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Effects of estrogen and progesterone on spatial memory consolidation in aged females

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Abstract

Interpretation of data illustrating that estrogen, with or without progestin, is detrimental to memory in post-menopausal women is complicated by the fact that little is known about the effects of progestins on memory. The present study examined if estrogen, alone or with progesterone, affects spatial memory consolidation in ovariectomized aged female mice. Mice received eight training trials in a spatial Morris water maze followed immediately by injection of water-soluble 17β-estradiol (E_2; 0.2 mg/kg) or vehicle. Mice were re-tested 24 h later. All mice learned to find the platform on Day 1. On Day 2, the performance of control, but not E_2 mice, deteriorated, suggesting that E_2 enhanced memory for the platform location. In a second experiment, mice were injected with E_2 and 10 or 20 mg/kg water-soluble progesterone. The 10 mg/kg dose of progesterone did not affect estrogen's ability to enhance spatial memory consolidation, but 20 mg/kg blocked this effect. These data indicate that estrogen can improve spatial memory consolidation in aged females and that this effect can be attenuated by progesterone.

Keywords: Spatial memory; Reference memory; Water maze; Aging; Post-training; Ovarian hormones; Progestin; Mouse; Cyclodextrin

1. Introduction

The substantial loss of estrogen experienced by menopausal women has been linked to memory loss in both normal aging and dementia. Estrogen treatment in healthy menopausal women has been shown to improve spatial working memory [3], object memory [4,19], and verbal memory [3,35]. Other studies, however, have reported little or no mnemonic benefits from estrogen treatment in women [14,45]. Indeed, recent data from the Women's Health Initiative Memory Study (WHIMS) indicates that estrogen, given alone or with a synthetic form of progesterone, actually increases the risk of cognitive decline in post-menopausal women [36,37]. These data sharply contrast with previous reports that estrogen benefits memory function in menopausal women, and highlights the need to understand the ways in which ovarian hormones modulate memory in aging females.

Animal models have been instrumental in elucidating the effects of ovarian hormones on the brain and behavior, and thus, can help shed light on this issue. In female rodents, estrogen has profound effects on brain regions that are critical to learning and memory, such as the hippocampus. For instance, elevated estrogen levels in young female rats have been associated with enhanced hippocampal long-term potentiation [42], neurogenesis [39], and CA1 dendritic spine density [43,44]. The most biologically active form of estrogen, estradiol, also increases dentate gyrus spine density in aged female rats [21] and synaptic protein levels in aged female mice [9]. Furthermore, estradiol improves spatial memory in aged male [17] and female [11] rats. In middle-aged and aged female mice, chronic estradiol treatment significantly improves performance in spatial reference memory and object memory tasks, and increases hippocampal levels of the presynaptic protein synaptophysin [6,9]. Although these data suggest that estradiol improves memory in aging rodents, this evidence

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is confounded by the fact that treatments were given prior to behavioral training. As such, these treatments may have affected non-mnemonic aspects of task performance, such as arousal, motivation, or sensorimotor function, in addition to memory. In contrast, administering estradiol after training allows specific effects on memory consolidation to be observed in the absence of these non-mnemonic confounds. Post-training peripheral administration of 0.2 mg/kg estradiol improves spatial memory consolidation in young female rats [29], but such treatment has never been attempted in aging females. Thus, it remains unclear whether estradiol treatment specifically improves memory in aged females.

Despite the ability of estradiol to consistently improve performance in memory tasks among aged rodents, studies examining the effects of progesterone or of hormone therapy consisting of estradiol plus progesterone have produced more equivocal results. For example, acute estradiol and progesterone administered to young female rats impairs spatial reference memory in the Morris water maze [2]. Similarly, young female mice receiving estradiol learned a footshock avoidance task significantly faster than mice receiving estrogen plus progesterone or progesterone alone [5]. When administered alone, progesterone and its metabolite, allopregnanolone, have also been shown to impair spatial working and reference memory in young rats [10,15]. On the other hand, treatment with estrogen and progesterone has been shown to reduce spatial reference and working memory impairments induced by the cholinergic agonist scopolamine [38] and protect against memory impairments induced by intra-hippocampal administration of the neurotoxin colchicine [41]. Furthermore, in middle-aged and aged female rats, chronic estradiol plus progesterone improves spatial reference and working memory [11,20]. As is the case for estradiol, all of these studies administered progesterone prior to training, and thus, it is unclear if the effects of this hormone are due to specific changes in memory consolidation or to non-mnemonic performance factors. This issue is particularly important for progesterone because this hormone binds to GABA-A receptors and may, therefore, reduce arousal [16].

The goal of the present study was to determine if estradiol alone or estradiol plus progesterone affect spatial memory consolidation in aged (22 months) ovariectomized female mice. In order to pinpoint effects of the hormones on memory, in the absence of confounding effects on motivation or sensorimotor function, water-soluble hormone preparations were given immediately after training (post-training) in a spatial Morris water maze task. Consolidation was tested by measuring retention of the platform location 24 h later. Because these water-soluble hormones are metabolized within 24 h [30,40], retention is tested in the absence of hormones. Mice in Experiment 1 were injected intraperitoneally with 0.2 mg/kg 17β-estradiol (E_2) or vehicle immediately after water maze training. In young female rats, 0.2 mg/kg E_2 injected immediately, but not 2 h after training, significantly improves retention of the platform location, suggesting that this dose enhances spatial memory consolidation in the water maze [29]. The 0.2 mg/kg dose also improves object memory consolidation and spatial working memory in young female mice [13]. Thus, we hypothesized that 0.2 mg/kg estradiol would also improve spatial memory consolidation in aged females. In Experiment 2, a different group of mice was injected after water maze training with 0.2 mg/kg E_2 combined with 10 or 20 mg/kg progesterone. Because this is the first study to utilize water-soluble progesterone, these doses were based on previous studies using progesterone dissolved in oil [44]. As in those studies, our low progesterone dose was 50 times that of estradiol. The higher dose was given to approximate levels similar to those during proestrus [1]. Given that levels of both estradiol and progesterone are greatly reduced by middle age in C57BL/6 mice [24], we hypothesized that the combination of both hormones would improve memory in aged females.

2. Methods

2.1. Subjects

Subjects were 22 months old female C57BL/6 mice obtained from the National Institutes on Aging colony at Harlan Sprague Dawley (Indianapolis, IN). Mice were housed up to five per shoebox cage in a room with a 12:12 light/dark cycle (lights on at 07:00), with all testing performed during the light phase. Mice had ad libitum access to food and water. Animals were handled for 5 min/day at least five times prior to ovariectomy surgery to habituate them to being picked up by the experimenter. All procedures were approved by the Institutional Animal Care and Use Committee of Yale University, and conformed to the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Ovariectomy

Although the estrous cycles of female mice have typically ceased by 22 months of age [8,25], all mice were ovariectomized to ensure similar endogenous levels of estrogens prior to treatment. Surgeries were conducted one month prior to behavioral testing as described previously [6,13]. Briefly, mice were anesthetized using 2% isoflourane gas in 100% oxygen. Bilateral dorsal incisions were made at the level of the pelvis, and the ovaries and tips of the uterine horns were isolated and removed. Uteri were then placed back into the body cavity, the muscle wall was sutured, and the skin closed with wound clips. Analgesia was provided by 300 mg/kg children's acetaminophen in the drinking water for one week post-surgery.

2.3. Morris water maze

Testing took place in a white circular tank (97 cm in diameter) filled with water (24 ± 2 °C). The water was made opaque