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Estradiol reduces anxiety- and depression-like behavior of aged female mice

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Abstract

Beneficial effects of the ovarian steroid, 17 β -estradiol (E₂), for affective behavior have been reported in young individuals, but less is known about the effects of E₂ among older individuals, and the capacity of older individuals to respond to E₂ following its decline. In the present study, the effects of acute E₂ administration to aged mice for anxiety-like and depression-like behaviors were investigated. Intact female C57BL/6 mice ($N=18$) that were approximately 24 months old were administered vehicle (sesame oil, $n=9$) or E₂ (10 μ g, $n=9$) subcutaneously 1h prior to behavioral testing. Mice were tested for anxiety-like behavior (open field, elevated plus maze, mirror chamber, light–dark transition task, Vogel conflict task) and depression-like behavior (forced swim task). To assess the role of general motor behavior and coordination in these aged mice, performance in an activity monitor and rotarod task, and total entries made in tasks (open field, elevated plus maze, light–dark transition task) were determined. Mice administered E₂, compared to vehicle, demonstrated anti-anxiety behavior in the open field, mirror chamber, and light–dark transition task, and anti-depressive-like behavior in the forced swim task. E₂ also tended to have anti-anxiety effects in the elevated plus maze and Vogel task compared to vehicle administration, but these effects did not reach statistical significance. E₂ did not alter motor behavior and/or coordination in the activity monitor, open field, or rotarod tasks. Thus, an acute E₂ regimen produced specific anti-anxiety and anti-depressant effects, independent of effects on motor behavior, when administered to aged female C57BL/6 mice.

Keywords

17 β -estradiol (E₂); Aging; Anxiety; Depression; Senescent; Motor activity

1. Introduction

The ovarian hormone, 17 β -estradiol (E₂), has pleiotropic effects in the brain and body. A situation in which these effects may be most evident is when individuals experience robust changes in ovarian function with aging and the onset of reproductive senescence, which can produce changes in quality of life (i.e. mood, anxiety, forgetfulness, sleepiness, etc.), as well as physical changes (i.e. changes in bone density, hot flashes, drying of mucosal membranes, etc.). Given that in many cases the source and target of E₂ are the same (e.g.

brain, ovaries), it can be difficult to parse out these effects and determine E₂'s mechanisms. A typical approach that has been taken to address this in the laboratory is to ovariectomize (OVX) rodents and replace them back with E₂ or E₂-mimetics, such as selective estrogen receptor modulators (SERMs), which have discrepant effects at different receptor targets. Although this has been a fruitful approach, differences in the timing of OVX and replacement can obscure some of the effects. For instance, there may be differences on anxiety measures in rats depending upon how long they are OVX, with or without E₂ replacement [1–3]. As well, the clinical relevance of using OVX as a model of reproductive senescence may be less than ideal. In support, differences in cognitive performance of women who have undergone natural, compared to surgical, menopause have been noted [4]. However, the functional effects of E₂ replacement to aged mice have not been well-characterized and are of interest.

Given that life expectancy of women has risen, the population is aging, and more women will live longer in a post-menopausal, E₂-deficient state [10], it is important to understand further the effects of E₂ in anxiety/depression among older individuals. Although most women do not become depressed during perimenopause, reproductive events may play a role in the onset of depression among some perimenopausal women [6]. Further, E₂ may be efficacious as a primary and/or adjunctive therapy for some perimenopausal women [5–7]. Indeed, some post-menopausal women have significantly higher depression scores, when evaluated, than do pre- and perimenopause women [8]. Some prospective, controlled studies show that E₂ can dose-dependently alleviate depressive symptoms [9]. As in the clinical literature, few studies reported in the animal literature have directly investigated the responses of older individuals to E₂ on affective measures.

