

Single Enrichment Variables Differentially Reduce Age-Related Memory Decline in Female Mice

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The authors sought to determine how different elements of enrichment, for example, cognitive stimulation and voluntary exercise, differ in their ability to improve memory throughout the lifespan. Young, middle-aged, and aged female C57BL/6 mice received 24-hr exposure in their home cages to toys alone (cognitive stimulation), running wheels alone (exercise), or both toys and running wheels (complex enrichment) for 4 weeks prior to and then throughout spatial water maze testing. As expected, spatial memory became progressively worse with age. Exercise alone improved spatial water maze performance in young mice, whereas both exercise alone and complex enrichment improved spatial maze performance in middle-aged mice. All enrichment treatments improved spatial maze performance in aged mice. These data suggest that exercise is the most effective element of enrichment in young female mice and that both cognitive stimulation and exercise are necessary to reliably improve spatial water maze performance in aging female mice.

Keywords: environmental enrichment, aging, reference memory, spatial memory, Morris water maze

Environmental enrichment provides animals with access to social, cognitive, and physical stimulation. Typically, enriched environments consist of toys, small dwellings, tubes, running wheels, and cage-mates. Early work in this field has established that enrichment can produce numerous cognitive and neural benefits in developing rodents (Green & Greenough, 1986; Greenough, West, & DeVoogd, 1978; Greenough, Wood, & Madden, 1972). Subsequent work in adult rodents has demonstrated that enrichment can enhance learning and memory (Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999; Williams et al., 2001) and protect against learning and memory deficits induced by such genetic manipulations as deletion of NMDAR1 receptor subunits from hippocampal CA1 (Rampon et al., 2000). Enrichment in adulthood also increases hippocampal CREB immunoreactivity (Williams et al., 2001), synaptophysin levels (Lambert, Fernandez, & Frick, 2005), and long-term potentiation (Duffy, Craddock, Abel, & Nguyen, 2001). Further, enrichment can alleviate spatial reference memory deficits observed in an APP23 mouse model of Alzheimer's disease and increase hippocampal brain-derived neurotrophic factor and neurotrophin (NT-3) levels relative to those of APP23 controls (Wolf et al., 2006).

Recent work has demonstrated that enrichment also protects against cognitive decline in aging animals. Studies utilizing rats and mice have shown that enrichment in middle age can signifi-

cantly reduce spatial memory deficits in the Morris water maze (Frick, Stearns, Pan, & Berger-Sweeney, 2003; Kempermann, Kuhn, & Gage, 1998; Pham et al., 1999), improve learning in the Hebb–Williams maze (Cummins, Walsh, Budtz-Olsen, Konstantinos, & Horsfall, 1973), and enhance cortical and hippocampal plasticity (Green, Greenough, & Schlumpf, 1983; Kempermann et al., 1998). In aging rodents, enrichment increases cortical thickness (Diamond, Johnson, Protti, Ott, & Kajisa, 1985), reduces hippocampal gliosis (Soffie, Hahn, Terao, & Eclancher, 1999), and increases neurogenesis in the dentate gyrus (Segovia, Yague, Garcia-Verdugo, & Mora, 2006). Enrichment in aging rodents also improves spatial memory in the Morris water maze (Frick & Fernandez, 2003) and radial arm maze (Bennett, McRae, Levy, & Frick, 2006), and increases incidental learning and food-seeking behaviors (J. M. Warren, Zerweck, & Anthony, 1982).

Together, these studies demonstrate that enrichment is an effective way to improve learning and memory in adult and aging animals. However, because environmental enrichment typically exposes animals to a combination of physical, cognitive, and social stimulation, it is difficult to determine how each type of stimulation contributes to its beneficial effects. Indeed, it seems likely that the interaction between multiple enriching factors may be necessary to produce an observable effect on memory and neural function. For example, social stimulation alone is not sufficient to enhance memory or to produce the cerebral changes observed in rodents enriched in complex environments (Rosenzweig, Bennett, Hebert, & Morimoto, 1978; Williams et al., 2001). Further, only direct interaction with an enriched environment, not indirect observation of the environment, provides sufficient stimulation to increase cerebral weights in rats (Ferchmin & Bennett, 1975). However, voluntary exercise alone can produce changes in the brain that are similar to those observed after complex enrichment. For instance, voluntary exercise alone increases hippocampal synaptophysin levels (Chen, Chen, Lei, & Wang, 1998), muscarinic receptor density (Fordyce & Farrar, 1991), and hippocampal

