The Different Sensitivity of Dendritic Degeneration in Hippocampal and Cortical Neurons to Estrogen Decline in Female Aging Mice

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Abstract

Aging is the most important risk factor for Alzheimer's disease (AD). Epidemiological studies reported that women have higher incidence of AD than men, which is associated with estrogen deficiency post menopause. The integrity of dendrites ensures normal neuronal function, and dendrite degeneration is one of the hallmarks in AD. However, the contribution of estrogen deficiency in dendritic remodeling during female aging is still unclaried. In the present study, female 18- and 22-month-old mice showed an age-dependent cognitive decline. Interestingly, female 18- and 22-month-old mice induced dendritic degeneration in both hippocampus and cortex, wherein the dendritic degeneration of hippocampal CA1 pyramidal neurons and cortical nonpyramidal neurons were more obvious in 18-month-old mice and that would not deteriorate in 22-month-old mice. The female mice after ovariectomy (OVX) 5 months induced similar changes in female 22-month-old mice indicating by cognitive decline and dendritic degeneration, however, dendritic degeneration of the hippocampal DG granular cells was less bad than that in female 22-month-old mice. Besides, estradiol (E2) level, and estrogen receptor α and β (ERα and ERβ) in hippocampus and cortex decreased in aged female and OVX mice. Importantly, E2 application rescued these changes induced by OVX. In conclusion, the female aging-induced dendritic remodeling existed regional differences, wherein hippocampal CA1 pyramidal neurons and cortical nonpyramidal neurons were the more vulnerable to onset of estrogen deficiency, while DG granular cells were more sensitive to age.

Introduction

It is well known that aging is the largest risk factor for neurodegenerative diseases including Alzheimer's disease (AD) and vascular dementia. Epidemiological data revealed that the incidence of AD in women over 65 years old is much higher than that of men at the same age [1]. Estrogen deficiency in women after menopause is closely related to this phenomenon [2]. However, the association between hormone replacement therapy and risk of AD is controversial [3, 4]. Estradiol (E2) is not only synthesized in ovaries but also in nonreproductive tissues, including liver, heart, muscle, bone, and brain that is consistent with a diversity of estrogen actions [5]. It has been reported that the frontal cortical E2 level reduced in female AD patients [2], and the decline of hippocampal E2 is associated with the cognitive deficits in female aging rats [6]. As per these studies, E2 deficiency might facilitate female dementia during aging. However, how E2 affects brain function in the process of female aging is still unclear.

Synaptic plasticity is considered as the basic neural mechanism of learning and memory and the reduction of that was found in aging [7]. It has been reported that E2 is involved in the learning and memory process by regulating presynaptic neurotransmitter release, postsynaptic receptors expression and distribution, and spine outgrowth [8–10]. Whereas, whether E2 participates in dendritic plasticity remodeling is not fully understood. The structural diversity of dendrites ensures neurons to perform the circuitry function [11]. It is well known that hippocampal excitatory pyramidal neurons encode spatial navigation information and episodic memory [12], while hippocampal DG granular cells are critical to spatial memory [13]. In the cortex, both the pyramidal and nonpyramidal neurons participate in
information processing and memory formation [14, 15]. Importantly, axonal and dendritic degeneration was commonly found before neuronal loss in various neurodegenerative diseases [16]. For example, the reduction of dendritic complexity of hippocampal and cortical neurons was found in APP/PS1 transgenic AD mice [17]. It needs to note that degeneration of neuron dendrites occurs in the aging state. For instance, it has been reported that the length of total and basal dendrites and the number of the apical dendrites of the pyramidal neurons were significantly reduced in the prefrontal cortex of the elderly rhesus monkey [18]. Moreover, male aged rats decreased the spine density and dendritic complexity of hippocampal CA1 apical dendrites [19, 20], as well as the length and intersection number of dendrites in the medial prefrontal cortex [21]. The abovementioned research demonstrated obvious dendritic degeneration in hippocampus and cortex in aged male animals. At present, the reports about the dendritic remodeling in female animals are limited. Our previous work indicated that female 18-month-old mice induced mitochondrial damage, lipofuscin deposition, and microtubule degradation [22]. However, the dendritic degeneration following female aging and the contribution of E2 deficiency in this process is unclear.

