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Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice

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Abstract

The present study tested whether environmental enrichment can reduce age-related spatial reference memory deficits and alter synaptic protein levels in aged female mice. Female C57BL/6 mice, (4 or 27–28 months), were tested in spatial and cued Morris water maze tasks. Prior to (14 days) and during testing, a subset of aged females was exposed to rodent toys and running wheels for 3 h per day. The remaining aged females were group housed but were not exposed to enriching objects. At the conclusion of testing, levels of the presynaptic protein synaptophysin were measured in hippocampus and frontoparietal cortex. Enrichment improved spatial memory acquisition; relative to young controls, aged enriched females performed similarly, whereas aged control females were impaired. Enrichment also accelerated the development of a spatial bias in spatial probe trials. In contrast, the cued task was not significantly affected by enrichment. Hippocampal and cortical synaptophysin levels were increased in aged enriched females relative to young and aged controls. These data suggest that environmental enrichment can be a potent cognitive enhancer for aged females and suggests a potential neurobiological mechanism of this effect.

Keywords: Environmental enrichment; Spatial reference memory; Synaptophysin; Hippocampus; Frontoparietal cortex; ELISA

1. Introduction

Memory decline is a common feature of aging in humans [19,30,67]. It is thought that this decline is the result of deterioration in brain regions critical for memory, such as the neocortex, hippocampus, and basal forebrain. The discovery that non-human animals, from rodents to primates, experience similar memory and neural dysfunction to that of humans has spurred decades of animal research designed to develop treatments to alleviate age-related memory loss (see [2,3] for reviews). Most often, these treatments involve administration of drugs that alter the function of various neurotransmitter or hormone systems. In contrast, few investigations have utilized behavioral manipulations to reduce or prevent age-related memory loss. Emerging evidence suggests that one such intervention, environmental enrichment, may be a particularly effective cognitive enhancer. For example, intellectual abilities in humans are maintained longer in people who continue to engage in intellectually stimulating activities throughout their lives [64,69]. Although animal models afford the ability to understand the neurobiological basis of this benefit, relatively few studies have examined the effects of this treatment in aging animals.

The impact of environmental stimulation on cognitive and neural development has been known for several decades. Seminal work in the 1960s and 1970s revealed that the cerebral cortices of rats raised in enriched environments (group housed, exposed to stimulus objects) were profoundly altered compared to the brains of rats raised in impoverished conditions (singly housed, no stimulation). Alterations in environmentally enriched rats included increased enzyme levels, cortical thickness, dendritic spines and branching, synaptic contacts and transmission, and neuron size (e.g. see [14,16,28,31,34–36,49,62] for review). Further, enriched rats exhibited enhanced learning and memory abilities relative to impoverished littermates [9,37,73,80].

Subsequent work indicates that this effect is not limited to early development. Environmental enrichment appears to benefit cortical plasticity and learning abilities at any point in the lifespan [32,62,71]. For example, enrichment can completely protect against learning and memory deficits in adult mice with a conditional knockout of the NMDAR1 receptor subunit in the hippocampal CA1 region [61]. Moreover, enrichment can enhance the proliferation of new neurons [47] and dendritic spines [61] in the hippocampus of adult mice. Particularly relevant to aging are studies

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examining enrichment-induced alterations in middle-aged and aged rodents. In rodents examined at middle-age, enrichment initiated during middle-age increases cortical dendritic branching [32], forebrain weight [13], neurotrophin levels [38,60], and hippocampal neurogenesis [48], as well as enhances learning of a Hebb-Williams maze [13] and the spatial reference memory version of the Morris water maze [23,48,60]. In rodents examined at old age, enrichment initiated either at weaning or during old age, results in a variety of alterations in the brain including increased Purkinje cell dendritic branching [33], increased cortical thickness, RNA content, and presynaptic vesicle number [15,57,76], reduced aging-induced hippocampal gliosis [71], and increased cortical and hippocampal levels of the presynaptic protein synaptophysin [63]. Furthermore, enrichment in aged male rodents improves several types of learning, reverses short-term memory deficits, increases spontaneous alternation, and increases food-seeking behaviors [71,75,76,79]. However, the benefit to behavior is not universal; enrichment in aged male rodents does not increase reactivity to spatial novelty or general motor activity, nor does it improve performance in the Lashley III maze or a brightness discrimination [75,76]. Nevertheless, the data in aging rodents suggest that environmental enrichment can profoundly alter neural structure and improve several types of behavior.

An interesting feature of the research to date examining enrichment in aging rodents is that the vast majority of these studies utilized males as their subjects. This discrepancy may be important, as numerous studies have reported sex differences in both the brain and behavior of young rodents in response to environmental enrichment. For example, sex differences in rats have been reported in cortical and hippocampal dendritic branching [42,43], myelinated axons in corpus callosum [45], and exploratory behavior [41], despite the fact that male and female rats interact with the enriched environment similarly [46]. Spatial memory, a type of memory particularly vulnerable to decline in aged humans (e.g. [19,64,67]), is enhanced by enrichment in both young males and females [44,66,73]. However, no study has thus far examined whether enrichment can improve spatial memory or alter synaptic plasticity in aged females. Spatial reference memory, as tested in the Morris water maze, is enhanced by enrichment in middle-aged females [23,48], as is cortical dendritic branching [32] and hippocampal neurogenesis [48], suggesting that the female brain retains plasticity well into aging. Thus, we hypothesized that enrichment in aged females would reduce spatial memory decline, perhaps by augmenting synaptic plasticity.