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This project was sponsored by a Claude D. Pepper Older Americans Independence Center at Yale University School of Medicine (P30AG21342) grant to Karyn M. Frick. We would like to thank Jennifer C. Bennett, Wen Fan, and Suzanne McGoey for assistance with data collection.

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bound protein kinase C (Fordyce & Wehner, 1993), and enhances long-term potentiation and neurogenesis in the dentate gyrus (van Praag, Christie, Sejnowski, & Gage, 1999). Voluntary exercise alone also improves spatial memory in the Morris water maze (van Praag et al., 1999), a water maze place learning-set task (Fordyce & Wehner, 1993), and the radial arm maze (Anderson et al., 2000).

These data suggest that exercise may be the most critical component in cognitive function enhancement. In fact, a recent article from our laboratory demonstrated that 3 hr/day of exercise alone, but not of cognitive stimulation or acrobatic training, can significantly improve spatial working memory in a water-escape motivated radial arm maze (Lambert et al., 2005). In that study, young female mice were exposed to running wheels alone, toys alone, or acrobatic training for 6 weeks prior to and throughout radial arm maze testing. Results indicated that only running wheels enhanced working memory (Lambert et al., 2005). This finding seems to suggest that exercise is more critical to improving cognitive function than cognitive stimulation, but it raises several key questions about the individual components of enrichment. For instance, because our previous study did not include a complex enrichment group (i.e., exercise plus cognitive stimulation), it is unclear whether the benefits of exercise alone are as great as those produced by complex enrichment. It is also unclear whether spatial memory in the Morris water maze, a task that is sensitive to the effects of complex enrichment (Bennett et al., 2006; Frick & Fernandez, 2003; Frick et al., 2003; Kempermann et al., 1998), is differentially affected by exercise and cognitive stimulation. Further, it is possible that more exposure to cognitive stimulation alone (e.g., 24 hr/day rather than 3 hr/day) would yield a beneficial effect greater than what we have previously reported (Lambert et al., 2005). Finally, of importance to the study of aging, it is unknown whether exercise alone similarly benefits memory in older mice, and whether cognitive stimulation or exercise alone can enhance memory as much as complex enrichment in aging mice.

The present study was designed to address these questions by testing the effects of cognitive stimulation and voluntary exercise, alone and in combination, on spatial memory in female mice throughout the lifespan. For 4 weeks prior to behavioral testing and then throughout testing, young (3 months), middle-aged (15 months), and aged (21 months) female C57BL/6 mice received 24-hr exposure in their home cages to toys alone (cognitive stimulation), running wheels alone (exercise), or both toys and running wheels (complex enrichment). Enriched mice were compared with nonenriched controls. The Morris water maze was used to test spatial reference memory because this task is sensitive to the beneficial effects of enrichment in aging mice (Bennett et al., 2006; Frick & Fernandez, 2003; Frick et al., 2003; Kempermann et al., 1998). We predicted that mice of all ages enriched in complex environments would show greater improvements in spatial memory than those enriched in single-element environments consisting of only running wheels or toys. This prediction was based on a previous study from our lab that reported enhanced spatial reference memory in aged male mice exposed to sophisticated complex enrichment environments in their home cages, relative to mice exposed to less sophisticated complex enrichment environments for 3 hr/day (Bennett et al., 2006). Because we have previously reported that exercise alone, but not cognitive stimulation alone, enhanced working memory in young female mice (Lambert et al.,

2005), we expected that exercise in the present study would benefit spatial memory in young mice more than cognitive stimulation alone. Relative to young mice, however, we expected that the greatest benefits of enrichment treatments would occur among aged mice, because typical age-related learning and memory decline would leave the most room for improvement (Frick, Burlingame, Arters, & Berger-Sweeney, 2000). The present study is the first to directly compare the ability of different elements of enrichment to improve memory in young, middle-aged, and aged rodents.