In animal models, E₂ to young individuals can have anxiety- and depression-reducing effects [2,11]. Although the majority of these studies have been done in rats, there is accumulating evidence that similar effects are observed in mice. For example, when tested in the high (behavioral estrous), versus the low (diestrus), steroid hormone phase of the estrous cycle, mice show less anxiety behavior in the plus maze [9,12,13]. Physiological dosing with E₂ reverses OVX-induced increases in depressive behavior in the forced swim test and in anxiety behavior in the elevated plus maze of young adult mice [2,14–18]. How alterations in ovarian function with aging of mice alter affective behavior is of interest. To this end, we investigated the effects of E₂ administration to aged female mice in a variety of behavioral tasks to assess its functional effects to test the hypothesis that aged mice would respond favorably to E₂ administration.

2. Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at the University at Albany-SUNY.

2.1. Subjects and housing

Intact, female mice ($N=18$, mean age 24 months, range 20–28 months), were bred in the Social Sciences Laboratory Animal Care Facility at SUNY-Albany, on a congenic C57BL/6 background. Mice were group-housed, on a 12/12 hour light/dark cycle (lights on 0800h) with free access to Purina Rodent Chow and water in their home cages.

2.2. Hormonal milieu

The pattern of female reproductive aging in mice is similar, but not analogous to that of women [19,20]. Although there is considerable variation at midlife, ovarian follicles become depleted and steroid production decreases to levels akin to that of OVX mice during reproductive aging. By 20–26 months of age, mice without pituitary tumors have

gonadotropin levels which are similar to that of OVX mice. As such, mice without pituitary tumors (as determined at autopsy) were utilized intact to prevent attrition from surgery. Mice were randomly assigned to receive E₂ (10 µg/0.2cm³; *n*=9) or vehicle (sesame oil; *n*=9) by SC injection 1h prior to behavioral testing. This E₂ regimen produces concentrations of E₂ in plasma of young female mice [21] and hippocampus of senescent female mice [22], akin to that of young, intact mice in behavioral estrus.

2.3. Behavioral testing

Mice were tested approximately once weekly until all tasks, described below, were completed. It was important to test mice across several tasks to account for potential age-related differences in locomotion and/or coordination in these affective tasks and control tasks. As such, in addition to reporting on the effects on anxiety and depression tasks, performance of mice for locomotor behavior in the activity monitor and open field and motor coordination in the rotarod were determined. A description of the tasks that mice were tested in is as follows. Mice were tested in one task per day, in the order that tasks are described below. Although the authors acknowledge that this order of testing may have confounded results, it was deemed necessary in a study such as this using a small number of aged individuals that may be particularly sensitive to the physical demands, or stress of being exposed to a shock stimulus, in the forced swim test and Vogel conflict task, respectively. Experience in these tasks may have altered typical performance in the other behavioral tasks utilized. As such, mice were tested in the following tasks in the order indicated.

2.3.1. Activity monitor—A Digiscan Optical Animal Activity Monitor (39×39×30 cm; Accuscan Instruments Inc) recorded the number of horizontal beam breaks in 5 min [23]. The number of beam breaks made is utilized as an index of general motor behavior.

2.3.2. Open field—Entries into central and peripheral squares of an open field (39 cm×39 cm×30 cm), with 16 squares on the grid floor, was recorded for 5 min [23]. The total and central square entries made in the open field are utilized as indices of general motor and anti-anxiety-like behavior, respectively.

2.3.3. Rotarod—The latency of mice to fall (3 min max, a 48 cm fall height) from The Accurotor Rotorod Apparatus (Accuscan Instruments Inc), with a 70 mm drum, set to rotate at accelerating speeds (0–60 rpm/min), was recorded [23]. The latency to fall from the rotarod is utilized as a measure of motor coordination.

2.3.4. Elevated plus maze—The time spent by mice in the 2 open (5×40 cm) or closed (5×40×20 cm) arms was recorded for 5 min [24]. The time spent in the open arms of the plus maze is utilized as a measure of anti-anxiety-like behavior in this task. Measures of motor activity in this task are the number of entries to the open arms and the total number of arm entries made.

2.3.5. Mirror chamber—The time spent by mice in the mirrored chamber (30 cm×30 cm×30 cm) or the adjoining alleyway (30 cm×5 cm×30 cm) without mirrors was recorded for 5 min [23]. The time spent in the mirrored chamber is considered an index of anti-anxiety-like behavior in this task. General motor activity in this task was assessed by determining the number of entries mice made to the mirrored chamber.