The purpose of this study is to clarify the changes of neuronal dendrites and cognitive ability during female aging and how much E2 deficiency contributes to that. In our study, we found that female mice decreased the cognitive function with age and degenerated the hippocampal and cortical dendrites, wherein the hippocampal CA1 pyramidal neurons and cortical nonpyramidal neurons were more vulnerable to the onset of menopause but not deteriorate with the time of E2 deficiency. For the hippocampal DG granular cells, the dendrites age-dependently retrograded in female aging mice, while dendritic degeneration of cortical pyramidal neurons did not deteriorate with age. Long-term E2 deficiency induced cognitive decline and neuronal dendrite remodeling in hippocampus and cortex, however its effect on hippocampal DG area was weaker than that of natural aged mice. Hippocampal and cortical E2, and ERα and ERβ might involve in the process. Our findings provided a dendritic degeneration pattern in the hippocampus and cortex of peri-menopausal and post-menopausal female mice and demonstrated the effect of E2 deficiency on neuronal dendritic degeneration is different in specific areas.

Materials And Method

Animals

Female C57BL/6 mice used in the experiments were separated into three groups: mice at the age of 6-month-old were used as young group, mice at the age of 18-and 22-month-old were designed as middle-aged group and aged group, respectively. The animals were raised at 23 ± 1°C and maintained on 12 h dark light artificial cycle (lights on at 07:00 A.M.) with food and water available ad libium. All animal procedures were approved by the ethic committees of Harbin Medical University and the Institute of Laboratory Animal Science of China (A5655-01). Moreover, the protocols complied with the guidance for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).
OVX Surgery and E2 Supplementation

The OVX surgery was prepared according to previous studies [23]. Female C57BL/6 mice at age of 3M were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). The adequacy of anesthesia was monitored considering the disappearance of the corneal reflex. After the ovaries were exposed, ovaries were permanently bilaterally ligated with sutures and removed. The sham operated group underwent anesthesia but ligated the adipose tissue only. All the female mice were raised for 5 months and randomly separated into 3 groups: sham operated (Sham), OVX, and OVX treated with 17β-E2 delivery (OVX+E2). The 17β-E2 (Sigma, USA) dissolved in peanut oil was subcutaneously injected into mice with a dosage of 3.5 μg/kg every three days for 5 months. The selection of the dosage of E2 was based on a previous study [22].

Passive Avoidance Tasks

The passive avoidance test was performed in the apparatus consisting of light and dark chambers connected via a sliding door. (PAT-8, China). In the acquisition trial, mice were individually placed into the light compartment and adapted for 30 s. The sliding door was opened, and the mice could enter into the dark compartment with a continuous electrical foot shock (2 V). Then the mice might return to the bright chamber immediately. The time taken to enter the darkroom (for a maximum of 120 s) was recorded, and mice that failed to enter the dark chamber within 90 s were excluded. After 24 h, during the retention trial, the electrical stimuli were removed from the dark chamber. Mice were placed into the light compartment, and the step-through latency was measured [24, 25].

Active Avoidance Tasks

The apparatus (STT-100, China) was divided into two identical shuttle-compartment of the same size (14.3 cm × 15.5 cm × 21 cm) connected by a gate (4 cm × 3.7 cm). A conditioned stimulus (CS, coincident presentation of a 3.6 W light and a 90 dB sound) was delivered 10 s before the unconditioned stimulus (US, a 2 V electrical foot shock) and overlapped it for 5 s. At the end of the stimulus presentation, both the CS and US were terminated, and the cycle began in the other compartment. Mice were subjected to five daily 30-cycle sessions with a 20 s cycle interval. On the sixth day, the test was performed without electric foot shock. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 10 s after the onset of the CS [24].

ELISA

Mice were intraperitoneally anesthetized (sodium pentobarbital, 60 mg/kg) before blood extraction from the left ventricle and standing at room temperature for 3 h, then the serum was collected after centrifugation (3000rpm, 5 min), and stored at -80 °C. The hippocampi and cortices were removed quickly and stored at -80 °C. The estradiol concentration was detected using the mouse estradiol ELISA kit (KGE014, R&D system, USA), following the manufacturer's instructions.