The present study was designed to test this hypothesis. Aged group-housed female mice were exposed to an enriched environment for 3 h per day both prior to and during testing in the spatial and cued (non-spatial) versions of the Morris water maze. Their performance was compared to that of young and aged controls who were also group-housed, but not exposed to the enriched environment. Aged female mice, like aged male mice and aged rats, are typically impaired in the spatial version of the Morris water maze, but not the cued version ([20,22,25,26,29,50,52], but see [10,39]). Thus, enrichment was predicted to affect the spatial, but not the cued, water maze task. Synaptic alterations were examined by measuring levels of the protein synaptophysin in the hippocampus and neocortex, brain regions critical for spatial memory. Synaptophysin is a 38-kDa calcium-binding glycoprotein found in the membranes of neurotransmitter-containing presynaptic vesicles [40,78]. In non-demented humans, synaptophysin decreases with age in the hippocampus and various cortical regions [17,51,55]. In patients with Alzheimer's disease, synaptophysin immunoreactivity is significantly reduced in frontal cortex and hippocampus, and is correlated with impaired cognitive abilities [51,72,74,82]. In rodents, some studies have found similar age-related reductions in cortical or hippocampal synaptophysin [11,63], whereas other studies report no age-related changes in this protein [10,59] or reductions restricted to specific hippocampal subregions [70]. Despite these discrepancies, several studies report significant correlations between synaptophysin levels and spatial memory in the water maze, such that more synaptophysin is associated with better spatial memory [10,11,70]. In addition, findings in aged male rats indicate that environmental enrichment can significantly increase presynaptic vesicle number in the frontal cortex [57] and synaptophysin expression in the hippocampus and several cortical regions [63]. Thus, modulation of synaptophysin may play a role in enrichment-induced alterations in memory. The present study will be the first to examine this potential relationship. Our findings suggest that environmental enrichment, even if initiated during old age, can significantly accelerate spatial memory acquisition and increase cortical and hippocampal synaptophysin levels.

2. Methods

2.1. Subjects

Subjects were 8 young and 14 aged female C57BL/6 mice obtained at the ages of 3 months and 26–27 months from Hilltop Lab Animals, Inc. (Scottdale, PA). Upon arrival at Yale, the mice were quarantined at our medical school animal facility for 3 weeks. They were then moved to an animal facility adjacent to the laboratory in the Department of Psychology, and handled for five days prior to testing to habituate them to being picked up by the experimenter. Thus, at the beginning of behavioral testing, the mice were approximately 4 months or 27–28 months of age. Mice were housed up to 4 per shoebox cage in a room with a 12:12 light/dark cycle (lights on at 06:00 h) and behavioral testing was performed during the light phase of the cycle. Food (Purina LabDiet 5P00 ProLab RMH 3000) and water were provided ad libitum. All procedures conformed to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the

Institutional Animal Care and Use Committee of Yale University.

2.2. Environmental enrichment

Two large Rubbermaid containers (HiTop Storage Box, 56.5 cm long × 41.5 cm wide × 22 cm high) were used for enrichment sessions. The bottom and sides of the chambers were constructed of translucent plastic and the removable covers were made of a white opaque plastic. During enrichment sessions, the covers were placed on top of the chambers to prevent escape. Between 75 and 80 small holes (6 mm in diameter) were drilled in the covers to allow for air circulation. Also, during the sessions, the floors of the chambers were covered with bedding and hosted both food (3–4 pieces of rodent diet) and water (in a medium-sized weigh boat). The two chambers were placed next to each other in a well-lit room with various visual cues including a table, computer boxes, shelves, a T-maze and a plus maze. Various objects were present in the chambers during each enrichment session. A running wheel (11.5 cm in diameter) was always present in each chamber, but its location changed each day. Other objects included rodent toys (a plastic toy in the shape of a boat, balls, seesaw, Critter Trial Puzzle Playgrounds in several configurations), PVC pipe fittings in different shapes, and a toy rope suspended in the cage in various ways. For each session, these objects were presented in different combinations and in different locations in the chamber. In any given session, 3–4 toys plus the running wheel were present. See Fig. 1 for a typical configuration.

The mice were divided into three treatment groups: young control ($n = 8$), aged control ($n = 6$), and aged enriched ($n = 8$). Control mice were group housed but were never exposed to the enrichment chamber or stimulus objects. Aged enriched mice underwent environmental enrichment prior to (14 days) and during water maze testing (during testing, the mice were tested in the morning and enriched in the afternoon). Each enrichment session lasted 3 h. Four mice were placed in the chamber at the same time. Thus, the mice were allowed to interact with both the objects and their cagemates. Both cages of aged enriched females were enriched in the same room simultaneously. By the end of behavioral testing, the mice had been enriched for 23 days.

