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ARTICLE

Weak if any effect of estrogen on spatial memory in rats+

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ABSTRACT In a number of species, males appear to have spatial abilities that are superior to those of females. The favored explanation for this cognitive difference is hormonal: higher testosterone levels in males than in females. An alternative explanation focuses on the role of varying levels of estrogens in females during the estrus cycle; females perform as well as males on days of low estrogen, but more poorly on days of high estrogen. Other investigators have reported that estrogens improve both types of memory processes, which depend on the striatal (nonspatial navigation) and hippocampal (spatial) memory systems. Additionally, estrogens have been found to protect the working memory. These contradictory results initiated the present study, in which ovariectomized female rats were trained to escape in a Morris water maze. The daily trials were preceded by estradiol application in low doses (Experiment I) or in higher doses (Experiment II). In Experiment I, no differences at all were found between the latencies of the treated and control groups to reach a submerged platform in a Morris water maze. In Experiment II, however, the animals treated with the higher dose of estradiol showed a small deficit in the acquisition of the Morris water maze task. This study indicates that estradiol at around the physiological level has no effect on spatial learning and memory functions.
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KEY WORDS

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One of the clinical symptoms reported by menopausal and postmenopausal women is a deficit in memory and cognitive functions (Kopera 1973; Brown 1976). There has recently been mounting evidence that estrogen receptors (ER) are also involved in "nonreproductive" behaviour, including that associated with the hippocampal spatial discrimination function (McEwen et al. 1997). For example, during proestrus, or following estradiol injections, female rats exhibit a poorer performance in the spatial version of the water escape task, which is sensitive to the hippocampal function (Korol et al. 1994; Berger-Sweeney et al. 1995; Frye 1995). Furthermore, the hippocampal anatomy and physiology are altered by these estradiol level or by estrogen treatment, suggesting possible neurobiological substrates for estrogenic effects on spatial performance and memory (Warren et al. 1995).

There are several putative mechanisms through which estrogens might affect memory, including the following: 1) the potential of estradiol to alter the glutamate sensitivity of the hippocampal neurons (Weiland 1992a); 2) the estradiol-induced activation of a subset of hippocampal GABA neurons (Weiland 1992b); 3) the putative action of estradiol on choline acetyltransferase (Luine et al. 1980); 4) the ability of estradiol to increase cyclic adenosine monophosphate (cAMP) levels in the hypothalamus (Gunaga et al. 1974); and 5) the effects of estrogens on neuronal plasticity, which has been well documented in several regions of the central nervous system (Chung et al. 1988; Párducz et al. 1993; Langub et al. 1994; Holstege 1997; VanderHols and Holstege 1997; Wooley 1998; Horváth et al. 2002).

As indicated above, the effects of estrogens in "nonreproductive" behaviour are well documented. However, the effects of estrogens on the learning and memory functions are rather controversial. Some researchers observed no effects of estrogens on the learning and memory functions (Healy et al. 1999; Wilson et al. 1999), whereas others reported a negative effect (Fuger et al. 1998; Chesler and Jaruska 2000), and some laboratories found estrogens to exert enhancing action in spatial memory tasks (Rissanen et al. 1999; Gibbs 2000).

In order to determine whether estrogen pretreatment has any effect on a spatial learning task, the present study was carried out in a Morris water maze.

Materials and Methods

Subjects and surgery

A total of 40 adult Sprague-Dawley rats were raised with access to water and food pellets (Altromin) *ad libitum*.

At the age of 3 months, all the animals were ovariectomized (OVX) by means of bilateral dorsal incisions. All the surgical procedures were carried out under deep Ketamine/xylazine anaesthesia (Ketamine 10.0 mg/100 g and xylazine 0.8 mg/100 g body weight, i.p.).

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+Dedicated to Professor Otto Fehér on the occasion of his 75th birthday.

Hormone administration

Experiment I: Fourteen days after ovariectomy, 22 rats were divided into two groups: 1) those (12) that received a single dose of 17β-estradiol (20 μg/kg in sesame oil, i.p.) and 2) those (10) that received an injection of vehicle alone. The first injection was given 28 h before the experiments, and the subsequent injections 4 h before the first trial of each day.

Experiment II: Eighteen rats were divided into two groups (10 and 8, respectively), and an experiment similar to that described above was carried out, except that the dosage of estradiol was higher (100 μg/kg).

Behaviour

Rats were tested with the spatial version of the Morris swim task, starting 2 weeks following gonadectomy. The animals were trained in a circular pool (160 cm in diameter) located in an artificially-lighted room containing an assortment of two- and three-dimensional cues (posters, lamp, etc.). The pool water (40 cm deep) was maintained at room temperature (22 ± 0.5ºC) and a white escape platform (10 cm in diameter) was situated approximately 1.5 cm beneath the water level. The escape platform was positioned at the center of one of the four quadrants of the pool and remained in the same location throughout testing.

In order to acclimatize the rats to the task, they were first placed into the water for a 30-min "free swim" and then assisted onto the platform from three different directions. A 30-s rest on the platform was then permitted before the first training trial was administered. The training consisted of three blocks of four trials per day for 4 consecutive days. Intertrial intervals were 20 s in duration while interblock intervals lasted approximately 15 min. In each trial, the rat was placed in the water from one or other of the four equally spaced start locations (N, S, E and W). A period of 60 s was available for the rat to escape during each trial. If it did not escape within that time, it was gently guided to the platform, and a time of 60 s was allocated as the escape latency. Between trials, the rats remained on the platform. After each block and between blocks, the rats remained in their home cages.

