Published in final edited form as:
Brain Res. 2005 March 2; 1036(0): 101–108. doi:10.1016/j.brainres.2004.12.014.

Estradiol to aged female or male mice improves learning in inhibitory avoidance and water maze tasks

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Abstract

Although 17β-Estradiol (E_2) improves cognitive performance of aged female mice, its mnemonic effects when administered post-training to aged male mice have not been examined. E_2 (10 μg, SC) or oil vehicle was administered to intact, 24-month-old female or male congenic (primarily C57BL/6 background) mice immediately after training in the inhibitory avoidance or water maze tasks. Following behavioral testing, effects of 1 or 24 h of E_2 exposure on hippocampal levels of E_2 and brain-derived neurotrophic factor (BDNF) were examined. Female and male mice administered E_2 showed significantly better performance in the inhibitory avoidance task than did vehicle-administered mice. When tested 24 h after training, mice that received E_2 had significantly longer latencies to cross-over to the shock-associated side of the chamber than did vehicle-administered mice. Female or male mice administered E_2 showed significantly better performance in the reference memory aspect of the spatial water maze task. When tested 30 min after training, mice administered E_2 had shorter latencies to, and spent longer swimming in, the quadrant that the hidden platform had previously been located in. E_2 administration produced physiological levels of E_2 in the hippocampus 1 and 24 h after E_2. BDNF levels in the hippocampus were decreased following 1 h of E_2 exposure compared to vehicle. These findings suggest that E_2 to female and male mice may overcome age-related deficits in reference memory in an emotional or spatial learning task.

Keywords

Estrogen; Aging; Memory; Hippocampus

1. Introduction

17β-estradiol (E_2) can enhance cognitive performance in a number of populations. Although hormone therapy has recently come under fire [82], E_2 may have some beneficial effects on cognitive function. E_2 replacement attenuates impaired cognitive performance associated with menopause [77,80]. Women receiving E_2 following menopause demonstrate enhanced verbal, short-term, and long-term memory, as well as logical reasoning, compared to women not receiving E_2 replacement [76,78,79]. These improvements appear to occur as a function of age. E_2 to older, physiologically-menopausal women produces greater improvement in

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verbal memory compared to that seen in younger, surgically-menopausal women with fewer verbal memory deficits [6,76]. E_2 also enhances cognition in a population with more profound cognitive deficits. Women with Alzheimer's disease receiving E_2-replacement therapy showed significant improvement on a number of cognitive measures compared to age-matched controls [1,62]. However, not all studies find beneficial effects of E_2. Hormone therapy that results in very high or very low levels of E_2 produces impaired spatial ability, whereas mid-level E_2 is associated with improvement on tests of verbal, visual, and semantic memory compared to that of post-menopausal women given placebo [1,34,45]. Thus, beneficial effects of E_2 among women seem to be most readily observed in aging individuals that receive replacement with moderate E_2 concentrations.

E_2 may also have beneficial effects among men for enhanced cognitive performance. Young men with high levels of E_2 performed better on two measures of visual memory than did men with normal but lower E_2 levels [38]. Among older men, low levels of E_2 were associated with decreased cognitive function [75]. Further, treatment of elderly men with testosterone enhanced spatial and verbal memory in association with increased testosterone and E_2 levels [9]. However, it should be noted that other studies have reported either no relationship [11,18,40] or a negative association between E_2 and cognition among men [15,37]. Thus, the effects of E_2 to modulate cognition are not well-understood and require further investigation.

Several factors may influence E_2's effects on cognitive performance in animal models. Among young rodents, variable performance during behavioral estrus (when endogenous E_2 levels are higher relative to estrus) is associated with E_2 concentrations and task demand [4,14, 26,31,41,83,84,95]. Findings from extirpation and E_2 replacement studies in younger rodents also suggest that better performance is related to moderate E_2 levels and task difficulty [5,10,13,21,24,25,27,30,33,35,44,49,63–65, 72,74]. In support, previous data have shown that proestrous rats exhibit increased motor behavior compared to rats in other phases of the estrous cycle [2,29]. As such, proestrous rats may appear to perform more poorly on tasks that involve more gross motor responses as a result of their increased locomotor activity. In contrast, performance in cognitive tasks without great motor demand is improved by physiological regimen of estrogen [81,96]. Thus, E_2's profound effects on activity and emotional arousal [55,56,68] may contribute to some of the different effects of E_2 reported in cognitive tasks that diverge in the activity and/or arousal required for optimal performance. Because of these possible non-mnemonic factors, the present studies utilized a physiological concentration of E_2 (10 μg, SC) that was administered after training in two tasks that have low (inhibitory avoidance) or high (water maze) motor demand.