Immunofluorescence Staining
PFA-fixed brain was dehydrated and cryoprotected in 30% sucrose and then sectioned into slices. Brain slices at a thickness of 30 μm were permeabilized and blocked with 1% Triton X-100 and 10% goat serum, and then incubated with the primary antibodies (ERα, Santa Cruz, USA; ERβ, Abcam, USA) overnight at 4 °C, followed by secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 594 (Molecular Probes, USA) as well as DAPI in the next day. Finally, the fluorescence signals were visualized using a microscope (Zeiss) and recorded the positive signals cell number.

**Golgi Staining and Sholl Analysis**

The fresh brains were removed and immediately processed using the FD Rapid Golgi Stain Kit (FD Neurotechnologies, Columbia, SC, USA) according to the manufacturer’s protocol. For image collection, bright-field microscopy was used with an ×20 objectives using a Zeiss Axio Scope A1 microscope. Neurons in the CA1 and DG regions of the hippocampi and cortices in each group were randomly selected from 4 brains, and 3 slices/brain were quantified. The selected neurons were traced and reconstructed after switching to 8-bit using Image-Pro Plus software. The dendritic complexity was determined by Sholl analysis, wherein, with the neuronal body as the origin, concentric circles 10 μm apart were drawn until reaching the end of the farthest dendrite, and the dendrite crossings on every circle were then recorded.

**Statistical Analysis**

Data are described as the mean ± standard error of the mean (SEM). Each data set was analyzed for the equality of the variance. The independent sample test was calculated using the Levene variance equality test. If $P > 0.05$, an independent student's $t$-test was used for the comparison between two groups; if $P < 0.05$, the Kruskal-Wallis rank sum test was performed. Statistical analysis was performed using one-way ANOVA with post hoc tests by using Fisher’s test of least significant difference (LSD-t) for comparisons of more than two groups. For Sholl analysis, the effects of group and distance from the soma on dendritic complexity were analyzed by using the general linear model (GLM), with repeated measures for distance from the soma, followed by LSD-t. $P < 0.05$ was considered statistically significant. SPSS22.0 was used for all statistical analyses, and graphs were generated using GraphPad Prism 8.0 software (La Jolla, CA, USA).

**Results**

**E2 Deficiency Involves in Age-dependent Cognitive Decline in Female Aging Mice**

Female C57BL/6 mice at age of 18-month-old are close to the average age of menopause in women, 22-month-old approximately correspond to the human at age of 70 years old [26]. Therefore, female 18- and 22-month-old mice were selected to investigate the effects of short-term and long-term E2 deficiency on cognitive function and neuronal dendrite plasticity. It has been reported that the reduction of serum E2 involved in the process of cognitive decline [27]. However, the relation between serum E2 level and risk of
AD in women is still controversial in clinic [2, 28]. To ascertain the association between E2 and cognitive function of female aging mice, we detected the E2 level in serum and brain by ELISA and found that the serum E2 level was age-dependently decreased in female aging mice (Fig. 1a). Compared to the serum E2 level, the content of E2 in brain is much higher that is consistent with the clinical findings [2]. Interestingly, although the hippocampal E2 level was also age-dependent reduced but this phenomenon is not happened in the cortex (Fig. 1b). Then, we investigated the short-term memory and context-dependent memory by passive avoidance task and active avoidance task, separately. Compared to the young mice, the latency of female 18- and 22-month-old mice was shorter with age in the passive avoidance task (Fig. 1c). In the active avoidance task, female 18- and 22-month-old mice decreased the percentage of active avoidance task response (Fig. 1d) and prolonged the latency (Fig. 1e) during the training period. In the test phase, the latency of active avoidance was age-dependently increased (Fig. 1g). However, although female 22-month-old mice reduced the successful active avoidance response, female 18-month-old mice did not change that compared with 6-month-old female mice (Fig. 1f), suggesting the age-dependent decline of the context-dependent learning and memory.

Considering sex difference, male mice are not the good choice to clarify the contribution of E2 in cognition during female aging, so we chose female mice at the age of 3-month-old underwent OVX surgery and raised for 5 months to mimic the time of E2 deficiency of female 22-month-old mice. Surprisingly, OVX successfully induced the decline of E2 level in serum, hippocampus and cortex, and E2 treatment could powerfully reverse that (Fig. 1h and i). Accordingly, the latency of OVX mice significantly reduced in the passive avoidance task (Fig. 1j). Similarly, in the active avoidance test, OVX decreased the percentage of successful avoidance (Fig. 1k, m) and prolonged the latency (Fig. 1l and n). Importantly, the administration of E2 reversed the decline of cognitive function in OVX mice (Fig. 1k-n).