Analyses

To assess the acquisition of the spatial task, latency measures (the time to reach the platform) were composed across blocks of trials, using repeated measures analysis of variance (ANOVA).

Results

Experiment I

Figure 1 illustrates the mean latencies of the Morris water task for the rats treated with 20 μg/kg estradiol (n=12) or oil (n=10). A decrease in escape latency over the course of training is routinely used as an indicator that the animals have acquired a successful strategy of escaping from the pool. As Figure 1 shows, both groups acquired the successful strategy, and the analysis did not reveal any significant difference in performance between them.

Experiment II

In this experiment, the animals treated with 100 μg/kg estradiol also acquired the strategy of escaping from the pool. On the first day, the performances of the animals in the two groups were very similar, the difference between them not being significant. During the following days, the animals treated with estradiol did not show such good progress in acquiring the spatial discrimination test as did the control group. However, the analysis indicated a slight but significant difference between the two groups only on the third day (Fig. 2).

Discussion

Since the literature on the effects of estrogens on spatial learning and memory functions is rather controversial, we carried out two series of experiments, in which the effects of two different doses of estradiol were compared.

In Experiment I, we used a dose of estradiol comparable to the blood level found in mice (Rissanen et al. 1999) and to the dose administered to rats in behavioural studies (Vongher and Frye 1999; Chesler and Juraska 2000). The higher dose in Experiment II is comparable to that applied by others (Fugger et al. 1998; Isgor and Sengelaub 1998) or by ourselves in an electrophysiological study (Kis et al. 1998).

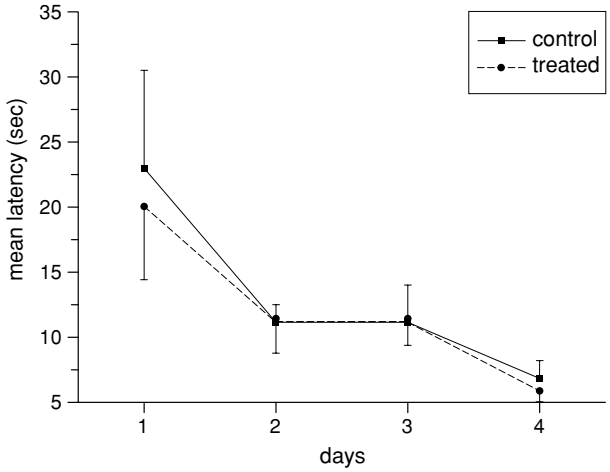

Figure 1. Experiment I: Maze performances of controls and animals treated with 20 μg/kg estradiol. As the results show, both groups acquired the successful strategy to escape. The analysis did not reveal any difference in performance between them. Bars represent mean (± SEM) latencies.

In the present study, at the low dosage (comparable to the physiological level), we found no difference between the performances of the treated and control groups.

Previous researches suggested that, during the breeding season (high estradiol), female voles show poor performance as compared to males in the spatial version of the water escape task (Galea et al. 1995). Our result is in accord with that observation; after the high-dosage estradiol treatment, the animals displayed a somewhat poorer performance in the water maze task than did the controls. These results are consistent with the findings in some other studies (Fugger et al. 1998), but seem to contradict those which found an improved performance related to estradiol treatment (Packard and Teather 1997a, b).

The reason for this discrepancy might be that, in addition to an impaired acquisition following acute estradiol treatment, when given over longer time periods, this estrogen can act through other mechanisms to improve memory functions. In fact, an improved memory can be observed in gonadectomized rats following chronic estrogen treatment (O'Neal et al. 1996).

Though it is possible that chronic estrogen treatment mediates an additional set of neuronal modifications, which in turn facilitate memory, our present study shows that acute estradiol treatment with a dosage within the physiological range has no effect on the spatial learning and memory functions. Treatment with a higher dosage of estradiol tends to impair the performance of the treated animals.

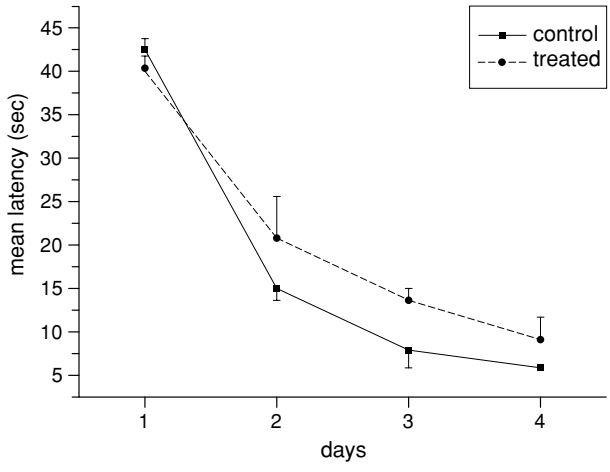

Figure 2. Experiment II: Maze performances of controls and animals treated with 100 μg/kg estradiol. The results demonstrate a small deficit in the acquisition of the Morris water maze task; however, the difference between them was significant only on the third day (t=2,3, p=0.0355). Bars represent mean (± SEM) latencies.

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