2.3. Morris water maze

A white circular tank (97 cm in diameter) was filled with water (24 ± 2 °C) and was surrounded by a variety of extramaze cues. The tank was divided into four quadrants, and four start positions were located at the intersections of the quadrants. Data were recorded using an HVS 2020 automated tracking system (HVS Image, Hampton, England). Prior to water maze testing, all mice were habituated to the water using a four-trial shaping procedure in which a smaller ring (55 cm) was inserted inside of the larger 97 cm ring to decrease the total swimming area. This procedure habituated the mice to the water and to taught them to escape from the water by climbing onto a platform. Each mouse was first placed on a visible red lucite platform (10 cm × 10 cm) for 10 s, and then placed at three progressively further distances from the platform where it was allowed 30 s to escape onto

Fig. 1. Typical environmental enrichment chamber setup.

the platform. No data were collected during this procedure. For water maze testing, two water maze tasks were conducted as follows.

2.3.1. Spatial task

In this task, the mice were trained to find a submerged platform using extramaze cues. A transparent lucite platform (10 cm × 10 cm) was submerged 0.5 cm underneath the water in the northeast quadrant of the tank, where it remained for the duration of the four spatial test sessions. The sequence of the four start positions in the tank (north, west, south, and east) varied each trial for each mouse. Six trials/mouse were conducted for four consecutive days (1 session per day). During the first five trials (platform trials), the platform was available to the mouse for escape. Each mouse was given 120 s to locate the platform, after which time the experimenter placed the mouse on the platform and allowed it to remain there for 10–15 s. Each mouse was dried with a cloth towel upon removal from the platform and placed in its home cage for an intertrial interval of approximately 20 min. The sixth trial was a variable-interval probe trial [54] in which the platform was collapsed, remaining under the water and unavailable for escape for either 20, 30, or 40 s. After this interval, the platform was raised and available for escape.

Multiple measures of water maze performance were recorded. *Swim time* (s), *swim distance* (cm), and *swim speed* (cm/s) were recorded during trials 1–5 of each session. For swim time and swim distance, lower numbers indicate better performance. During the probe trial (trial 6 of each session), *platform crossings* (the number of times/10 s that the mice crossed the exact location of the platform) were recorded, as was *quadrant time* (the percent time each mouse spent in each quadrant) and *proximity* (average distance (cm) to the platform; distances sampled 10 times/s). For quadrant time and platform crossings, higher numbers indicate better performance (more time spent in the correct quadrant and more crossings over the platform location), whereas lower numbers indicate better performance for proximity (i.e. shorter distances from the platform).

2.3.2. Cued task

In this non-spatial reference memory task, the platform was made visible using several local cues. In addition, the platform was located in a different quadrant for each trial. Thus, intramaze cues on the platform predicted platform location rather than extramaze cues. The platform was made visible by: (1) lowering the water level such that the platform was 0.25 cm above the surface of the water, (2) covering the platform with red tape, and (3) attaching the circular top of a plastic container (8 cm in diameter, 0.5 cm wide) to the platform oriented perpendicular to the surface of the platform and water. For each of three days, six platform trials were conducted. *Swim time*, *swim distance*, and *swim speed* were recorded. No probe trials were conducted. The intertrial interval was approximately 20 min.

2.4. Synaptophysin assay

Each mouse was sedated briefly with CO_2 and decapitated [4]. The brain was removed immediately, and frontoparietal cortex and hippocampus were dissected bilaterally on ice. Tissue samples were weighed and stored at −70 °C until the day of assay. Samples were resuspended in 0.02% Triton X-100 in 0.1 mM Tris, pH 7.4, sonicated with a probe sonicator, and centrifuged for 10 min at 10,000 × *g*. The supernatant was diluted 1:5 and designated as the crude extract. This crude extract was further diluted as described below. The protein content of the samples was measured using a Bradford protein assay [8].

Synaptophysin was measured using an enzyme-linked immunosorbent assay (ELISA) adapted from [65] for use in mice by Berger-Sweeney et al. [5]. Because purified synaptophysin was not available for use as a standard, synaptophysin levels in the samples are expressed as "equivalents" relative to synaptophysin immunoreactivity from whole mouse brain homogenate (termed "mouse brain standard" or "MBS"). An antibody sandwich ELISA assay using two different anti-synaptophysin antibodies (monoclonal anti-synaptophysin Clone SY 38 and polyclonal rabbit anti-synaptophysin, DAKO Corp., Carpinteria, CA) was used to determine the relative amounts of synaptophysin in the samples. Samples were diluted to 1:32,000 from the crude extract and were assayed in triplicate. Optical density was measured at a wavelength of 405 nm using a Labsystems Multiskan Plus microplate reader. The average absorbance of three wells containing no MBS was subtracted from each reading.

To calculate the relative amount of synaptophysin in the samples, the absorbance of each of four MBS concentrations was plotted versus the log of the total protein concentration. The equation of the straight line that resulted and the absorbance of each sample was used to determine the concentration of MBS which would have the absorbance exhibited by the sample. This apparent MBS concentration of the sample was divided by the total protein concentration of the sample (obtained from the protein assay described in the previous section) to yield the relative amount of synaptophysin in the sample versus the amount of synaptophysin in the MBS homogenate (termed "MBS synaptophysin equivalent").

