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# Spatial memory retention is enhanced by acute and continuous estradiol replacement

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**Abstract**

Estradiol replacement to ovariectomized female rats causes dramatic changes in hippocampal structure and function as well as in performance on hippocampally dependent tasks. Using a delayed matching-to-place version of the water maze, the present study examines the time course of estradiol-induced enhancements in memory retention as well as the effectiveness of acute and continuous patterns of replacement. One 10-μg injection of estradiol administered on each of two successive days resulted in significant improvements in memory retention that persisted for approximately 4 days following the second injection. When estradiol administration continued for 10 consecutive days, these improvements in memory retention persisted. These findings indicate that estradiol replacement can improve memory retention and that these improvements can be maintained by continuous replacement for at least 10 days.


*Keywords:* Water-maze; Estrogen; Progesterone; Hippocampus; Spatial memory; Memory; Retention; Hormone

Ovarian hormones have been shown to exert a powerful influence on learning and memory processes and their neural substrates under a variety of experimental conditions. The exact nature of these effects, however, appears to depend on a variety of factors including the timing, duration, pattern, and dose of hormone exposure. For example, numerous studies have examined learning during various phases of the estrous cycle. The results, however, have been rather equivocal with some studies suggesting no significant effect of cycle phase on spatial learning (Berry et al., 1997; Stackman et al., 1997) and other studies reporting that periods of high estradiol are associated with impairments in learning (Chesler and Juraska, 2000; Frye, 1995; Warren and Juraska, 1997). In these studies, the exposure to ovarian hormones is physiological, however, it is difficult to separate the effects of estradiol from other hormones that also vary across the cycle. Acquisition of spatial learning tasks also appears to be impaired following replacement estradiol in ovariectomized voles (Galea et al., 2002) and one strain of mice (Rissman et al., 2002). Thus, it is likely that high estradiol slows learning rate.

In contrast, there are many reports that low-physiologic levels of estradiol replacement (approximately 15–30 pg/ml) to ovariectomized female rats over periods ranging from 10 days to 2 months is associated with improved performance on a variety of tasks that assess trial-specific (episodic) memory (called 'working memory' by Olton and Papas, 1979) including the t-maze (Fader et al., 1998), delayed matching-to-place version of the water maze (O'Neal et al., 1996), and radial-arm maze (Daniel et al., 1997; Fader et al., 1999; Luine et al., 1998; Williams, 1996). Furthermore, estradiol improves performance on a water version of the radial-arm maze, particularly as the number of spatial locations to be remembered increased (Bimonte and Denenberg, 1999). In all these studies, circulating estradiol was continuously elevated for 10–60 days before behavioral evaluation, so it is difficult to determine when or how estradiol may be causing improved performance as well as the time frame over which estradiol-induced improvements persist during continuous treatment and following estradiol withdrawal.

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Recently, we have reported that estradiol administered at doses and times that have been shown to increase excitatory connectivity in the hippocampus improves spatial memory retention of ovariectomized rats (Sandstrom and Williams, 2001). In our study, rats received two injections of estradiol benzoate (10 μg, sc) or vehicle separated by 24 h. The rats were then behaviorally evaluated 48 h following the second injection using a matching-to-place version of the water maze. The memory demands of this task were varied by altering the retention interval between the train trial and the test trials. We found that on trials following estradiol treatment, rats were better at remembering the location of the escape platform at longer delays than on trials following vehicle treatment. That is, when the delay between training and testing was short, all rats, regardless of hormone treatment, performed equally well. Only at long delays (i.e., 100 min) was the effect of estradiol apparent. Using the same estradiol replacement paradigm, Woolley and McEwen (1993) found a transient increase in dendritic spine density of CA1 pyramidal neurons that peaks approximately 48 h following the second injection and returns to baseline levels over the course of a week. Progesterone administered 48 h following priming with estradiol initially causes a further increase in spine density followed by a rapid decline in spine density back to baseline levels within 12 h (Woolley and McEwen, 1993). Our improvements in memory retention were similarly affected by progesterone with enhancements in memory evident immediately following progesterone but not when testing occurred 24 h after progesterone administration (Sandstrom and Williams, 2001). Thus, the improvements in memory induced by estradiol may be due to alterations in excitatory connectivity to CA1 pyramidal neurons—a phenomenon that requires 24–48 h of estradiol exposure and can be rapidly reversed by progesterone administration. To date, we do not know whether estradiol-induced increases in spine density and connectivity of CA1 pyramidal cells are sustained when estradiol is administered continuously for many days or weeks.

One clue that the temporal characteristics of estradiol replacement may be critically important for its behavioral and neural effects is that in 18-month-old ovariectomized female rats, spine density of dentate granule neurons increases following two 10-μg injections of estradiol; yet, continuous replacement of estradiol for many months does not maintain spine density at high levels (Miranda et al., 1999). These findings suggest that the behavioral effects of acute and continuous estradiol replacement may be quite different.

The goal of the present study was to investigate the temporal characteristics of estradiol replacement on spatial memory retention of ovariectomized rats. In Experiment 1, we examine the time course over which two 10-μg injections of estradiol separated by 24 h alters memory retention. In Experiment 2, we compare the time course of effects on memory retention when rats are given an acute, 2-day, treatment with estradiol, 10 days of continuous replacement, or no replacement of estradiol. Both of these experiments utilize a repeated-measures design, in which each rat is tested over a series of days to examine the time course of changes in performance following various hormone manipulations. Furthermore, each rat is repeatedly tested in each of the different treatment conditions allowing each rat to serve as its own control. We report improvements in memory retention of ovariectomized female rats that persist for only 3–4 days following acute estradiol priming. Furthermore, when daily injections of estradiol continue over a 10-day period, the improvement in memory retention can be maintained.

## Materials and methods

### *Animals*

Twelve female Sprague–Dawley (CD strain) rats were ovariectomized at 4 months of age. Rats were anesthetized for surgical procedures using a cocktail of 60 mg/kg ketamine (Fort Dodge Laboratories, Fort Dodge, IA) and 3.3 mg/kg xylazine (Sigma, Inc., St. Louis, MO) administered intraperitoneally. The ovaries were removed through a small midline incision on the abdomen. All surgical procedures were performed using aseptic techniques and in accordance with Duke University's Institutional Care and Use Committee guidelines. All rats had free access to Purina Rat Chow and were maintained on a 12-h light–dark cycle with lights on at 7:30 a.m. Behavioral training began at approximately 8 months of age and was performed during the light phase of the cycle, between 8:30 a.m. and 4:00 p.m.

### *Apparatus*

A circular black plastic pool (diameter = 164 cm, height = 50 cm) was filled to a level of 35 cm with water (22–24 °C) colored black with nontoxic black paint. A circular escape platform (diameter = 10.8 cm; height = 33 cm) with a nonskid surface was hidden just under the surface of the water. The water maze was in a rectangular testing room with a variety of extramaze cues including a computer, holding cages, cabinets, door, etc. Swimming behavior was recorded by a video camera connected to a tracking system (HVS Image, Buckingham, UK) and each trial was recorded on a computer for later analysis.

### *Hormone administration*

Hormones were dissolved in sesame oil (Sigma) and injected subcutaneously. Estradiol benzoate and progesterone (Steraloids, Inc., Newport, RI) were dissolved to concentrations of 0.2 and 10 mg/ml, respectively. Oil, estradiol (10 μg), and progesterone (500 μg) were injected