Beneficial effects of E_2 may be clearer in aging rodents. First, despite considerable differences between strains in age-related deficits in water maze performance [89,90], decline in spatial memory typically occurs with ageing. In general, this decline occurs earlier in female (~17 months), as compared to male (~24 months), mice and among females decline seems to coincide with cessation of ovarian function [24,51,60,61]. Second, E_2 to senescent female or male rats improves spatial performance [32,48] and E_2 to aged female mice enhances spatial and non-spatial performance [23,25,53,91]. However, whether the aforementioned effects of E_2 were attributable to mnemonic- or performance-enhancing effects of E_2 was not dissociated because E_2 was present during training and testing. Given the ageing population, ascertaining the efficacy of treatments and the responsive populations, in which E_2 may minimize age-related cognitive decline is becoming increasingly important [52,54,57]. As such, effects of post-training E_2 administration to aged female or male mice (that have a varied genetic background) on spatial and non-spatial memory was investigated.

Another factor that may contribute to some of the variable effects previously reported for E_2 on learning are its diverse mechanisms of action. E_2's ligand-dependent actions, via traditional intracellular E_2 receptors (ERs) that have been localized to the hippocampus and forebrain [39], typically take hours to days to occur [67]. However, E_2 can also have rapid effects via actions at neuronal membranes [88]. Some of E_2's mnemonic effects may be due in part to its modulation of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF). BDNF affects neuronal survival, differentiation, and synaptic plasticity, and the gene for BDNF has an estrogen response element [47,87]. BDNF has been implicated in learning and memory and Alzheimer's Disease [58,97]. Notably, BDNF is abundant in the hippocampus and is altered by E_2 exposure [3,8,46]. For these reasons, E_2's effects on BDNF levels in the hippocampus were examined.

The present studies utilized female and male mice to examine effects of E_2 on learning. We hypothesized that if E_2 can produce mnemonic effects via either rapid or sustained actions in aged mice, then post-training administration of E_2 would be expected to improve learning in the inhibitory avoidance and water maze tasks.

2. Materials and methods

Procedures were pre-approved by The Institutional Animal Care and Use Committee at SUNY-Albany.

2.1. Subjects and housing

Subjects were 24-month-old congenic mice (having primarily a C57BL/6J background; n = 16 females, n = 14 males), raised and group-housed in the Laboratory Animal Care Facility at The University at Albany [12,93].

2.2. Hormonal milieu

Mice were left intact, as male mice would be expected to have low endogenous E_2 concentrations and aged female mice would be expected to have E_2 levels that have reached nadir [60,66]. Further, surgical procedures in mice of this age are contraindicated due to attrition that would be produced.

Regimen of E_2 ranging from 0.5 to 10 μg have been utilized previously to produce physiological concentrations of E_2 [16]. There are strain differences in responsiveness to gonadal hormones, which may be more apparent in congenic mice [85]. Further, older animals may be less responsive to E_2 administration. For these reasons, we employed the highest known regimen that has been utilized to produce physiological E_2 levels in mice (10 μg). Immediately following training in the behavioral tasks, mice were administered SC E_2 or vehicle (sesame oil). Our lab, and others', have previously demonstrated that exposure to E_2 within 1 h of training is critical for consolidation [50,65,70].

2.3. Procedure

Mice were randomly assigned to receive E_2 or vehicle injections. All mice were first trained and tested in the inhibitory avoidance task and then in the water maze task, as described below. After testing, some mice were re-administered E_2 or vehicle for tissue collection for measurement of hippocampal E_2 and BDNF levels. Mice were kept in the same condition for both tests and tissue collection. There was a 7- to 10-day washout period between each E_2 injection. Although this is a sufficient washout period for younger animals, whether aged animals can clear E_2 as efficiently is not known. Thus, there may have been carryover effects of E_2 administration.