2.5. Data analysis

For the water maze, platform trial measures (swim time, swim distance, and swim speed) and platform crossings from the probe trials were averaged within a group for each session and analyzed using a one-way repeated-measures analysis of variance (ANOVA) with Group as an independent variable (SuperANOVA, Abacus Concepts, Berkeley, CA). Quadrant time and proximity during the probe trials were calculated for each of the four quadrants, and each session was analyzed separately using one-way repeated measures ANOVAs with Group as the independent variable

and Quadrant as the repeated measure. For quadrant time, a spatial bias was qualitatively assessed as a predominance of time spent in the correct quadrant relative to the incorrect quadrants (chance = 25%). For the proximity measure in quadrants not containing the platform, proximity distances were calculated relative to virtual "platforms" located in the center of each quadrant, mirroring the location of the actual platform in the correct quadrant (these "platforms" were generated by the computer software). Spatial bias was suggested by shorter proximities to the actual platform than the virtual "platforms". To examine between-group differences in memory for the platform location during the probe trials, *t*-tests (independent, two-tailed) were performed for quadrant time and proximity in the correct quadrant for sessions in which a significant group × quadrant interaction was observed (Statview, SAS Institute Inc., Cary, NC). Synaptophysin data were analyzed using separate one-way ANOVAs without repeated measures for each brain region. Fisher's protected least significant difference posthocs were performed to delineate between-group differences for synaptophysin and the water maze platform trial measures.

3. Results

3.1. Subjects

All mice appeared in good health, although alopecia was evident in most mice. One aged control female and two aged enriched females were excluded from the experiment due to difficulty swimming or inability of the tracking system to detect them consistently (due to alopecia). Thus, the final samples sizes for the behavioral analyses were: young control ($n = 8$), aged control ($n = 5$), and aged enriched ($n = 6$). Two of the young controls were perfused for histological analysis of their brains (data not shown), and thus, the sample sizes for the synaptophysin assays were: young control ($n = 6$), aged control ($n = 5$), and aged enriched ($n = 6$).

3.2. Spatial water maze

Environmental enrichment significantly improved the acquisition of the spatial task. The main effect of group was significant in swim time ($F(2, 16) = 8.9$, $P < 0.01$), swim distance ($F(2, 16) = 6.8$, $P < 0.01$), and swim speed ($F(2, 16) = 4.2$, $P < 0.05$). Posthoc tests conducted on the swim time and swim distance measures (Fig. 2A and B) revealed that the aged control mice exhibited significantly greater swim times and distances relative to both young control females (Ps < 0.01) and aged enriched females (Ps < 0.05). In both measures, no significant difference was observed between young control females and aged enriched females. Aged control females also swam more slowly than young control females ($P < 0.05$, Fig. 2C). Although aged enriched females tended to swim more slowly than young females, this difference was not significant ($P = 0.08$). Session effects were significant in all three measures (Fs$(3, 48) = 7.8$–18.5, Ps < 0.001), indicating improvement among the groups. No session × group interaction was significant.

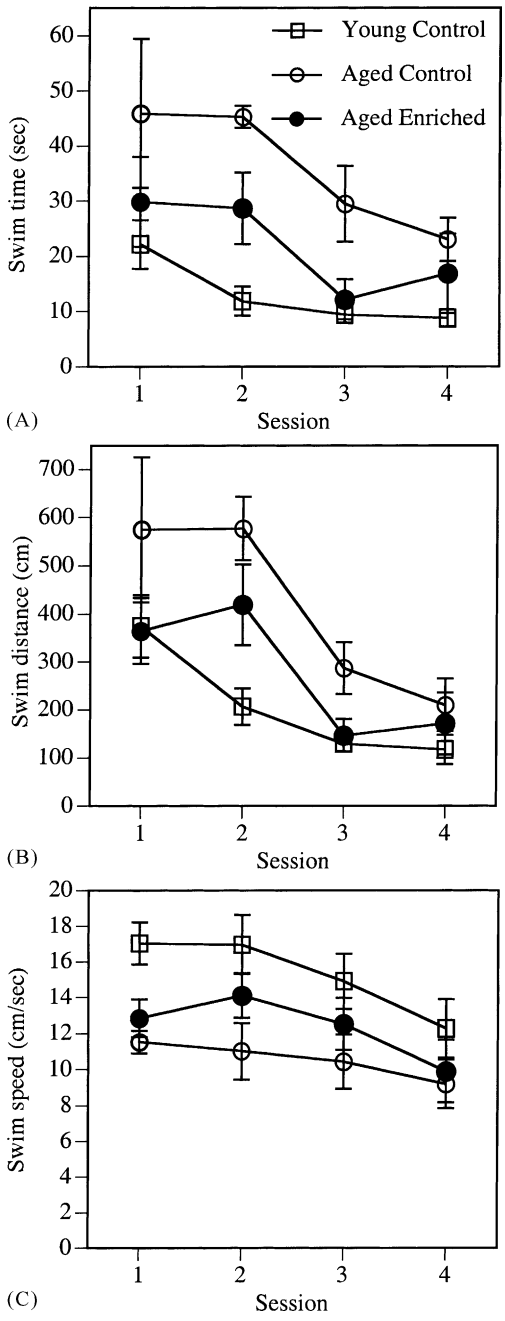

Fig. 2. Environmental enrichment significantly improved acquisition of the spatial task. Swim time (A), swim distance (B), and swim speed (C) were recorded during the platform trials of the spatial water maze task (trials 1–5 of each session). Each point represents the mean ± standard error of the mean (S.E.M.) of each group for trials 1–5. Aged control females exhibited significantly slower swim times and swim speeds, and longer swim distances than young controls. In contrast, aged enriched females were not significantly different from controls in any measure.