2.3.6. Light–dark transition task—The time spent by mice in the light or the dark compartments of the testing chamber (24.5 cm×23.5 cm×35 cm) were recorded for 5 min [23]. The time spent in the light compartment is considered an index of anti-anxiety-like

behavior in this task. The number of entries made to the light side and the total number of entries made in this task were utilized as indices of general motor activity in this task.

2.3.7. Vogel conflict task—The number of licks made by water-deprived (24h) mice of an electrified water bottle that delivered a shock every 20 licks was recorded for 15 min [23]. The number of licks that are made in this task are utilized as an index of anti-conflict/anti-anxiety-like behavior in this task. The number of times that mice approached and began licking from the spout (“bouts”) was utilized as an index of general motor activity.

2.3.8. Porsolt forced swim task—The duration of immobility when mice were placed in a glass cylinder (20.5 cm diameter, 21.5 cm depth), which contained 18 cm of 25 °C water was recorded for 5 min [25]. The time spent immobile is considered an index of anti-depressive-like behavior of mice in this task. Other measures that were assessed in this task was the amount of time spent swimming (paddling in the chamber) and struggling (making movements in which paws break the surface of the water, generally on the sides of the chamber, as an attempt to climb out of the chamber).

2.4. Data analyses

One-way analyses of variance (ANOVAs), with Fisher’s *post hoc* tests, were used to evaluate effects of hormone condition (E₂ or vehicle) on behavioral indices. The α level for statistical significance was $p < 0.05$ and a statistical tendency was considered when $p < 0.10$.

3. Results

3.1. Activity monitor

There was no significant difference between vehicle and E₂ to mice for general motor activity in the Activity monitor. Mice administered E₂ or vehicle had a similar number of beam breaks in this task (Table 1).

3.2. Open field

There was a statistically significant main effect of condition for the number of central squares entered by mice $F(1, 16) = 65.35$, $p < 0.01$. Mice administered E₂ entered more central squares than did mice administered vehicle (Fig. 1). However, the total number of square entries was not significantly different for E₂- or vehicle-administered mice (Table 1).

3.3. Rotarod

There was no significant difference between conditions for fall latencies in the rotarod. Mice administered E₂ or vehicle had similar short fall latencies in this task (Table 1).

3.4. Elevated plus maze

Although the data were not significant, the time spent on the open arms of mice tended to be greater for E₂ (170±24s) than for vehicle (118±16s) administered mice $F(1, 16) = 3.22$, $p < 0.09$. There were no differences between mice administered vehicle or E₂ for the open arm entries made or the total arm entries made in this task (Table 1).

3.5. Mirror chamber

There was a significant main effect of condition for the time spent in the mirrored chamber $F(1, 16) = 9.97$, $p < 0.01$. Mice administered E₂ spent significantly more time in the mirrored chamber than did vehicle controls (Fig. 2).

3.6. Light–dark transition

There was a significant main effect of condition for the time spent in the light chamber in the light–dark transition task $F(1, 16)=33.62, p<0.01$. E_2 -administered mice spent significantly more time in the light compartment than did vehicle mice (Fig. 3). There were no differences in the total entries made in the task to both chambers, or to the light chamber (Table 1).

3.7. Vogel conflict task

Although the main effect was not statistically significant, the number of punished licks tended to be greater for E_2 - (222±15) than for vehicle- (158±32) administered mice $F(1, 16)=3.31, p<0.08$. There were no differences between these groups for the number of drinking bouts in this task (Table 1).

3.8. Forced swim task

E_2 -administered mice had significantly $F(1,16)=12.3; p < 0.01$ shorter duration of immobility than did vehicle-administered mice (Fig. 4). There were no differences between groups in the time spent swimming in this task (Table 1). There was no statistically significant difference for time spent struggling, but mice administered E_2 tended to spend more time struggling than mice administered vehicle $F(1, 16)=4.03, p<0.06$ (Table 1).