2.4. Behavioral testing

2.4.1. Inhibitory avoidance—During week 1, mice were trained and tested in the inhibitory avoidance task, using modified methods described in previous reports [28,30,69]. Briefly, mice were placed in the two-chambered inhibitory avoidance apparatus that is equally partitioned by a sliding door (one side white and brightly-lit; one side black and not illuminated). 20 min later, mice were placed on the light side of the chamber and the door was lifted. When the mouse crossed to the dark side, the door was closed and a shock was administered (0.25 mA, 5-s duration). Immediately after training, mice were administered 10 μg SC E_2 or oil vehicle. 24 h after training, mice were placed on the light side of the chamber, the door was lifted, and the latency to cross-over was recorded (maximum latency, 300 s).

2.4.2. Water maze—During week 2, mice were trained and tested in a water maze task using methods previously described [24,25,94]. On day 1, mice were placed on the hidden platform (8 cm in diameter) in the water maze (97 cm in diameter) and then allowed to swim freely in the maze for 1 min. On day 2, mice were given twelve trials, organized into three blocks of 4 trials (one trial/start position within a block). The four trials within a block were separated by 10 s. Each block of trials was separated by 30 min. Immediately following the last trial (trial 12), mice were administered 10 μg SC E_2 or vehicle. 30 min later, mice were given a 1-min probe trial in which the platform was not available for escape. The start point for the probe trial was randomly selected for each subject. The latency to cross where the platform had been, and the duration of time spent in the quadrant where the platform was during training, were recorded. To address possible non-mnemonic effects of E_2 on performance, 20 min following the probe trial, a cued task was conducted. A visible platform was raised above the surface of the water and placed in a different quadrant for each of the four trials. The latency to reach the platform in each trial was recorded.

2.5. Biochemical measures

2.5.1. Tissue collection—One week following water maze testing, some mice (n = 6/group) were put into the same hormonal conditions as they were in for behavioral testing. One or 24 h after E_2 or vehicle administration mice were killed by cervical dislocation and brains were rapidly removed and placed on dry ice followed by storage at −70 °C until measurement.

2.5.2. Tissue preparation—Immediately prior to measurement of E_2 and BDNF levels, unilateral hippocampus were dissected on ice and weighed. Tissues were homogenized in a glass/glass homogenizer in 2 ml of distilled water. These homogenates were then utilized to measure E_2 (radioimmunoassay) and BDNF (ELISA) levels in the hippocampus.

2.5.3. Radioimmunoassay for E_2 levels in hippocampus—For measurement of hippocampal levels of E_2, 600 μl of the above preparation was extracted twice with ether by snap freezing. Following chromatographic separation, TMP was evaporated, and pellets were reconstituted in PBS (pH = 7.4). The standard curve was prepared in duplicate (12.5–1000 pg/0.1 ml). Standards were added to PBS, with E_2 antibody (Dr. Niswender, #244, Colorado State University, Fort Collins, CO), and [^{3}H] E_2 (NET-317, 51.3 ci/mmol; New England Nuclear, Boston, MA, 8000 dpm/100 ml). Assay tubes were incubated at room temperature for 50 min. Separation of bound and free occurred with dextran-coated charcoal and centrifugation at 3000 × g for 10 min following a 10-min incubation on ice. Supernatant was counted using a Tri-Carb 2000CA Liquid Scintillation Analyzer. Unknowns were interpolated from the standard curve using Assay Zap, a program for RIA analyses.