Enrichment also appeared to accelerate the development of a spatial bias in the spatial probe trials (Figs. 3

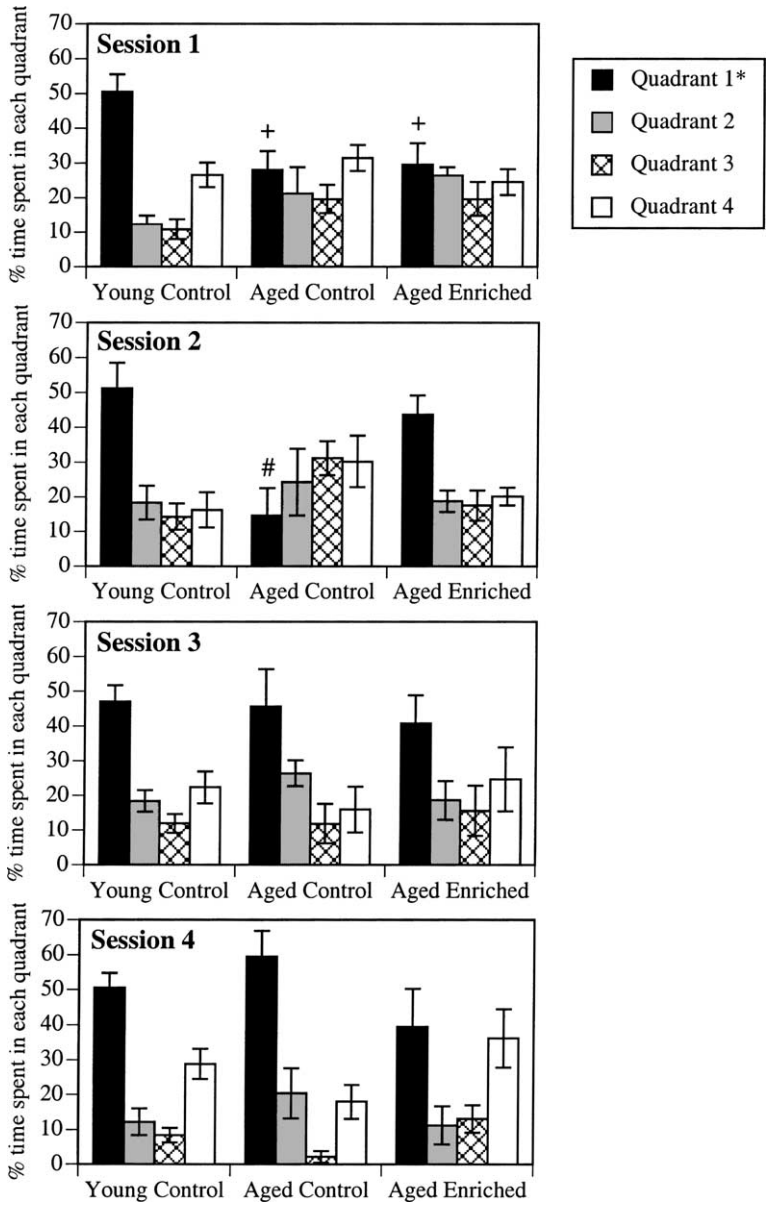

Fig. 3. Environmental enrichment accelerated the development of a spatial bias in the probe trials. Percent time spent in each quadrant is presented for Session 1 (top), Session 2 (second), Session 3 (third), and Session 4 (bottom). Each bar represents the mean ± S.E.M. of each group for one quadrant. Quadrant 1 contained the platform (indicated by "∗"). The patterns of time spent in the four quadrants indicate that young controls displayed a spatial bias by Session 1, aged enriched females by Session 2, and aged controls by Session 3. All aged females spent significantly less time in the correct quadrant during Session 1 than young controls ("+" indicates $P < 0.02$ relative to young controls). In contrast, aged controls were impaired relative to both young and aged enriched groups during Session 2 ($P < 0.05$ indicated by "#"). None of the groups differed in Sessions 3 and 4.

and 4). For both quadrant time and proximity, significant group × quadrant interactions were observed in Sessions 1 (Fs$(6, 48) = 3.0$ and 2.6, respectively, Ps < 0.05) and 2 (Fs$(6, 48) = 3.6$ and 4.1, respectively, Ps < 0.01), but not Sessions 3 and 4. Examination of Figs. 3 and 4 (top) reveals the presence of a significant spatial bias in the young control group during the probe trial of Session 1. Young controls spent more time in the correct quadrant (50%) than the other quadrants and swam closer to the actual platform than the virtual "platforms" in the incorrect quadrants, indicating the development of a spatial bias. In contrast, neither aged group displayed such a bias; both spent a similar amount of time in all quadrants and swam a similar distance from all "platforms". Indeed, young controls spent significantly more time in the correct quadrant than either aged controls ($t(11) = 3.03$, $P < 0.02$) or aged enriched females ($t(12) = 2.7$, $P < 0.02$). The two aged groups did not differ. Similarly, the proximity of young controls was significantly closer to the actual platform than that of either aged group ($t(11) = -2.4$, $P < 0.05$; $t(12) = -2.4$, $P < 0.05$), whereas proximities of the aged groups did not differ from each other. During Session 2, however, the aged

