesterone combinations reveal different results in memory consolidation in MWM experiments using aged rats; higher progesterone doses are reported to impair performance[29].

Other studies indicate that estradiol has been found to both facilitate and disrupt avoidance learning. Shingh et al.[30] showed that estradiol (E2) replacement in female rats facilitates the acquisition of active avoidance in a two-way avoidance task, particularly after long-term chronic estradiol replacement. This was also supported in a high estradiol and progesterone state in another study[31]. In a T-maze shock avoidance paradigm, ovariectomized rats treated with estradiol learned faster than those with progesterone or progesterone plus estrogen implants[32]. In contrast, estradiol has been found to reduce the acquisition of two-way active avoidance compared to ovariectomized non-replaced rats[33]. Also, in a follow-up study low doses of estradiol (0.2 μg/rat) disrupted, while high doses (20 μg/rat) enhanced acquisition[34].

Using 5 mg/kg tamoxifen, 2.5 mg/kg medroxyprogesterone and 50 μg/kg estradiol in our study, the number of correct responses, indicating learning and memory performance became significantly higher across days and the highest scores were obtained in normal animals (tamoxifen and controls). These experiments suggest an important role for gonadal hormones in active avoidance learning.

From this study, it was drawn the idea that spatial learning seems not to have any relationship with hormone replacement and tamoxifen. On the other hand, active avoidance learning deteriorates when gonadal hormones are absent. ■

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