2.5.4. ELISA for BDNF levels in hippocampus—BDNF levels in the hippocampus were examined utilizing the Promega BDNF E_{max} Immunoassay System (Promega, Madison, WI). Briefly, 200 μl Promega lysis buffer was added to 100 μl aliquots of previously prepared samples. Samples were sonicated with a microtip at power level 4 for 15 s. Samples were then centrifuged at 16,000 × *g* for 30 min. Twenty-five μl aliquots of the resulting supernatant were removed and diluted with 100 μl of DPBS buffer. 100 μl anti-BDNF monoclonal antibody (mAB) diluted 1:1000 in carbonate coating buffer was applied to a 96-well polystyrene plate and incubated overnight at 4 °C. Unabsorbed mAB was removed and plates were washed once with TBST wash buffer. Plates were blocked using 200 μl Promega 1× Block and Sample buffer followed by incubation, without shaking, for 1 h at room temperature. Plates were then washed once using TBST wash buffer. Two hundred μl of each standard (0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 pg/ml) were added in duplicate to plates. One hundred μl of samples were added in duplicate to plates. Plates were incubated for 2 h with shaking at room temperature. Plates were then washed five times with TBST wash buffer. Anti-human BDNF polyclonal antibody (pAB; 100 μl diluted 1:500 in 1× Block and Sample buffer) was added to each well and plates were incubated for 2 h with shaking at room temperature. Plates were washed five times with TBST wash buffer. Anti-Ig Y horseradish peroxidase conjugate (100 μl diluted 1:200 in 1× Block and Sample buffer) was then added to each well and plates were incubated for 1 h with shaking at room temperature. Plates were emptied again and washed five times with TBST wash buffer. Finally, plates were developed using 100 μl Promega TMB One Solution and the reaction was stopped at 10 min using 100 μl 1 N HCl. Protein was measured using Bradford's method [7]. Absorbance was measured at 450 nm. BDNF levels are reported as ng/mg tissue.

2.6. Statistical analyses

Effects of E_2 administration on inhibitory avoidance and water maze learning were compared across groups using two-way analyses of variance (ANOVAs) using hormone (E_2 or vehicle) and sex (female or male) as factors. As there were no effects of sex on behavior and tissues collected from a subset of animals (which would yield a small number of observations using sex as a variable), sex was not considered a factor when analyzing biochemical measures. One-way ANOVAs were used to examine effects of vehicle, and 1 or 24 h of E_2 exposure on E_2 and BDNF levels in the hippocampus. Alpha level for statistical significance was $P < 0.05$. Where appropriate, Fisher's post hoc tests were used to determine group differences.

3. Results

3.1. Inhibitory avoidance

E_2 enhanced inhibitory avoidance learning of female and male mice. There was a main effect of hormone on cross-over latencies on test day ($F(1,26) = 12.44$, $P < 0.05$). Female and male mice administered E_2 had significantly longer latencies to cross-over to the dark, shock-associated side of the chamber compared to their counterparts administered vehicle (Fig. 1). There was no main effect of sex on cross-over latencies on test day ($F(1,26) = 0.58$, $P > 0.05$). The better performance of E_2-administered mice was not due to differences in latencies on training day. There were no main effects of sex ($F(1,26) = 0.5$, $P > 0.05$) or hormone ($F(1,26) = 0.04$, $P > 0.05$) on cross-over latencies on training day.

3.2. Water maze

Learning of female and male mice in the water maze was improved by E_2. There was a main effect of hormone, but not sex, on the latency to cross the hidden platform ($F(1,26) = 13.91$, $P < 0.05$ and $F(1,26) = 3.25$, $P > 0.05$, respectively) and duration of time spent in the

quadrant where the platform had been during training ($F(1,26) = 13.27$, $P < 0.05$ and $F(1,26) = 0.44$, $P > 0.05$, respectively). Female or male mice administered E_2 had significantly shorter latencies to cross the hidden platform and spent significantly more time in the quadrant where the platform had been compared to their vehicle-administered counterparts (Fig. 2). Training latencies to find the hidden platform were not different among the groups. There was no main effect of hormone ($F(1,26) = 0.05$, $P > 0.05$) or sex ($F(1,26) = 1.21$, $P > 0.05$) on latencies to find the platform across training trials.

3.3. Hippocampal E_2 levels

E_2 administration increased hippocampal E_2 levels ($F(2,15) = 4.29$, $P < 0.05$). E_2 levels in the hippocampus were significantly higher 1 h following E_2 administration and tended to be higher 24 h after E_2 administration compared to vehicle (Fig. 3, top).

3.4. Hippocampal BDNF levels

BDNF levels in the hippocampus were altered by E_2 administration ($F(2,15) = 3.75$, $P < 0.05$). One hour following E_2 administration, BDNF levels in the hippocampus were decreased compared to vehicle. There were no differences in BDNF levels in the hippocampus 24 h after E_2 administration compared to vehicle (Fig. 3, bottom).