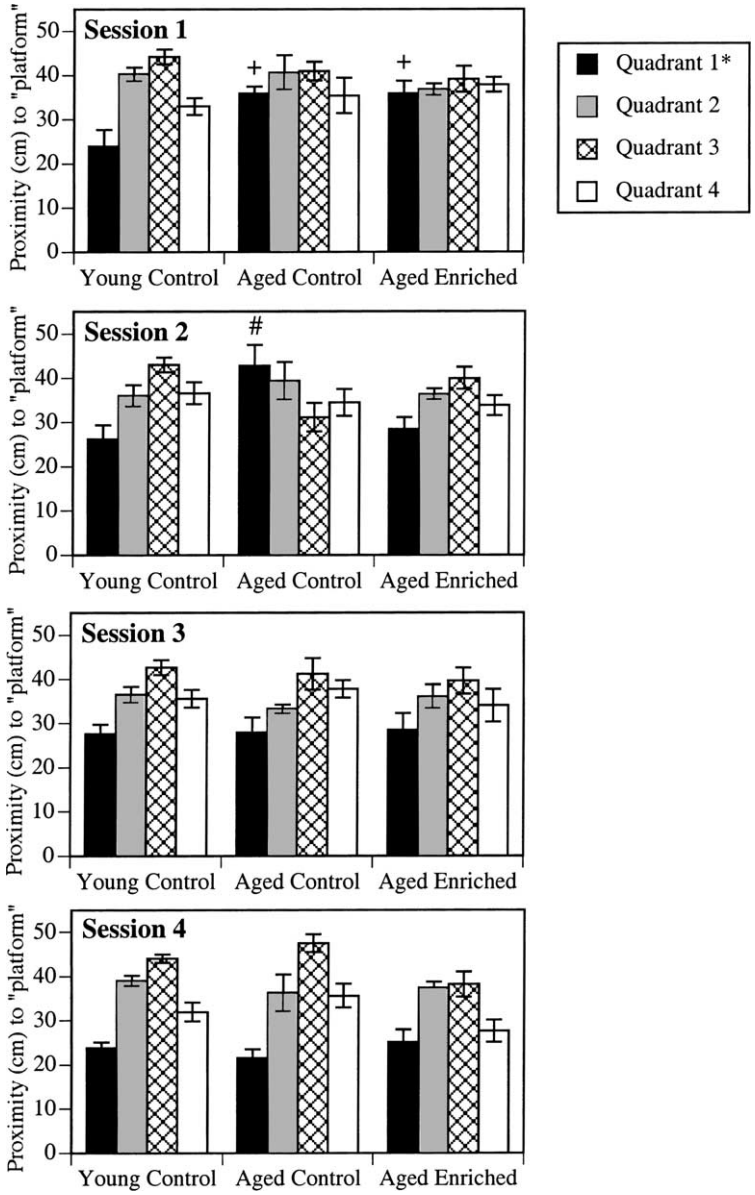

Fig. 4. Enrichment significantly improved proximity values. Proximities to each "platform" (actual and virtual) are presented for Session 1 (top), Session 2 (second), Session 3 (third), and Session 4 (bottom). Each bar represents the mean ± S.E.M. of each group for one platform. Quadrant 1 contained the platform (indicated by "∗"). All aged females swam significantly further from the actual platform during Session 1 than young controls ("+" indicates $P < 0.02$ relative to young controls). In contrast, aged controls were impaired relative to both young and aged enriched groups during Session 2 ($P < 0.05$ indicated by "#"). None of the groups differed in Sessions 3 and 4.

enriched group demonstrated significant improvement. As illustrated in the figures (second graph), the aged enriched group, but not the aged control group, joined the young controls in displaying a spatial bias. In this session, the aged controls spent significantly less time in the correct quadrant and swam a further distance from the platform than both the young control (ts(11) = 3.3 and −3.0, respectively, Ps < 0.02) and aged enriched (ts(9) = −3.1 and 2.7, respectively, Ps < 0.03) groups. The young control and aged enriched groups did not differ from each other. Finally, in Sessions 3 and 4, the performance of the aged control group indicated a spatial bias (Figs. 3 and 4, bottom two graphs). Neither aging nor enrichment affected the platform crossings measure (data not shown).