E2 Deficiency Contributes to Female Aging-induced Dendritic Degeneration of Hippocampal CA1 Pyramidal Neurons and DG Granular Cells

To investigate how E2 affects hippocampal dendritic remodeling during female aging, we quantitatively compared dendritic number and length of hippocampal CA1 pyramidal and DG granular neurons by Golgi staining. We found that, compared with female 6-month-old mice, 18- and 22-month-old mice dramatically reduced the total length and number of dendrites of hippocampal CA1 pyramidal neurons and that did not show age-dependent reduction (Fig. 2a-c). The same phenomenon was displayed in the primary, secondary and tertiary dendrites (Fig. 2a-c). Neuronal dendritic complexity reflects the function of the neural network. Next, we analyzed the dendritic complexity by Sholl analysis and found that the intersection number of dendrites in hippocampal CA1 pyramidal neurons was dramatically reduced in 18- and 22-month-old mice and that did not exacerbate in 22-month-old mice (Fig. 2d).

Apical and basal dendrites raising from different sides of the cone-shaped soma of pyramidal neurons ensure that pyramidal neurons could receive synaptic inputs from different afferent sources [29]. Therefore, we evaluated apical and basal dendrites separately. Compared with the young group, female 18- and 22-month-old mice decreased the number and length of all basal dendrites and reduced the
dendritic intersection number (Fig. 2e-g). For the hippocampal CA1 apical dendrites, the number and length of the total, secondary and tertiary dendrites and the dendritic complexity were significantly decreased, however, the primary apical dendrites did not changed in female 18- and 22-month-old mice (Fig. 2h-j). Like the total dendrites, the degeneration of apical and basal dendrites did not show age-dependent decline in female aging mice (Fig. 2e-i). These results suggested that dendrites of hippocampal CA1 pyramidal neurons degenerated at onset of menopause, and that would not further aggravate with age.

In the hippocampal DG region, axons of granular cells form mossy fibers play a critical role in spatial memory [13]. In the present study, the number and length accompanied by dendritic complexity of total dendrites of hippocampal DG granular cells were significantly reduced in both female 18- and 22-month-old mice (Fig. 2k-n). However, although the number and length of primary and secondary dendrites dramatically reduced in 22-month-old group that did not changed in 18-month-old mice (Fig. 2l, m). The intriguing results implied that age deteriorates the dendrites degeneration of hippocampal DG granular cells post menopause.

To ascertain how much effect of E2 deficiency contributes to the dendrite degeneration of hippocampal CA1 pyramidal neurons and DG granular cells, OVX mice were used to evaluate the same indexes in female aging groups. Surprisingly, similar to female 22-month-old mice, the number and length of all dendrites, as well as the dendrite intersection number of hippocampal CA1 pyramidal neurons were dramatically decreased in OVX mice (Fig. 3a-d). Apart from apical primary dendrites, OVX mice decreased all the number, length, and intersection number of apical and basal dendrites (Fig. 3e-j). The data indicated that E2 deficiency plays an important role in the dendritic remodeling of hippocampal CA1 pyramidal neurons in female mice during aging. Interestingly, in the hippocampal DG granular cells, E2 deprivation induced by OVX failed to mimic the degeneration of primary dendrite that observed in female 22-month-old mice (Fig. 3l-m). As predicted, the E2 application reversed all the above-mentioned changes (Fig. 3a-n). These results suggested that long-term E2 deficiency leads to severe degeneration in hippocampal CA1 pyramidal neurons relative to DG granular neurons.

**E2 Deficiency Involves in Dendrite Degeneration of Cortical Pyramidal and Nonpyramidal Neurons in Female Aging Mice**

The cortical pyramidal and nonpyramidal neurons play essential roles in emotion control and receiving, modulating sensory information to memory formation [14, 15]. Next, we quantified the dendritic number and length by tracing pyramidal and non-pyramidal neurons in cortex. Different from hippocampal CA1 pyramidal neurons, although female 18- and 22-month-old mice reduced the overall number and length of total, secondary and tertiary dendrites, as well as the dendritic intersection number of cortical pyramidal neurons, whereas the number and length of primary dendrites did not change (Fig. 4a-d). Similar results were also observed in apical dendrites (Fig. 4e-g). For the basal dendrites, we found that female 18-month-old mice decreased the number and length of total and tertiary dendrites excepting primary and secondary dendrites, while, female 22-month-old mice reduced that of total, secondary and tertiary
dendrites but not primary dendrites (Fig. 4h-j). Intriguingly, the length and number of nonpyramidal neuronal dendrites and the dendrite intersection number were significantly decreased in female 18- and 22-month-old mice (Fig. 4k-n). These results demonstrated that female aging from menopause induced dendritic degeneration of cortical pyramidal and nonpyramidal neurons in an age-independent manner.