4. Discussion

These results support the hypothesis that E_2 has enhancing effects on memory of aged female and male mice. Previous studies have demonstrated age-related decline in learning of male and female mice and that E_2 to female mice can overcome age-related deficits in cognitive performance [22,24,25]. The present results extend these prior studies in several important ways. First, E_2 had similar beneficial effects when administered to aged female or male mice (with a variable genetic background) as has been previously reported for aged female C57BL/6 mice [24,25]. Second, in previous studies, beneficial effects of E_2 were observed when E_2 was present during training and testing. The present effects of post-training E_2 demonstrate that E_2 can have mnemonic effects in aged mice. Third, exogenous E_2 produced particularly rapid effects (within 30 min) in the water maze, but also effects that were sustained and salient neurorestorative effects in the inhibitory avoidance task 24 h later in these aged mice. Given that the E_2 regimen employed in the present studies produced modest E_2 levels in the hippocampus 1 and 24 h after E_2 administration, these results suggest that physiologically-relevant concentrations of E_2 may have important cognitive-enhancing effects.

Mnemonic effects of E_2 were evident in both the inhibitory avoidance and water maze tasks, which suggests that some of E_2's beneficial effects may involve the hippocampus. First, the hippocampus mediates spatial and explicit memory function and is integral to performance in the inhibitory avoidance and water maze tasks [17,36]. Second, E_2 produces changes in hippocampal physiology and morphology that has recently been related to cognitive performance [86]. E_2 to ovariectomized C57BL/6J mice enhances performance in the object placement task, a spatial episodic memory task, and increases the number of dendritic spines with mushroom shapes in the dorsal hippocampus [44]. Although E_2 can have actions in the hippocampus for its mnemonic effects, this does not preclude the involvement of actions of E_2 in other brain areas for enhanced learning.

The mechanisms for E_2's mnemonic effects have not been identified. E_2 may have actions in the hippocampus to improve learning via traditional ligand-dependent actions at intracellular ERs [19,20,42]. In support, E_2 administered to ERβ knockout mice does not enhance spatial learning compared to vehicle [73]. Further, there may be ER specific effects of E_2 to

enhance learning. Ligands with specific activity at ERa or ERβ can enhance learning [43,50,71]. However, these data do not preclude non-ER mediated effects of E_2 to enhance learning. For example, ER antagonists fail to block E_2's effects on inhibitory avoidance. Systemic administration of tamoxifen or intra-hippocampal ICI 182,780 does not attenuate the mnemonic effects of intra-hippocampal E_2 in the inhibitory avoidance task of rats [28,30]. Further, other models have demonstrated that membrane actions of E_2 may propagate later actions at ERs [92]. The present results that post-training exposure to E_2 for 30 min is sufficient to enhance learning in the water maze, and post-training E_2 for 24 h improves learning in the inhibitory avoidance task is consistent with times frames required for membrane (latencies within seconds) and ER-mediated actions (latencies within 15 min) [67]. Although timeframe does not directly address E_2's mechanism, to our knowledge, these results demonstrate the most rapid mnemonic effects of E_2 that have been reported. Further, more rapid effects are unlikely to be demonstrated, as a 1-h post-training consolidation period is necessary for E_2's mnemonic effects.

To more directly address E_2's mechanism, BDNF levels in the hippocampus were examined. One hour following E_2 administration, BDNF levels in the hippocampus were decreased compared to vehicle. It is possible that E_2 may have enhanced learning by down-regulating BDNF. E_2 down-regulates BDNF in cultured hippocampal neurons, which decreases inhibition and increases excitatory tone in pyramidal neurons, leading to a 2-fold increase in dendritic spine density [59]. Thus, in the present studies, reduced BDNF following 1 h of E_2 exposure may reduce GABAergic neurotransmission, leading to increased spine growth and enhanced memory. Notably, BDNF following 24 h of E_2 exposure was not different than that seen following vehicle. However, previous data have demonstrated that the critical timeframe for consolidation of learning is within 1 h of training [50, 65,70]. Given the relevance of E_2's neurorestorative actions for aging populations, the mechanisms associated with E_2's rapid and sustained effects need to be investigated further.