3.3. Cued water maze

Cued data are presented in Fig. 5. The main effect of group was not significant for swim time or swim distance ($F(2, 16) = 2.4$, $P > 0.05$), although there was a trend in swim time ($F(2, 16) = 2.9$, $P = 0.08$). Because of this trend, a posthoc analysis was performed on the group effect from swim time. This analysis revealed a trend for young controls to exhibit faster swim times than both aged groups,

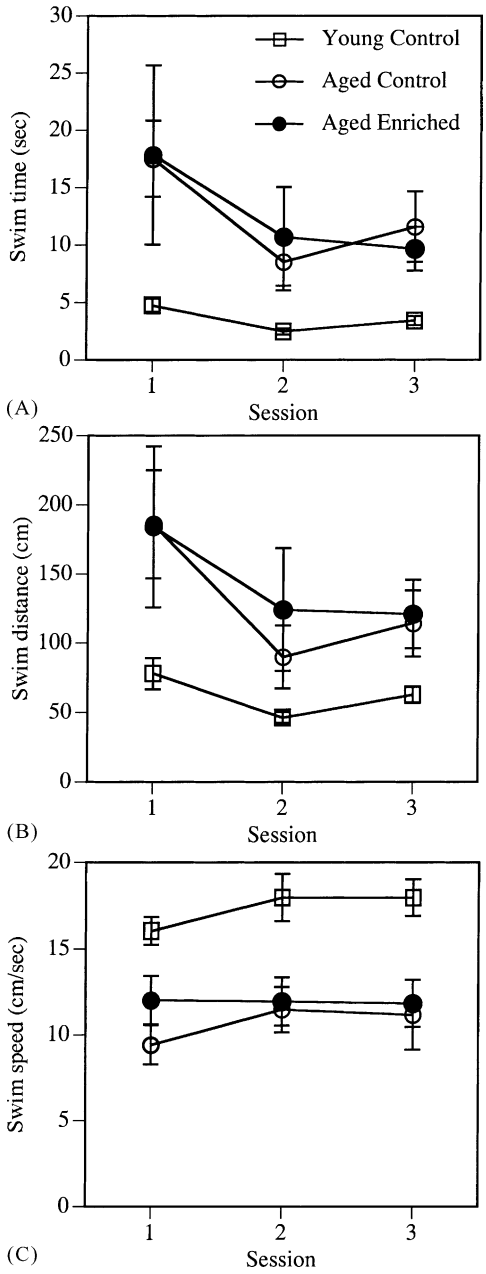

Fig. 5. Environmental enrichment did not affect acquisition of the cued task. Swim time (A), swim distance (B), and swim speed (C). Each point represents the mean ± S.E.M. of each group for trials 1–6. No enrichment-related differences were found, however, young controls swam significantly faster than aged females in both groups.

but the *P*-values were not significant (*P*s = 0.06 for both comparisons). Like the spatial task, the group effect was significant for swim speed ($F(2, 16) = 10.9$, $P < 0.001$). However, in this case, both aged groups swam significantly slower than young females (*P*s < 0.01). Session effects were significant in swim time ($F(2, 32) = 7.1$, $P < 0.01$) and swim distance ($F(2, 32) = 11.1$, $P < 0.001$), but not swim speed. Session × group interactions were not significant for any measure.

Table 1
Synaptophysin data for each treatment group

Brain region	Young control	Aged control	Aged enriched
Frontoparietal cortex	0.34 ± 0.02	0.39 ± 0.02	0.53 ± 0.02*
Hippocampus	0.46 ± 0.04	0.38 ± 0.01	0.59 ± 0.03*

Values represent mean ± S.E.M. "MBS synaptophysin equivalents" expressed as sample immunoreactivity relative to that of an equal amount of mouse brain standard.

* $P < 0.01$ relative to young and aged controls.

3.4. Synaptophysin

Synaptophysin data are presented in Table 1. Environmental enrichment significantly increased synaptophysin immunoreactivity in both the hippocampus ($F(2, 14) = 11.8$, $P < 0.001$) and frontoparietal cortex ($F(2, 14) = 23.5$, $P < 0.0001$). In both brain regions, aged enriched females exhibited higher synaptophysin values than either young or aged controls (*P*s < 0.01). The young and aged control groups did not differ from each other in either brain region, although a trend was evident in the hippocampus ($P = 0.08$).

4. Discussion

The results of the present study demonstrate for the first time that environmental enrichment, initiated in old age, can significantly improve spatial memory and increase synaptic protein levels in females. Aged females in this study were exposed to only 14 days of environmental enrichment prior to testing in the water maze. The findings suggest that this enrichment improved acquisition of a spatial reference memory task in aged females. This conclusion is supported by the swim time and swim distance measures from the spatial task in which aged enriched females, but not aged control females, performed similarly to young controls. In addition, the quadrant time and proximity measures indicate the earlier acquisition of a spatial bias (i.e. memory for the platform location) in aged enriched females relative to aged controls. In contrast, enrichment did not significantly affect performance in the cued task, suggesting a specific effect on spatial reference memory. This effect was associated with increased synaptophysin immunoreactivity in both the hippocampus and frontoparietal cortex, suggesting that altered presynaptic plasticity may contribute to memory enhancement in aged females.

Our finding that spatial memory is improved by enrichment in aged females is consistent with previous reports in enriched middle-aged female mice tested in the same task [23,48,60]. Thus, the present data suggest that enrichment can benefit spatial memory in females at nearly any age. These findings are also consistent with some previous reports in aged males in which enrichment improved learning, memory, alternation, and food-seeking behaviors [71,75,76,79]. However, this improvement is inconsistent with data from

other behavioral tasks demonstrating no enrichment-induced improvement of spatial novelty, maze learning, and discrimination learning in aged male rats and mice [75,76]. This discrepancy may suggest that enrichment effects are memory or task specific. Given that all brain regions involved in learning and memory are not likely altered by enrichment to the same extent, it may not be surprising to find that some types of learning and memory are affected more than others. Nevertheless, the present data suggest that at least some types of learning and memory can be improved by enrichment in aged females and it remains to be seen whether this finding extends to other types of memory.