Method

Subjects

Subjects were 160 female C57BL/6 mice obtained from the National Institute on Aging colony at Harlan Sprague Dawley (Indianapolis, IN). At the time of arrival, mice were 3 months ($n = 48$), 15 months ($n = 56$), and 21 months ($n = 56$) of age. Control mice were housed up to 5 per shoebox cage, and enriched mice were housed up to 8 per large enrichment cage. All subjects had ad-lib access to food (Harlan 2018 18% Protein Rodent Diet) and water and exposure to a 12:12-hr light-dark cycle (lights on at 0700). All behavioral testing was performed during the light phase of the cycle. Mice were handled for 5 min/day for 5 days to habituate them to being picked up by the experimenter before behavioral testing. All procedures were approved by the Institutional Animal Care and Use Committee of Yale University and conformed to the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Environmental Enrichment

Mice were randomly assigned to either control or enriched housing conditions. There were a total of 12 treatment groups: young control ($n = 12$), young toy ($n = 12$), young wheel ($n = 12$), young complex enrichment ($n = 12$), middle-aged control ($n = 14$), middle-aged toy ($n = 14$), middle-aged wheel ($n = 14$), middle-aged complex enrichment ($n = 14$), aged control ($n = 14$), aged toy ($n = 14$), aged wheel ($n = 14$), and aged complex enrichment ($n = 14$). Hereafter, the four enrichment treatments are referred to as follows: control, toy, wheel, and complex.

Mice were housed in either control or enriched conditions for 4 weeks prior to and then throughout behavioral testing (7 weeks total). Control mice were housed in standard shoebox cages and had no access to toys or running wheels. Enriched mice were housed as previously described (Bennett et al., 2006) in large transparent plastic bins (Ancare, Bellmore, NY; 66 cm long \times 46 cm wide \times 38 cm high) covered with removable transparent lids. Two large ventilation holes (30 cm long \times 18.5 cm wide) on the top of the bins were fitted with wire feeding racks and covered by ventilation lids from the standard shoebox cages. Food pellets were placed in a stainless steel food bowl on the floor of each bin, and a water bottle (Ancare, Bellmore, NY) was mounted on the side of the bin. Bins were cleaned twice per week, and new enrichment objects in different configurations were presented after each cleaning. Examples of object configurations for each treatment are illustrated in Figure 1. For the toy treatment, cognitive stimulation was provided by an assortment of toys and rodent dwellings

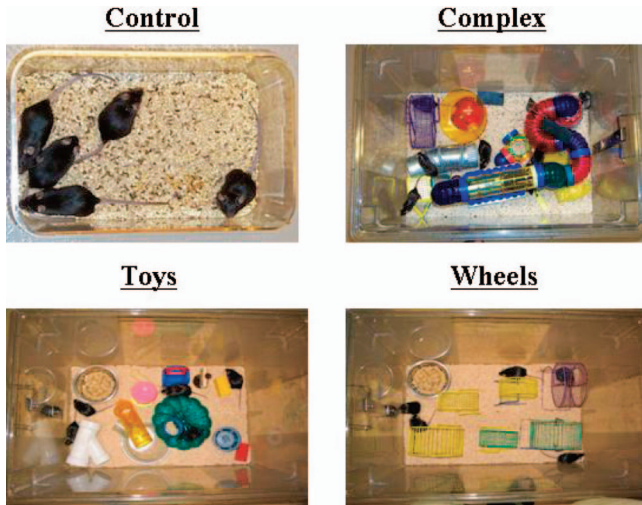


Figure 1. Photographs of enrichment housing conditions.

without vertical climbing aspects. For the wheel treatment, six running wheels provided the opportunity for voluntary exercise. For the complex enrichment treatment, cognitive stimulation and exercise were provided by an assortment of objects including 2 running wheels, 2 rodent dwellings, 4–6 miscellaneous toys, and a large plastic tube configuration with vertical climbing aspects.