Acknowledgments

We appreciate the grant support provided by The National Science Foundation (DBI 00-97343, IBN03-16083), The National Institute of Mental Health (MH067698), and SUNY-Albany.

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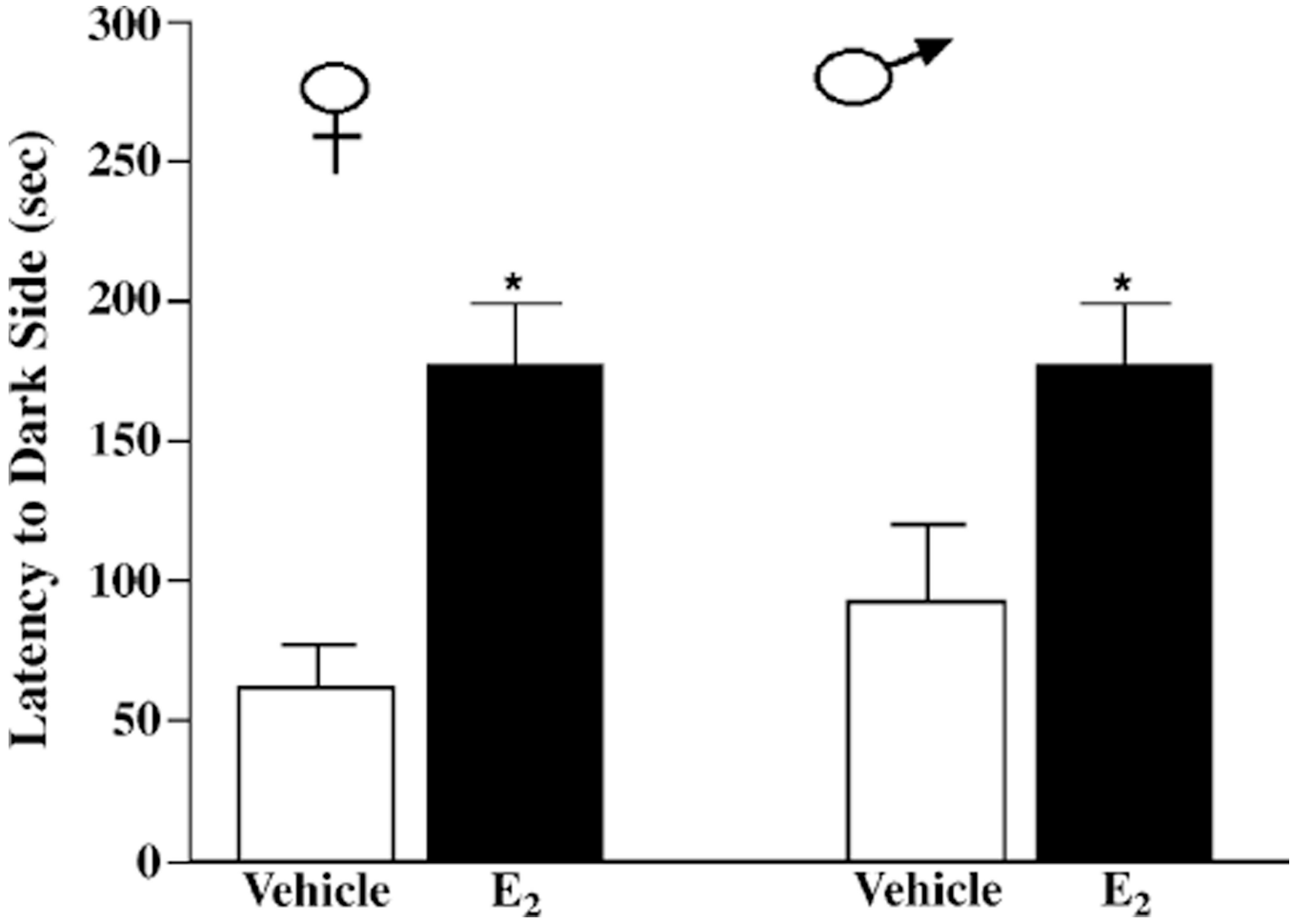

Fig. 1.