Although the aged mice in the present study were not statistically worse than young controls in the cued task, a trend was evident. This is surprising, given than aged mice are not typically impaired on the cued task [22,29,50,52] and that both aged groups in this study eventually learned to find the hidden platform by session four. Perhaps the aged mice found it difficult to switch from navigating using extramaze cues to a singular intramaze cue. Alternatively, absent an explicit intramaze cue to guide the mice to the platform, enriched mice may have been more likely to explore the tank (perhaps due to familiarity with novelty) and thus, learned the spatial task more quickly. These possibilities invite further research aimed at addressing this issue.

Because mice in this study were provided with mentally stimulating objects as well as objects that encourage aerobic exercise (e.g. running wheels), it is impossible to determine whether the beneficial effect on spatial memory was the solely the result of intellectual stimulation. For example, good physical fitness in older humans has been associated with better memory (e.g. [12]), and exercise in rats has been reported to improve acquisition of a spatial working memory task [1]. However, in the brain, motor learning reportedly increases synaptogenesis, whereas motor activity increases angiogenesis [7]. This latter finding, in combination with our synaptophysin data, would suggest that intellectual enrichment, rather than exercise, contributed primarily to the spatial memory improvement observed here. Future studies are being planned to examine the contribution of each to cognition enhancement in aging mice.

Synaptophysin immunoreactivity was increased by environmental enrichment in both the hippocampus and frontoparietal cortex. These findings are consistent with previous data from aged male rats in which enrichment prevented the age-related loss of hippocampal and neocortical synaptophysin immunoreactivity [63]. Because this and the present study measured synaptophysin protein using ELISAs, neither study can directly address whether the alterations observed were the result of an increased number of presynaptic boutons, increased numbers of vesicles in presynaptic boutons, or increased amount of synaptophysin in presynaptic vesicle membranes. Although increases in synaptophysin immunoreactivity have most often been interpreted as an increase in presynaptic terminals (e.g. [11,17,74]), a recent electron micrography study indicates that presynaptic terminals are not increased in response to environmental enrichment [57]. Instead, the number of vesicles per terminal is increased in aged rats in response to enrichment [57]. Because synaptophysin is a constituent of neurotransmitter-containing presynaptic vesicle membranes [78], an increase in synaptophysin may reflect an increase in neurotransmission. It may be this increase in neurotransmission that leads to improved spatial memory. However, relationships among synaptophysin levels, aging, and memory are not straightforward. Although this, and other, reports found no age-related changes in hippocampal and/or neocortical synaptophysin [10,59], other studies, particularly those examining discrete hippocampal subregions, indicate reductions in synaptophysin with age [11,63,70]. This is particularly evident when aged rats are subdivided into those that are impaired and unimpaired in water maze learning relative to young rats. In these cases, reduced synaptophysin in hippocampal CA3 and dentate gyrus, but not CA1, was correlated with poor spatial memory [10,70] (this suggests that we may have failed to detect an age-related reduction in synaptophysin because our examination of the entire hippocampus would have obscured regional differences). A similar relationship has been reported in frontoparietal cortex [11]. Unfortunately, due to limited sample size, a similar analysis of impaired and unimpaired aged controls could not be conducted in the present study. However, the data do suggest a positive relationship in aged females between synaptophysin and memory; enrichment in aged females resulted in improved memory and increased synaptophysin levels. This relationship will need to be explored further in future studies.

In recent years, it has become clear that ovarian hormones, such as estrogen and progesterone modulate regions of the brain involved in learning and memory (for reviews, see [27,56,81]). The loss of hormone cycling during middle-age has been associated with impaired memory in a variety of mammals including humans [68] and rodents [22,53]. In mice, this loss begins at 13–14 months of age and is complete by 24–25 months of age [58]. Because the aged females used in this study were 27–28 months of age, hormone cycling had already ceased in these mice. However, the young control mice were intact and presumably exhibited normal estrous cycling. In young rodents, the issue of whether cyclic fluctuations of ovarian hormones influence learning and memory has been a matter of debate. Of the four published studies examining the influence of estrous cycle stage on acquisition of the spatial water maze, one found no effect [6], whereas three found subtle, but conflicting, effects [21,24,77]. The inevitable conclusion from these studies, as well as those examining the menstrual cycle and cognition in humans [18], is that hormone cycling in young subjects has minimal, if any, influence on memory. Thus, it is highly unlikely that hormone cycling influenced the performance of the young mice tested in this study.

5. Conclusions

The present study indicates that short-term environmental enrichment initiated late in life can accelerate spatial memory acquisition and increase synaptic protein levels in aged females. Our behavioral findings are consistent with previous studies in humans that indicate that intellectual stimulation can delay or prevent age-related memory loss [64,69]. Our synaptophysin data suggest a potential neurobiological mechanism of this improvement. Thus, aged rodents may provide a relevant animal model to study the effects of enrichment on cognition and the brain.

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