Accordingly, we quantified dendritic number and length in the cortex of OVX mice. Surprisingly, OVX perfectly reproduced the result of age female mice at the age of 22-month-old (Fig. 5a-n). As shown in Fig. 5a-d, the number and length of the total, secondary and tertiary dendrites, as well as the dendrite intersection number of cortical pyramidal neurons in OVX group were significantly decreased without changing that of primary dendrites. Similar results were observed in apical dendrites (Fig. 5e-g) and basal dendrites (Fig. 5h-j). As expected, E2 treatment could powerfully reverse these changes (Fig. 5a-j). Same to the female 22-month-old mice, OVX group reduced the number and length of all dendrite, as well as the dendrite intersection number of cortical nonpyramidal neurons, and application of E2 could rescue that (Fig. 5k-n). These results demonstrated that long-term E2 deficiency could induce dendritic degeneration of cortical pyramidal and nonpyramidal neurons.

E2 Deficiency-induced Similar Decrease of ERα and ERβ in Female Aging Mice.

Most function mediating by estrogen works by ERα and ERβ [30]. It has been reported that the expression of both ERα and ERβ significantly decreased in the hippocampal CA1 region of female aging rats [31, 32]. However, whether long-term E2 deficiency alone could mimic the changes of ERα and ERβ in hippocampus and cortex of female aging mice is obscure. Using the immunofluorescence staining, we found that the number of ERα and ERβ positive cells was reduced in the hippocampal CA1 (Fig. 6a-c), CA3 (Fig. 6d-f) and DG (Fig. 6g-i) as well as in the cortex (Fig. 6j-l) of female 18- and 22-month-old mice. These results suggested that the level of ERα and ERβ in the hippocampus and cortex decreased from the early stage of menopause. Similar to the female 22-month-old mice, OVX mice decreased the number of ERα and ERβ positive cells in hippocampal subfields and cortex and that was effectively prevented by E2 treatment (Fig. 7). These results suggested that E2 deficiency-induced decreased expression of ERα and ERβ in the hippocampus and cortex may be involved in the cognitive deficits in female aging mice.

Discussion

It is well known that aging is the most risk factor for AD. Literatures reported that decline of estrogen levels after menopause may contribute to higher AD incidence in female [1, 2]. However, the role and molecular mechanism of estrogen in plasticity of dendritic morphology during female aging are largely unknown. In the present study, we found that the female aging-induced hippocampal and cortical neuronal dendrite remodeling existed regional difference, and CA1 pyramidal neurons and cortical nonpyramidal neurons were more sensitive to onset of menopause which would not aggravate with age. The dendrites of hippocampal DG granular cells degenerated after menopause in an age-dependent manner. The hippocampal and cortical E2 and ERα and ERβ might be involved in this process.
The decline of short-term memory and context-dependent memory are the main early clinical symptoms of AD [33]. It has been reported that the latency of passive avoidance task reflects the short memory [34]. Active avoidance task is used to assess the pavlovian conditional active avoidance behavior that depends on hippocampal functional integrity of context memory [35]. Female C57BL/6 mice at age of 18-month-old are used to mimic the human average age of menopause, 22-month-old approximately correspond to the human at age of 70 years old [26]. So, we selected female 18- and 22-month-old mice to investigate the effects of short-term and long-term estrogen deficiency on cognitive function and neuronal dendrite plasticity. It has been reported that male 14-month-old Balb/C mice, 16-month-old Swiss mice in both sexes, and female 18-month-old SD rats showed memory decline assessed by passive avoidance test [36–38]. Moreover, female 18-month-old SD rats also impaired performance in active avoidance tasks [38]. In our present study, we also provided evidence that female 18- and 22-month-old mice showed age-dependent cognitive impairment in passive and active avoidance tasks.