Latencies to cross-over to the dark, shock-associated side of the training apparatus of female (left) or male (right) mice administered post-training vehicle ($n = 8$ female, $n = 7$ male, white bars) or 10 μg E_2 ($n = 8$ females, $n = 7$ males, black bars). * indicates significantly different from vehicle ($P < 0.05$).

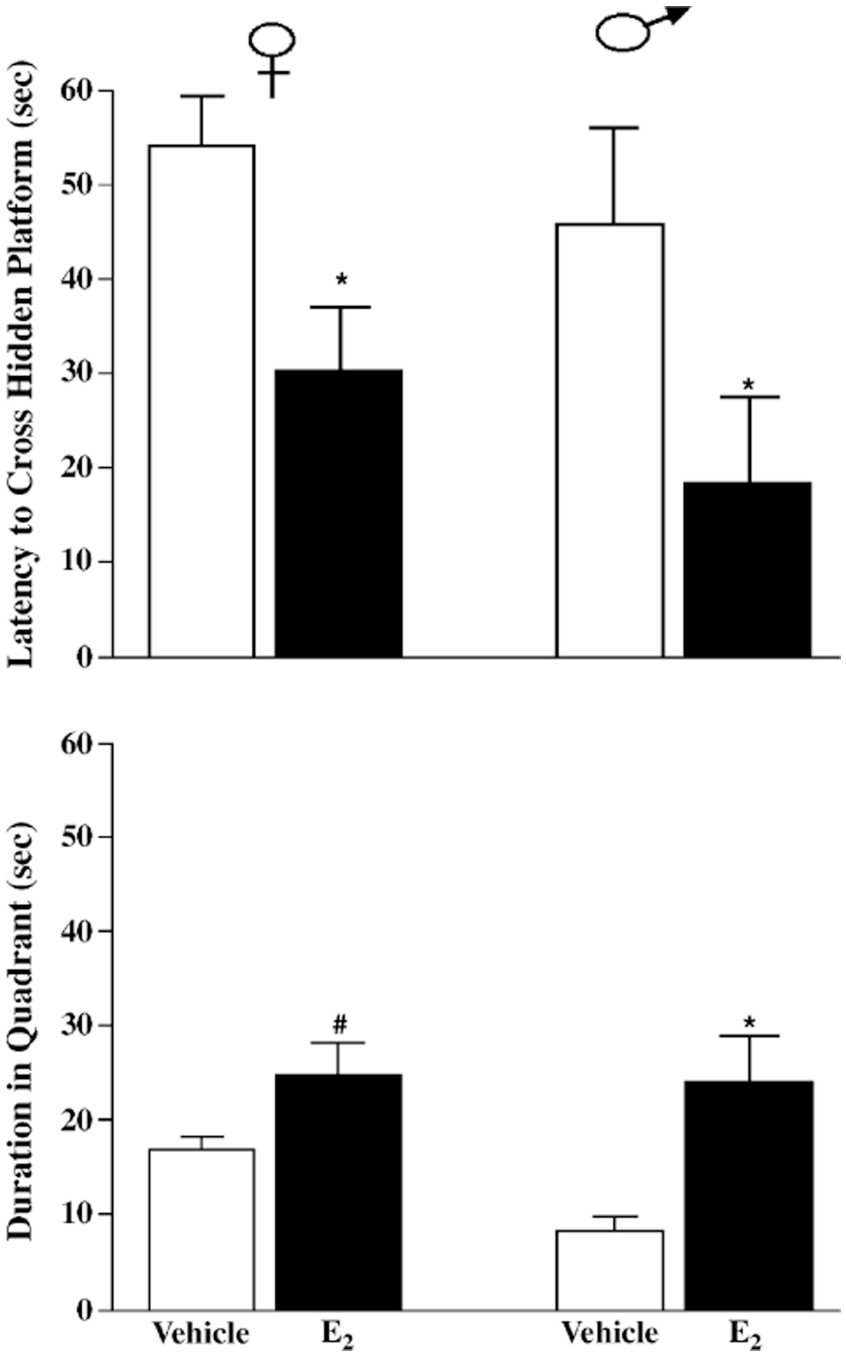

Fig. 2.