Morris Water Maze

The Morris water maze consisted of a white circular tank (97 cm in diameter) surrounded by various extramaze cues as previously described (Frick & Fernandez, 2003; Frick, Fernandez, & Bulinski, 2002). The tank was filled with water ($24 \pm 2^\circ \text{C}$) that was made opaque with white, nontoxic tempera paint. Data were collected by means of an HVS 2020 (HVS Image, Hampton, England) automated tracking system.

One day prior to testing, mice were shaped in a four-trial procedure in which a smaller ring (55 cm) was placed inside of the tank to decrease the total swimming area. Mice were first placed on a $10 \times 10\text{-cm}$ visible red platform for 10 s and then removed. They were then placed at three distances progressively further from the platform and allowed to swim to the platform. If the mouse did not find the platform within 60 s, it was led to the platform by the experimenter. No data were collected during shaping.

Spatial Water Maze

The spatial task consisted of six trials per day for 5 consecutive days and was conducted as previously described (Frick & Fernandez, 2003; Frick et al., 2002). During spatial trials, a transparent Lucite platform ($10 \times 10\text{ cm}$) was submerged just underneath the surface of the water and remained in the same location for all trials. For the first five trials, each mouse was placed in one of four start positions and was given 120 s to find the platform. The start positions varied between mice and for each trial. If the mouse did not find the platform after 120 s, the experimenter led the mouse to the platform, where it sat for 10 s. The mouse was then removed

from the testing room and returned to its home cage for an intertrial interval of approximately 20 min. During these first five trials, swim time (in seconds), swim distance (in centimeters), and swim speed (in centimeters per second) were recorded.

The sixth trial of each day was a variable-interval probe trial (Markowska, Long, Johnson, & Olton, 1993). During this trial, the platform was collapsed and made unavailable for 20, 30, or 40 s, and then raised and made available for escape. Regardless of how long the platform was collapsed, the total probe trial duration was 60 s. Quadrant time (the percentage of time that a mouse spent in the quadrant containing the collapsed platform) and platform crossings (the number of times a mouse crossed the platform location per 10 s that the platform was collapsed) were recorded. Quadrant time was recorded to provide a measure of how well the mice learned the general platform location, whereas platform crossings were recorded to provide a measure of how well the mice learned the exact location of the platform.

Cued Water Maze

The cued task was conducted as a measure of nonspatial memory and to control for non-mnemonic aspects of task performance (e.g., motivation, swimming ability, and visual acuity). Cued testing began 2 days after the completion of the spatial task. For six trials per day for 3 consecutive days, the platform was made visible with red tape and a white circular cue attached to the side of the platform (7.5 cm in diameter). The platform was also raised just above the surface of the water. The platform was moved to a different location in the tank for each trial. All other aspects of the procedure were the same as the spatial task. Swim time, swim distance, and swim speed were recorded as in the previous task.

Data Analysis

Spatial and cued Morris water maze measures were averaged within a group for each session and analyzed with a repeated measures analysis of variance (ANOVA), with age and enrichment as the independent variables and session as the dependent variable (SuperANOVA, Abacus Concepts, Berkeley, CA). Because subtle effects of the independent variables may be obscured in these large 12-group ANOVAs, separate one-way ANOVAs were also performed for young, middle-aged, and aged groups for each measure, with enrichment as the independent variable and session as the repeated measure.

Because all ANOVAs in the present study were performed on 4 or 12 treatment groups, we anticipated that significant enrichment-induced differences between pairs of groups may be obscured by nonsignificant effects of enrichment in other groups. Therefore, Fisher's protected least significant difference post hoc tests were performed a priori on all main effects of enrichment to compare the effect of the different treatment groups of the same age. An alpha level of .05 was used to reject the null hypothesis.

Results

Subjects

On arrival in the laboratory, mice appeared in good health and were randomly divided into treatment groups. All mice successfully completed behavioral testing.