4. Discussion

Our hypothesis that E_2 administration to aged female mice would decrease anxiety and depressive behaviors was generally supported. Administration of E_2 , in an acute regimen that produces physiological concentrations of E_2 (10 μg SC, 1h prior to testing), significantly decreased anxiety and depressive behavior in the open field, mirror chamber, light–dark transition and forced swim test, compared to that produced by vehicle. A similar pattern of effects was observed in the elevated plus maze and Vogel conflict task, but differences between conditions in these tasks did not reach statistical significance. Interestingly, these effects of E_2 on anxiety and depressive behavior occurred independent of changes in motor activity and/or coordination. With the exception of a tendency for differences in the duration spent struggling by mice in the forced swim test, there were no differences in the number of beam breaks in an activity monitor, latencies to fall from the rotarod, or any differences in the motor indices in the anxiety tasks or forced swim test. Thus, E_2 administration to aged female mice produces specific effects to reduce anxiety and/or depression behavior.

The present results confirm that E_2 administration to young, OVX mice can produce anti-depressant effects in the forced swim task and extend them to senescent intact mice. Young adult, OVX, Swiss-albino mice spent approximately 113s immobile in the forced swim task when administered vehicle compared to 50s when administered 100 $\mu\text{g}/\text{kg}$ E_2 [14]. These results are similar to that observed for young OVX, CD1 and 129S6 mice that spent ~100 and 75s immobile, respectively, when administered vehicle and 75 and 40s immobile when administered 100 $\mu\text{g}/\text{kg}$ E_2 [16]. Given that there are well known differences between mouse strains in response to steroid administration and for their behavioral effects [26], the effects of E_2 on depressive behavior seem very consistent and robust across studies. Our aged intact C57BL/6 congenic mice were immobile for ~70 and ~35s when administered vehicle and E_2 (10 μg , SC) 1h before testing, respectively. Although the pattern and magnitude of E_2 's effects are clearly consistent across these studies, whether the overall lower levels of immobility observed among our mice is attributable to E_2 dosing, aging and/or strain differences is unclear. Indeed, we have recently reported that young OVX mice on a C57BL/6 background have about a 33% reduction in immobility when administered 10 μg

E₂ 44–48h before forced swim testing [18]; whereas the older mice in the present study, and mice of a different strain in the aforementioned studies, had a greater (~100% reduction) response. Current studies are investigating the extent to which aging, strain differences, and/or exposure to other stressors are contributing factors to responsiveness of mice to physiological E₂ dosing.

Another factor to consider is the extent to which the duration of E₂ deprivation may have influenced the results. Indeed, given the results of recent basic research and clinical trials which suggest that beneficial effects of E₂ may be limited after prolonged periods of E₂ deprivation and/or neuronal compromise [3,27–29]. It is important to note that we do not know precisely for how long mice in the present study were steroid-deprived. It has been reported that about 80% of 17 month old C57BL/6 mice have irregular or absent cycles, whereas 100% of their 25 month old counterparts are acyclic [30]. These findings suggest that our mice may have experienced E₂ (progesterin and androgen) deprivation for up to 25% of their lifespan before administration of E₂ or vehicle and behavioral testing. As such, the favorable response to E₂ observed may suggest that anxiety and/or depressive behavior can be improved following E₂ deprivation among some aged individuals. This is in direct contrast to the effects that we observed on motor measures in these tasks. Although these effects support the notion that E₂'s functional effects were specific to the anxiety and depression-like behaviors, and not secondary to activity, among aged mice, E₂ has a well-known clear role in behavioral arousal and motor activity among young mice [31]. In support, over the estrous cycle, and with OVX and E₂ replacement, greater motor activity and exploration by mice of their homecage or novel environment is noted [32–36], and may be related to reproduction [37]. In the present study, aged mice did not have an increase in motor activity in any task following administration of E₂. Mice of this age were likely reproductively-senescent so these data lend some support to the notion that E₂'s enhancement of motor activity may subserve reproduction-related function.