Latencies to cross (top), and duration spent swimming in (bottom), the quadrant where the platform had been during training of female (left) or male (right) mice administered post-training vehicle ($n = 8$ females, $n = 7$ males, white bars) or 10 μg E_2 ($n = 8$ females, $n = 7$ males, black bars). * indicates significantly different from vehicle ($P < 0.05$). #indicates tendency for difference from vehicle (P, 0.10).

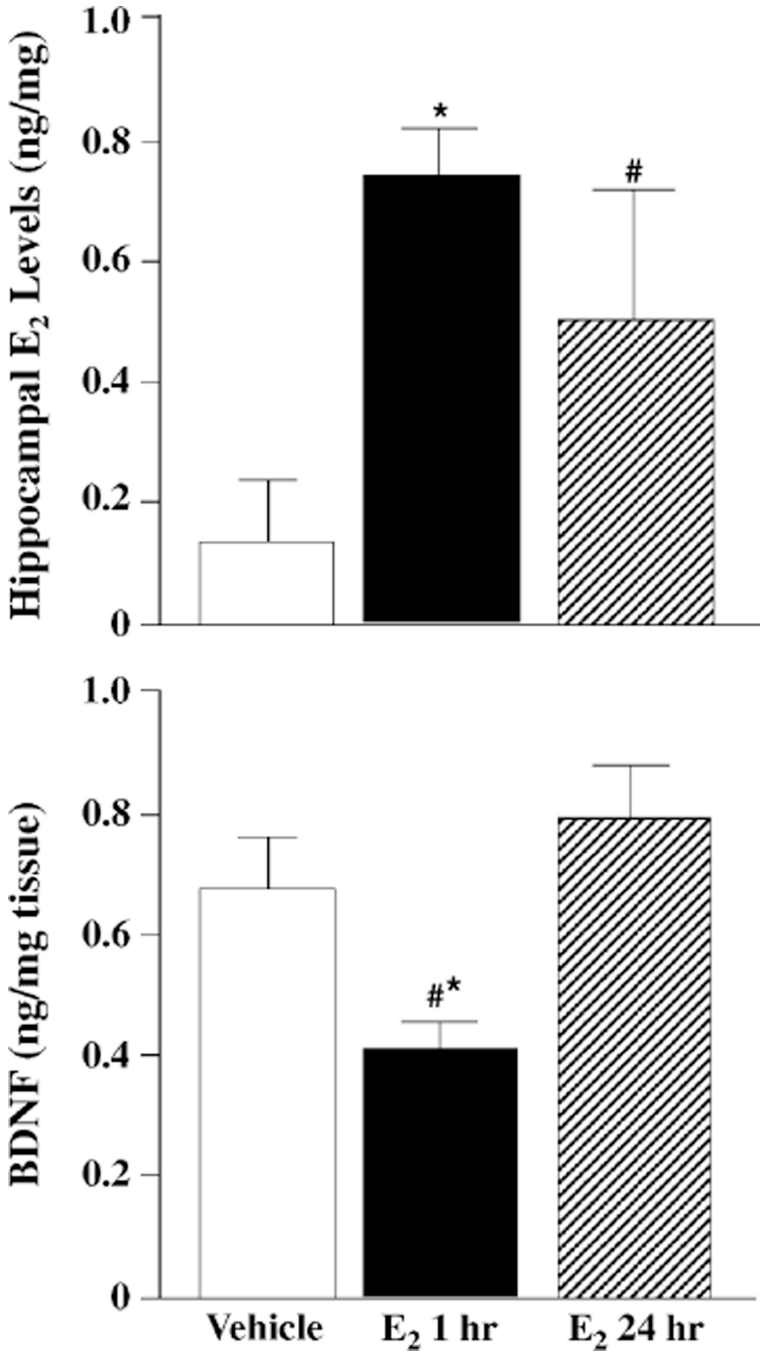

Fig. 3.

Hippocampal levels of E_2 (top) or BDNF (bottom) of mice exposed to vehicle ($n = 6$, white bars), 1 h ($n = 6$, black bars), or 24 h ($n = 6$, striped bars) of E_2. Top panel * indicates significantly different from vehicle ($P < 0.05$) and # indicates tendency for difference from vehicle ($P < 0.10$). Bottom panel * indicates significantly different from E_2 24 h and # indicates tendency for difference from vehicle.