Morris Water Maze

Spatial Task

Overall effects. A summary of the age-related impairments in the Morris water maze is presented in Table 1. All spatial task measures were significantly affected by age, $F_s(2, 148) = 3.05-10.96, p_s \leq .05$. Post hoc tests indicated that aged mice were significantly impaired relative to young and middle-aged mice in all measures but swim speed. Middle-aged mice were impaired relative to young mice in swim speed and platform crossings. All measures from the spatial task were also significantly affected by enrichment, $F_s(3, 148) = 2.82-8.13, p_s < .05$. Post hoc tests revealed that the toy, wheel, and complex groups performed significantly better than controls in the swim time, swim speed, and platform crossings measures. The wheel groups performed better than controls in swim distance and quadrant time. The toy groups also exhibited more quadrant time than controls.

Significant session effects in all five measures demonstrated overall learning throughout training, $F_s(4, 592) = 12.49-82.67, p_s < .05$. There was a significant Session \times Age \times Enrichment interaction for swim time, $F(24, 592) = 2.31, p < .05$ (see Figures 2A-2C). This appeared to be due, in part, to the fact that the aged control group decreased swim time most over the course of training (Figure 2C). A significant Session \times Age interaction for swim distance, $F(8, 592) = 2.23, p < .05$ (Figures 2E-2G), indicated that the performance of young mice improved more rapidly than that of older mice. There was also a significant Session \times Age \times Enrichment interaction, $F(24, 592) = 2.34, p < .05$, for swim distance. A significant Age \times Enrichment interaction for swim speed, $F(6, 148) = 4.03, p < .05$ (Figures 2I-2K), suggested that the enrichment treatments affected swim speed differently among the age groups. There were also significant Session \times Age, $F(8, 592) = 3.64, p < .05$, and Session \times Enrichment, $F(12, 592) = 5.50, p < .05$, interactions for swim speed. For platform crossings during the probe trial (see Figures 3E-3G), a significant Session \times Enrichment interaction was most likely driven by the fact that the toy, wheel, and complex groups increased platform crossings more rapidly than controls over the course of training, $F(12, 592) = 1.85, p < .05$. No interactions were significant for quadrant time (Figures 3A-3C). No other interactions were significant for any measure.

Table 1
Summary of Age-Related Impairments in the Morris Water Maze

Task	Measure	Middle-aged	Aged
Spatial	Swim time*	—	Xx
	Swim distance*	—	Xx
	Swim speed*	X	—
	Quadrant time*	—	Xx
	Platform crossings*	X	Xx
Cued	Swim time*	—	Xx
	Swim distance	—	—
	Swim speed*	X	X

Note. * indicates a significant main effect of age in overall analysis of variance ($p < .05$). X represents a significant impairment relative to young mice ($p < .05$). x represents a significant impairment relative to middle-aged mice ($p < .05$). — represents no significant difference from the young and middle-aged groups.

Young groups. Performance improved across training in all measures, as demonstrated by significant session effects for all five measures, $F_s(4, 176) = 4.33-44.42, p_s < .005$ (Figures 2A, 2E, and 2I; Figures 3A and 3E). There was a significant main effect of enrichment for swim speed only, $F(3, 44) = 3.17, p < .05$. Post hoc tests indicated that the young wheel group swam faster than the young toy and control groups ($p_s < .05$). A significant Session \times Enrichment interaction for swim speed (Figure 2I) appears to be due to faster swim speeds for the young complex and wheel groups during the first session, which decreased over the course of training, $F(12, 176) = 2.93, p < .05$. For swim time (Figure 2A) and swim distance (Figure 2E), significant Session \times Enrichment interactions, $F_s(12, 176) = 2.03$ and 2.54 , respectively, $p_s < .05$, were driven by the fact that the young complex group exhibited longer swim times and swim distances during the first testing session relative to the other young groups. When averaged across all sessions, the young wheel group had significantly shorter swim times (Figure 2D) than controls and significantly faster swim speeds (Figure 2L) than the control and toy groups (post hoc tests, $p_s < .05$). There were no significant main effects of enrichment or Session \times Enrichment interactions for quadrant time (Figures 3A and 3D) or platform crossings (Figures 3E and 3H). However, the young wheel group made significantly more platform crossings than the young control group ($p < .05$).