This study confirms results of prior investigations which demonstrate that E₂ has anti-anxiety effects in young rodents and extends them to show effects in aged mice across multiple behavioral assays, but not all tasks utilized. Effects of E₂ on anxiety behavior of young mice have been investigated primarily in the plus maze. Mice in behavioral estrous, which have elevated levels of E₂ and progestins, spend typically twice as long on the open arms of the elevated plus maze as do diestrous mice, which have low levels of E₂ and progestins [12]. However, when young adult, cycling mice were subsequently OVX and administered E₂, only modest effects of E₂ on plus maze behavior were observed [12] as was observed in the present study in aged mice. This was not a test decay effect, as controls were not different across experiments. Similar to the present study, other studies have demonstrated a robust effect of endogenous and exogenous E₂ in young to reduce anxiety-like responses in additional tasks (open field, elevated zero maze, light–dark transition, mirror maze, social interaction) [13,17,38]. Furthermore, the role of progesterone (P) should be considered in this context. P administration to young or aged wild type or progesterin receptor knockout mice decreases anxiety behaviors in all tasks, except the plus maze, in part through increasing formation of the P metabolite 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP) and its subsequent enhancement of GABA_A receptor function [23]. Consistent with this, P administration did not alter open arm time in the plus maze when administered to 5 α -reductase or wild type mice although effects on other anxiety measures were observed [25]. Thus, these data suggest that there may be differential sensitivity of mice to hormonal modulation of anxiety-like behavior across tasks.

An important question is whether some of the effects of E₂ for anxiety-related behavior may involve actions of 3 α ,5 α -THP. E₂ can increase activity of the 5 α -reductase enzymes [39] and enhance *de novo* formation of 3 α ,5 α -THP [40,41]. Indeed, we have found that E₂ to

OVX mice that do not express a molecular target of E₂, the estrogen receptor β (ER β), do not have increased 3 α ,5 α -THP in the hippocampus and enhanced cognitive performance as do their wild type counterparts [42]. In addition to other stress systems (e.g. cholinergic; [43]), the hippocampus is a likely central target for E₂'s anti-anxiety and anti-depressive effects. In support, administration of this same E₂ regimen to aged, intact, female C57BL/6 mice produces physiological concentrations of E₂ in the hippocampus compared to levels observed in aged intact female mice administered vehicle, which were at nadir [22]. Administration of E₂, compared to vehicle, subcutaneously or to the hippocampus of OVX rats similarly increases anti-anxiety and anti-depressive behavior [2,44]. As well, whether actions of E₂ at the β isoform of the E₂ receptor (ER β) in the hippocampus may underlie the anti-anxiety and anti-depressant effects observed herein was not established. Among young OVX rats and young, intact of OVX and E₂- or ER β -SERM-replaced mice, actions at ER β are sufficient to produce anti-anxiety and anti-depressant effects; but this effect is abrogated in ER β knockout mice [2,11,13,15–18,38,45]. We plan to address this critical question further by examining effects of E₂ administration to aged ER β knockout mice and their wild type counterparts. Thus, further investigation of how some of E₂'s functional effects may involve interactions between ER β and its metabolism to 3 α ,5 α -THP in the hippocampus in young and aged individuals is of interest.

In summary, our results demonstrate that aged female mice respond favorably to E₂ administration and exhibit greater anti-anxiety and anti-depressant behavior than is observed following vehicle administration. These effects on affective measures can be parsed out from general motor activity. Given the increases in anxiety and depression among post-menopausal women, these findings have clinical relevance. Further studies are needed to elucidate the mechanisms by which E₂ influences anxiety and depressive behavior in aged individuals.