Neuron death and dendrite degeneration is considered as the hallmarks of AD [16]. There are several studies described dendritic degeneration in aging process. Folarin reported that male 18-month-old BALB/c mice decreased the dendrite branches of hippocampal CA1 pyramidal neurons [39]. In rats, male 18-month-old rats reduced the dendrite length in the prefrontal cortex and hippocampal CA1, CA3, and DG subfields [40]. Similarly, male aged rats at age of 20-24-month-old or 18-20-month-old decreased dendritic complexity in apical dendrites of hippocampal CA1 pyramidal neurons, or length and intersection number of dendrites in the medial prefrontal cortex, separately [20, 21]. The abovementioned research demonstrated obvious dendrite degeneration of hippocampus and cortex in aged male rodents. There are limited reports describing the dendritic remodeling in female animals. For example, both male and female rats at age of 20-24-month-old or 19-month-old decreased the spine density of apical and basilar dendritic branches in the anterior cingulate cortex, or reduced the basilar dendritic length of pyramidal neurons in medial prefrontal cortex [21, 41]. Interestingly, reduction dendritic complexity of apical dendrites in medial prefrontal cortex was present in male rats but not in female [21]. Moreover, the decrease of length in total dendrite and basal dendrite, and the number of the apical dendrite of the pyramidal neurons have been found in the prefrontal cortex of the elderly rhesus monkey [18]. However, the sex of these rhesus monkey did not describe in the text. Systemic deciphering the cognitive decline and dendrite remodeling in the brain during female aging may provide more clues to clarify why women have higher AD risk.

The slow persistent changes in spike rate in neurons in the frontal cortex and related brain regions are associated with short-term memory [42], while the hippocampus contributes to context learning and memory [43]. Thus, we next elucidated the changes of neurons dendrite in these areas. In our study, significant degeneration of hippocampal CA1 pyramidal dendrites has been found in female 18-month-old mice indicating by a decrease of the dendrite number and length, as well as dendritic complexity in all dendrites, which did not deteriorate in 22-month-old female mice. However, for the hippocampal DG granular cells, 22-month-old female mice decreased all the number and length, while 18-month-old mice only reduced that of total and tertiary dendrites. Previous MRI studies showed that hippocampal CA1 atrophy is obvious at age of 50 years old and DG atrophy can be observed in older age [44]. Moreover,
hippocampal CA1 pyramidal neuron is the most sensitive to ischemia and hypoxia[45]. Our findings are consistent with these results that dendritic remodeling of hippocampal CA1 pyramidal neuron was more vulnerable to the onset of menopause, whereas DG granular cells were predominantly affected by aging. It has been reported that the nonpyramidal interneurons exhibited more pathological beading in dendrites than pyramidal neurons in prefrontal cortex under oxygen glucose deprivation and reoxygenation, suggesting higher vulnerability of nonpyramidal interneurons [46]. In our study, we found that female 18- and 22-month-old mice showed a decrease of number and length in all dendrites excepting primary dendrites of cortical pyramidal neurons. Like the hippocampal CA1 pyramidal neurons, all the dendrites of nonpyramidal neurons in cortex degenerated in both female 18- and 22-month-old mice in an age-independent manner. Our findings provided evidence that the dendritic remodeling of cortical nonpyramidal neurons is worse than pyramidal neurons during female aging.

The basal dendrites of hippocampal CA1 pyramidal neurons receive projection from CA3 via scheffer-collateral pathway, while apical dendrite tuft receives projections from the entorhinal cortex and thalamic nucleus via perforant path [29]. Morphological remodeling of these two regions would lead to different pathophysiological changes. A previous study has shown that the dendritic complexity of basal dendrites of pyramidal neurons showed a more prominent interhemispheric effect of the lesion than the apical dendrites in layer V of the caudal part of the supplementary motor area after unilateral spinal cord injury [47]. Besides, the spine density reduced in the basal dendrites without changing that in apical dendrites in hippocampal CA1 regions of two kinds of transgenic AD mice (Tg2576 and APP/Lo mice) [48]. However, whether the remodeling asymmetry of neuronal dendrites exists under the progress of female aging remains poorly understood. In the present study, we found that the degeneration of apical dendrites is more obvious than basal dendrites of cortical pyramidal neurons, while the basal dendrites are more vulnerable of hippocampal CA1 pyramidal neurons following female aging. These findings demonstrated that the dendrite degeneration of pyramidal neurons in cortex and hippocampal CA1 is asymmetric, and this phenomenon in these two areas is different. However, the mechanism underlying the dendrite remodeling asymmetry in female aging needs to be explored further.