Middle-aged groups. All middle-aged groups learned to find the platform, as indicated by significant session effects for all five measures, $F_s(4, 208) = 4.26-21.40, p_s < .005$ (Figures 2B, 2F, and 2J; Figures 3B and 3F). The main effects of enrichment were significant for swim time (Figures 2B and 2D), swim distance (Figures 2F and 2H), and swim speed (Figures 2J and 2L), $F_s(3, 52) = 4.70-7.91, p_s < .05$. For swim time, post hoc tests revealed that the wheel and complex groups performed significantly better than controls, and that the complex group also performed significantly better than the toy group ($p_s < .05$). For swim distance, both the wheel and complex groups performed significantly better than the control and toy groups ($p_s < .05$). Finally, for swim speed, the control and wheel groups swam more slowly than the toy and complex groups ($p_s < .05$). The wheel group also had longer quadrant times relative to controls ($p < .05$). Significant Session \times Enrichment interactions for swim time and swim distance, $F_s(12, 208) = 2.38-2.56, p_s < .05$ (Figures 2B and 2F), appear to be due to the fact that the performance of the middle-aged wheel group improved most rapidly during early training. A significant Session \times Enrichment interaction for swim speed (Figure 2J) indicated a differential change in swim speed among groups over training, $F(12, 208) = 2.31, p < .05$. Although the main effect of enrichment was not significant for platform crossings during the probe trial (Figure 3F), the Session \times Enrichment interaction was significant, likely driven by the fact that the middle-aged toy group increased platform crossings most over training, $F(12, 208) = 1.87, p < .05$. Neither the main effect of enrichment nor the Session \times Enrichment interaction was significant for quadrant time (Figures 3B and 3D).

Aged group. Significant session effects for all measures indicated that all aged groups could learn to find the platform, $F_s(4, 208) = 2.36-26.94, p_s \leq .05$ (Figures 2C, 2G, and 2K; Figures 3C and G). All three aged enrichment groups had significantly shorter swim times than aged controls, as indicated by a significant main effect of enrichment, $F(3, 52) = 3.20, p < .05$ (Figures 2C and

