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High levels of estrogen enhance associative memory formation in ovariectomized females

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Summary The ovarian hormone estrogen is presumed to modulate processes of learning and memory in adulthood. In this study, we examined the effects of short-term estrogen replacement on associative memory formation. Adult ovariectomized female rats received two injections of estradiol (10, 20 or 40 μg) 24 h apart and were trained 4 h following each injection on the hippocampal-dependent task of trace eyeblink conditioning. Only the highest dose of estrogen, which produced plasma estradiol levels >250 pg/ml, enhanced conditioned responding. One day after the last injection, estrogen treated rats continued to exhibit elevated levels of conditioning and extinguished responding when the conditioned stimulus was no longer presented. Exposure to estrogen did not alter pain sensitivity or activity levels, but did greatly increase uterine weight. These results provide additional support to the view that that ovarian steroids are beneficial to the performance of certain forms of learning and memory tasks, albeit at supraphysiological doses. They are discussed with reference to hormone replacement and its effects on cognitive processes.

1. Introduction

The ovarian hormone estrogen has a profound influence on the morphological and electrophysiological properties of the hippocampus, a brain region implicated in certain forms of learning and memory. It has been shown that exposure to estrogen either exogenously or endogenously during proestrus greatly enhances the density of dendritic spines in area CA1 of the hippocampus (Gould et al., 1990; Woolley and McEwen, 1993; Shors et al., 2001). Over the 5-day estrous cycle of the rat, spine density can fluctuate as much as 30% (Woolley et al., 1990). Moreover, these changes in the dendritic spines have been shown to reflect changes in synapse density and to be accompanied by changes in astrocytic volume (Woolley and McEwen, 1992; Klintsova et al., 1995). In addition to these structural alterations, hippocampal electrophysiology is also sensitive to estrogen (Wong and Moss, 1992). For example, both in vivo and in vitro studies have shown that estrogen affects hippocampal excitability, as well as the induction of LTP (Warren et al., 1995; Cordoba Montoya and Carrer, 1997) and LTD (Desmond et al., 2000).

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Such hormonally regulated changes in hippocampal synaptic plasticity may have important behavioral implications. Indeed, there are numerous studies that have examined the effects of estradiol on hippocampal-dependent learning. However, the results have been equivocal, with estrogen reported to impair, enhance or have no effect on performance. Studies examining performance in the spatial water maze task during the estrous cycle have reported small deficits (Frye, 1995; Warren and Juraska, 1997) or no difference (Berry et al., 1997) in females during exposure to proestrous levels of estrogen. In the radial arm maze, a test of spatial working memory, stable performance across the estrous cycle was demonstrated (Stackman et al., 1997). In contrast, our laboratory has observed that performance of both hippocampal-dependent and -independent types of classical eyeblink conditioning are enhanced during proestrus relative to other stages of estrus and to performance in males (Shors et al., 1998; Wood et al., 2001).

In hormone replacement studies, estrogen administration in ovariectomized rats enhances performance on some types of tasks but impairs performance on others. For example, estrogen improves spatial working memory aspects of the radial arm maze (Fader et al., 1999; Daniel et al., 1997; Luine et al., 1998; Bimonte and Denenberg, 1999; Gibbs, 1999; Korol and Kolo, 2002). Beneficial effects of estrogen replacement have been observed in spatial water maze tasks (O'Neal et al., 1996; Packard and Teather, 1997; Sandstrom and Williams, 2001). Estrogen has also been shown to adversely affect learning of conditioned place preference (Galea et al., 2001) and contextual fear memories (Markus and Zecevic, 1997; Gupta et al., 2001).

Although these studies differ with respect to route and duration of hormone administration, age of the animals and type of task used, circulating estradiol levels were within the physiological range, typically less than 100 pg/ml. However, there are several reports demonstrating that supraphysiological doses of estrogen have positive effects on cognition. For example, inhibitory avoidance performance is improved in rats treated with both physiological and supraphysiological doses of estradiol (Fugger et al., 2000; Frye and Rhodes, 2002). In humans, administration of estradiol after surgical menopause enhanced short-term memory when subjects were tested at a time when estradiol levels were supraphysiological (more than 4× that of their preoperative baseline) (Phillips and Sherwin, 1992). More recently, it was reported that postmenopausal women with Alzheimer's disease treated with a high dose of estrogen exhibited enhanced attention and memory as compared to placebo treated controls (Asthana et al., 2001). High levels of plasma estrogen have also been shown to have a positive effect on mood in postmenopausal women (Klaiber et al., 1979; Sherwin, 1991; but see Schleifer et al., 2002).

Given the various reports that exogenous estrogen can affect learning processes, we evaluated the effects of both physiological and supraphysiological acute doses of estrogen on associative learning. Following ovariectomy, females were injected with differing doses of estradiol and trained on the hippocampal-dependent task of trace eyeblink conditioning. This task was chosen because it is affected by sex differences and stages of estrus (Solomon et al., 1986; Moyer et al., 1990; Shors, 1998; Beylin et al., 2001).

2. Materials and methods

2.1. Subjects and surgical procedures

Adult virgin female Sprague–Dawley rats (250–300 g) were obtained from Zivic Laboratories and housed individually prior to and following surgery in the Department of Psychology animal facility, Rutgers University. Rats had unlimited access to water and Purina Lab Chow (Ralston-Purina, St. Louis, MO) and were maintained on a 12:12 h light–dark cycle with light onset at 07:00 h. After at least a 1-week acclimation period, animals were anesthetized with 30 mg/kg pentobarbital injected intraperitoneally supplemented with isoflurane inhalant and bilaterally ovariectomized (OVX). Ovaries were removed through a small midline incision on the ventrum. After the removal of the ovaries, the muscle wall and skin were closed with absorbable suture. Immediately following OVX, rats underwent eyeblink surgery. In this surgery, a headstage with four electrodes was anchored to the skull with screws and acrylic. The electrodes were implanted subcutaneously to emerge through the ridge of the eyelid: two were used to deliver a periorbital shock to the eyelid and two detected eyeblinks by transmitting electromyographic activity. After surgery, 0.3 ml of penicillin (250,000 units/ml) was administered intramuscularly, and the rat kept warm until recovery from anesthesia. Postoperatively, rats were provided with 24 h access to acetaminophen (32 mg/ml; IDE, Interstate, Amityville, NY) diluted 1:100 in drinking water for two days, and allowed at least five days of recovery before behavioral training.