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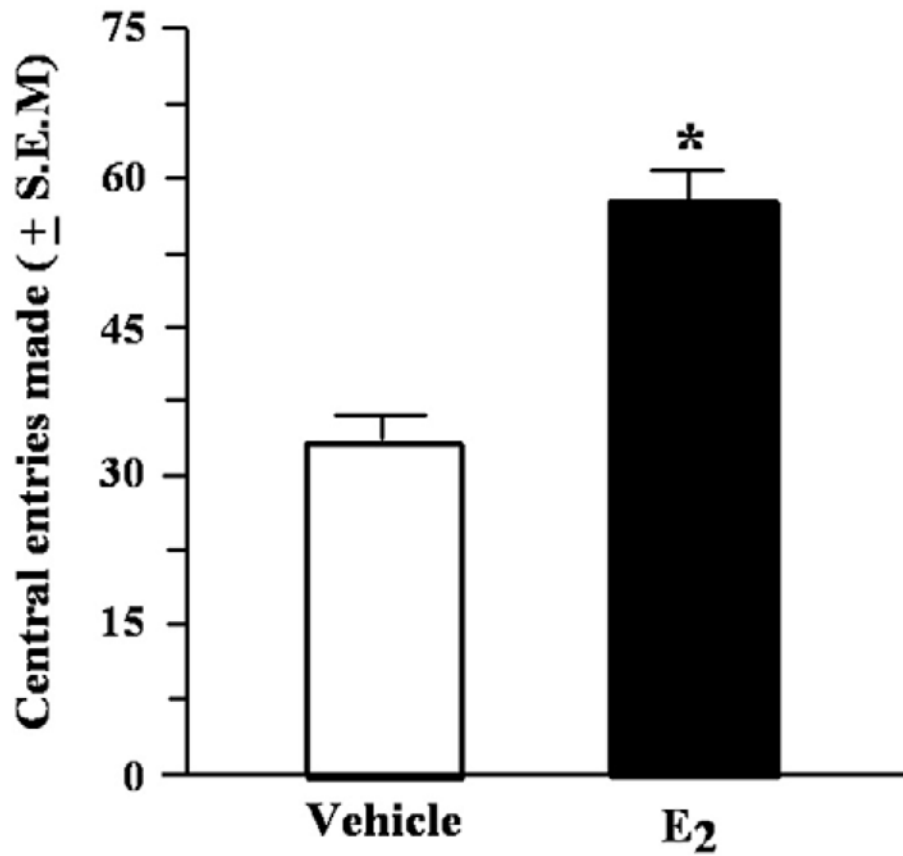


Fig. 1. Central entries (mean±S.E.M.) made in the open field of aged mice administered vehicle or estradiol (E₂) 1h before testing. *Indicates a significant difference from vehicle ($p < 0.05$).