Interestingly, we found that the degeneration of apical dendrites of hippocampal CA1 pyramidal neurons and dendrites of cortical pyramidal neurons was mainly observed in secondary and tertiary dendrite but not in the primary dendrite of female 18- and 22-month-old mice. A previous study found that chronic brain hypoperfusion induced dendritic degeneration displayed higher order dendrite (tertiary and secondary dendrite) remodeling but not primary dendrite, which was similar to the dying back process of axon degeneration [49]. Our results suggested that during the aging process, neuronal dendrite degradation may also begin to degenerate in a "dying-back" manner. However, the specific molecular mechanism is not yet clear, and further exploration is needed in the future.

It has been reported that the decline of E2 is associated with cognitive deficits in female aging rats [6]. A previous study reported that female 18-20-month-old mice and female mice in same age with long-term E2 deprivation all decreased neuronal density in both the arcuate nucleus and preoptic area [50]. However, the estrogen level of female mice after menopause decreases, so it is hard to judge how much effects of
Estrogen deficiency after menopause contributes to cognitive decline and other pathophysiological changes. To investigate the effects of the estrogen deficiency on cognitive function and dendrite remodeling, we constructed the OVX mice when mice is at the age of 3 months. Our study first reported that OVX induced a similar cognitive decline and the neuronal dendrite remodeling in hippocampi and cortices as female 22-month-old aging mice. However, the dendritic degradation of hippocampal DG granule cells in OVX mice is between female 18- and 22-month-old mice. In addition, supplementing E2 rescued the dendrite remodeling of OVX mice in hippocampal DG granule cells. These findings indicated that age rather than estrogen deficiency plays a pivotal role in the degeneration of hippocampal DG granule cell dendrites following female aging.

Estrogen synthesized in the brain involves in age-related diseases [5]. In our present study, we found that the hippocampal and cortical as well as serum E2 level of female 18- and 22-month-old mice were reduced. It has been reported that the hippocampal ERα and ERβ assessing by western blotting of female 20-month-old mice were decreased [51]. We also confirmed that the hippocampal and cortical ERα and ERβ decreased in female 18- and 22-month-old mice. Interestingly, OVX mice decreased the E2 level in serum and brain, and E2 application could reverse that. These is consistent with Khaksari's findings that E2 could reverse the decrease of ERα and ERβ mRNA in traumatic brain injury female rats [52]. Our findings suggested that E2 and ERα and ERβ in hippocampus and cortex might involve in cognitive decline and neuronal dendritic degeneration during female aging.

In summary, we reported that the hippocampal and cortical neuronal dendritic remodeling of female aging mice existed regional difference, wherein hippocampal CA1 pyramidal neurons and cortical nonpyramidal neurons were the most vulnerable to the onset of menopause. For the dendritic degeneration of hippocampal DG granule cells, the age powerfully aggravates that in female aging mice after menopause. Our findings provide a new theoretical basis for the cognitive protection of supplementing E2 in postmenopausal women. However, whether supplementing E2 directly increases E2 content in brain or promotes E2 synthesis in brain tissue requires further investigation. Moreover, the molecular mechanism of supplementing E2 to up-regulate the expression of ERα and ERβ remains to be studied.

**Declarations**

The authors declare that there are no conflicts of interest.

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**Conflicts of Interest**
The authors declare that there are no conflicts of interest.

**Availability of Data and Materials**

The data used in this study are available from the corresponding author upon reasonable request.

**Authors’ Contributions**

L.Y, S.Z, W.Y.R, T.T.L, and S.Y.H performed research. L.Y and S.Z analyzed data. L.Y wrote the manuscript. Y.Q and X.B.A edited manuscript. J.A conceived the study, supervised the progress of all experiments, interpreted the results, and edited and finalized the manuscript. All authors read and approved the final manuscript.

**Ethical Approval**

All experimental procedures were approved by the Ethics Committee of Harbin Medical University and the Institute of Laboratory Animal Science of China (A5655-01) in full accordance with the ethical guidelines of the National Institutes of Health for the care and use of laboratory animals.

**Consent to Participate**

Not applicable.

**Consent for Publication**

Not applicable.

**Code Availability**

Not applicable.

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