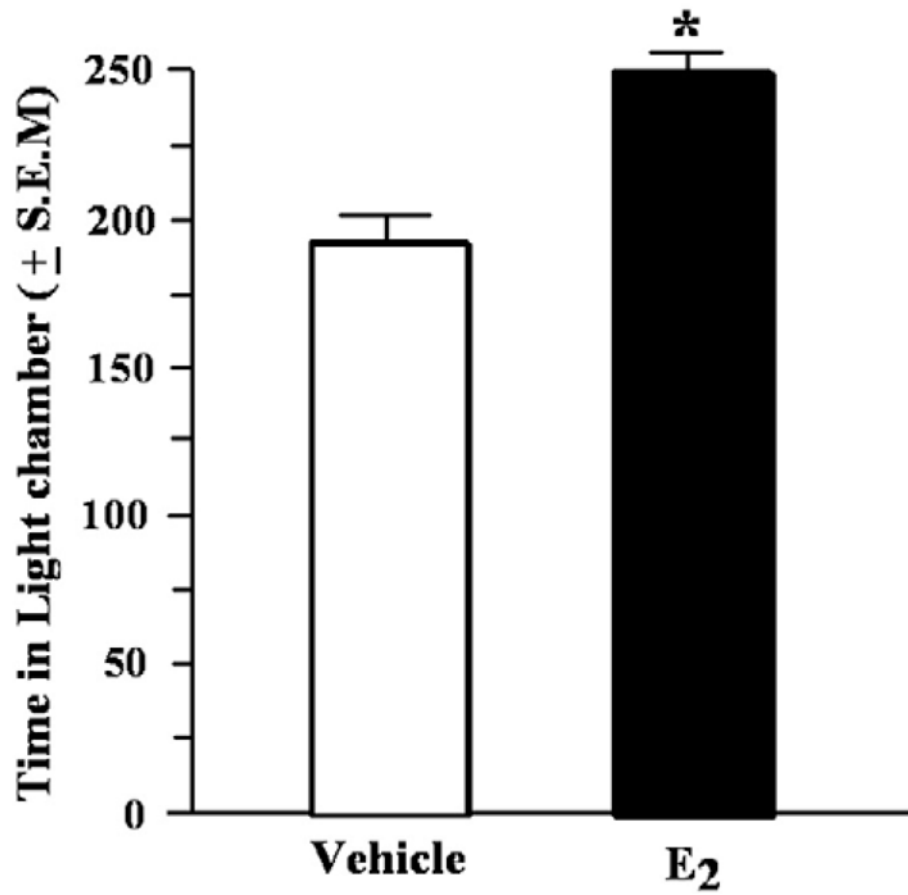


Fig. 2. Time spent in the mirrored chamber (mean in $s \pm$ S.E.M.) of aged mice administered vehicle or estradiol (E₂) 1h before testing. *Indicates a significant difference from vehicle ($p < 0.05$).

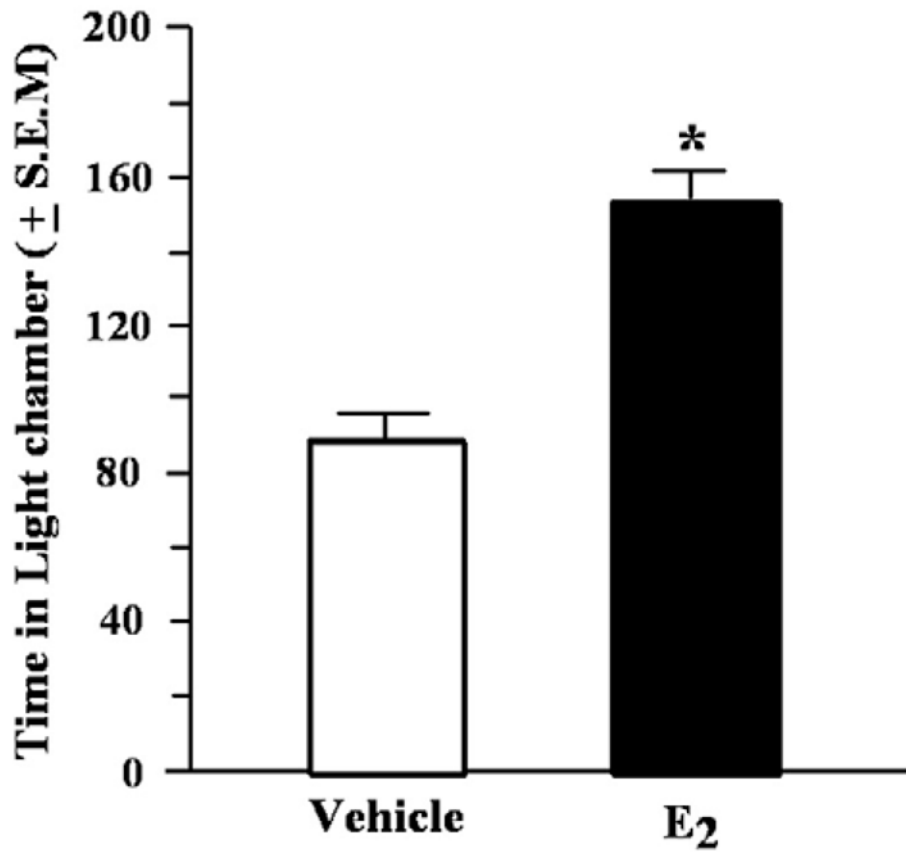


Fig. 3. Time spent in the light side of the light-dark transition task (mean in s ±S.E.M.) of aged mice administered vehicle or estradiol (E₂) 1h before testing. *Indicates a significant difference from vehicle ($p < 0.05$).

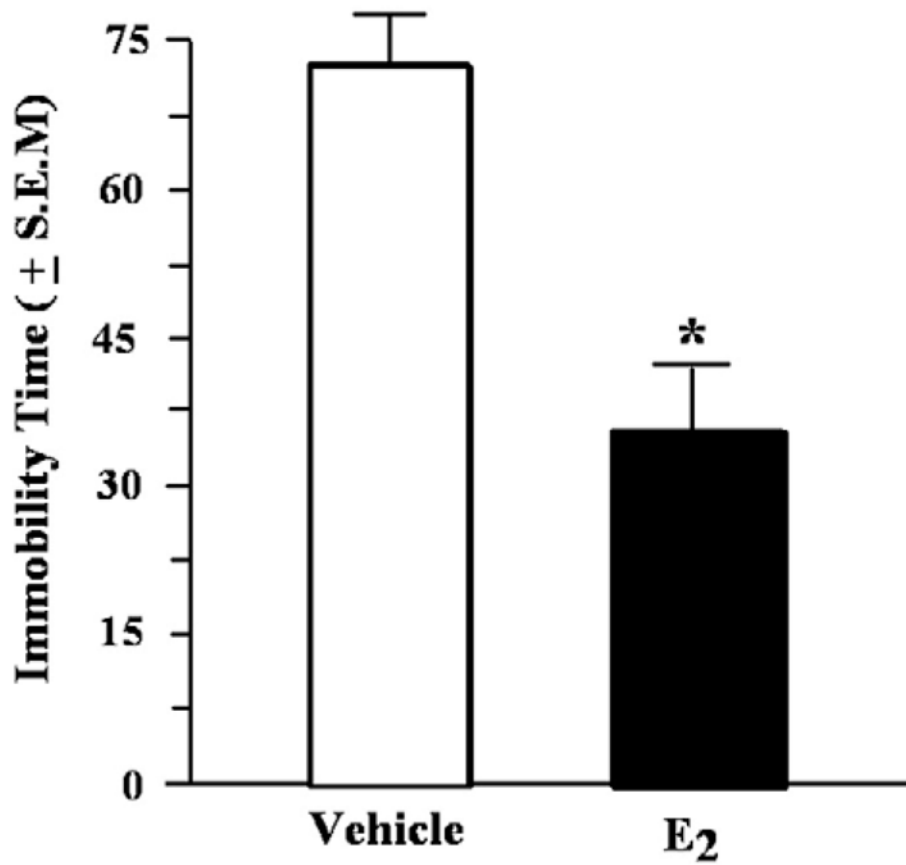


Fig. 4. Time spent immobile in the forced swim test (mean in $s \pm S.E.M.$) of aged mice administered vehicle or estradiol (E_2) 1h before testing. *Indicates a significant difference from vehicle ($p < 0.05$).

Table 1

Indices (mean±S.E.M.) of general motor activity (Activity monitor), coordination (Rotarod), and motor behavior in the tasks utilized (open field, elevated plus maze, mirror maze, light–dark transition, Vogel conflict task, forced swim test) of aged mice administered vehicle or estradiol (E₂) 1h before testing.

Measures	Condition	
	Vehicle	E ₂
Activity monitor — # of beam breaks	1276±128	1156±110
Rotarod — fall latency (s)	9±5	8±2
Elevated plus maze — open arm entries	6.3±0.5	6.9±0.6
Elevated plus maze — total arm entries	13.1±1.1	13.9±1.3
Mirror maze-total entries to mirrored chamber	7.0±0.9	7.1±0.8
Light–dark transition — light side entries	5.6±0.4	6.0±0.4
Light–dark transition — total entries	21.7±4.0	25.7±5.0
Vogel conflict task — total drinking bouts	6.2±1.0	6.6±0.4
Forced swim test — time spent swimming (s)	93.3±10.7	112.4±3.5
Forced swim test — time spent struggling (s)	18.0±4.8	31.9±4.9 ^a

^aIndicates a tendency for a difference from vehicle ($p=0.06$).