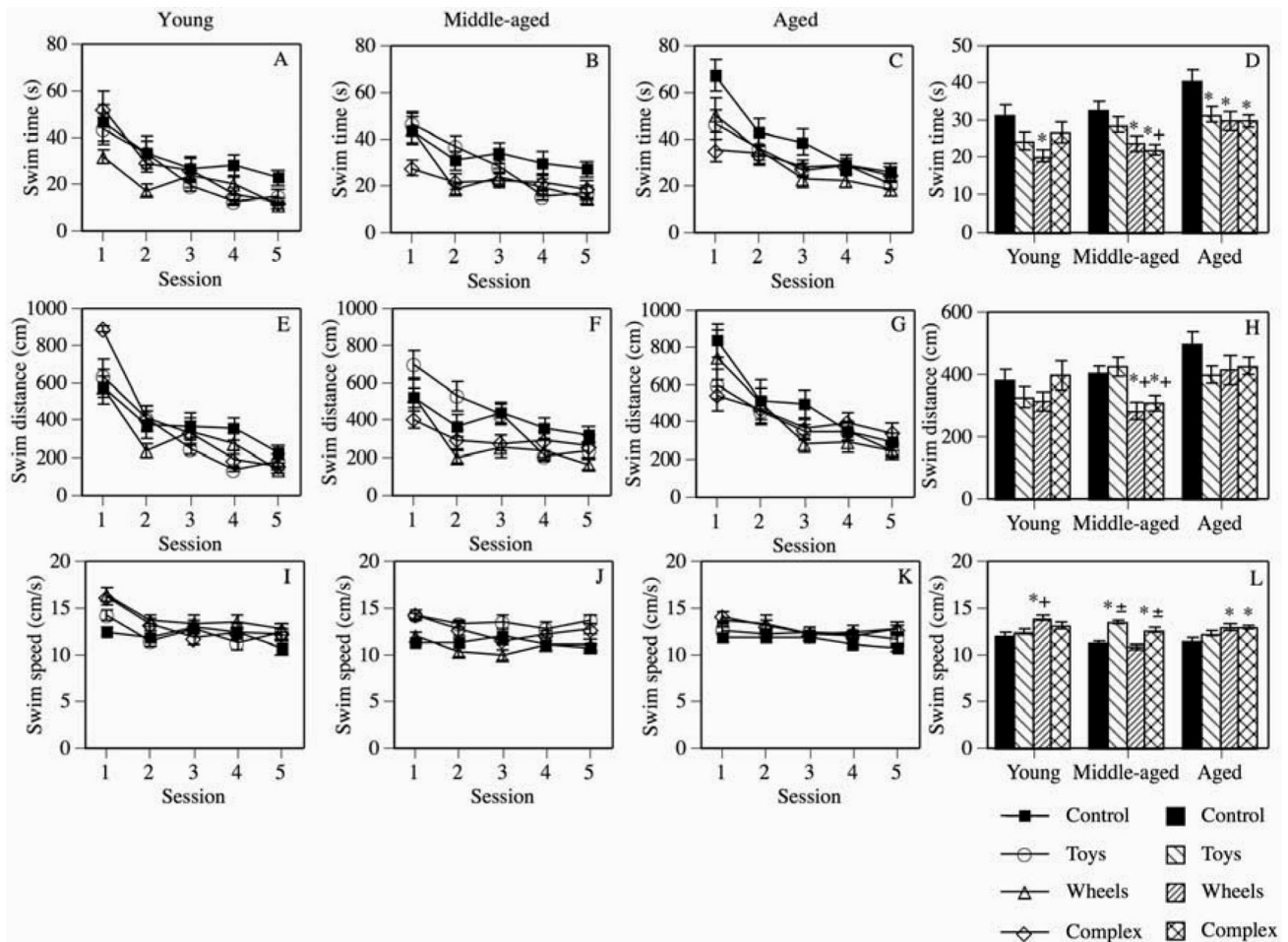


Figure 2. Performance in the spatial water maze task as illustrated for young (A, E, I), middle-aged (B, F, J), and aged (C, G, K) female mice. Each point represents the mean \pm the standard error of the mean (*SEM*) for each group during one test session. The bars in D, H, and L are means (\pm *SEM*) of all five sessions and illustrate differences among enrichment treatment groups of the same age in swim time, swim distance, and swim speed, respectively. * = significantly different from same-age controls, + = significantly different from same-age toy group, \pm = significantly different from same-age wheel group ($p < .05$).

2D), and post hoc tests ($ps < .05$). The main effect of enrichment was also significant for platform crossings, $F(3, 52) = 3.62, p < .05$ (Figures 3G and 3H), and post hoc tests indicated that aged mice in the complex group made significantly more platform crossings than aged controls during the probe trial ($p < .05$). Although the main effect of enrichment was not significant for swim speed, the aged complex and wheel groups swam significantly faster than aged controls ($ps < .05$). No other main effects of enrichment were significant for aged mice. The Session \times Enrichment interaction was significant for swim time, $F(12, 208) = 1.85, p < .05$, suggesting that aged controls had longer swim times during the first training session than the other groups. No other interactions were significant.

Cued Task

Overall effects. The main effect of age was significant for swim time and swim speed, $F_s(2, 148) = 3.01$ and 14.53, respec-

tively, $ps \leq .05$ (see Figures 4A–4C and 4I–4K), such that young and middle-aged mice had significantly shorter swim times than aged mice and young mice had significantly faster swim speeds than middle-aged and aged mice (Table 1). Swim distance was not affected by age (Figures 4E–4G). The main effect of enrichment was significant for swim speed, $F(3, 148) = 3.35, p < .05$, indicating that enrichment increased swim speed in the cued task. Performance improved throughout testing, as indicated by significant session effects for swim time, swim distance, and swim speed, $F_s(2, 296) = 108.95$ – $234.98, ps < .05$. There were significant Session \times Enrichment interactions for swim time and swim speed, $F_s(6, 296) = 3.14$ and 10.67, respectively, $ps < .05$, such that the toy groups decreased swim time and increased swim speed most over the course of training. No other interactions were significant.

Young groups. Enrichment did not affect the performance of young mice in the cued task, as indicated by nonsignificant main

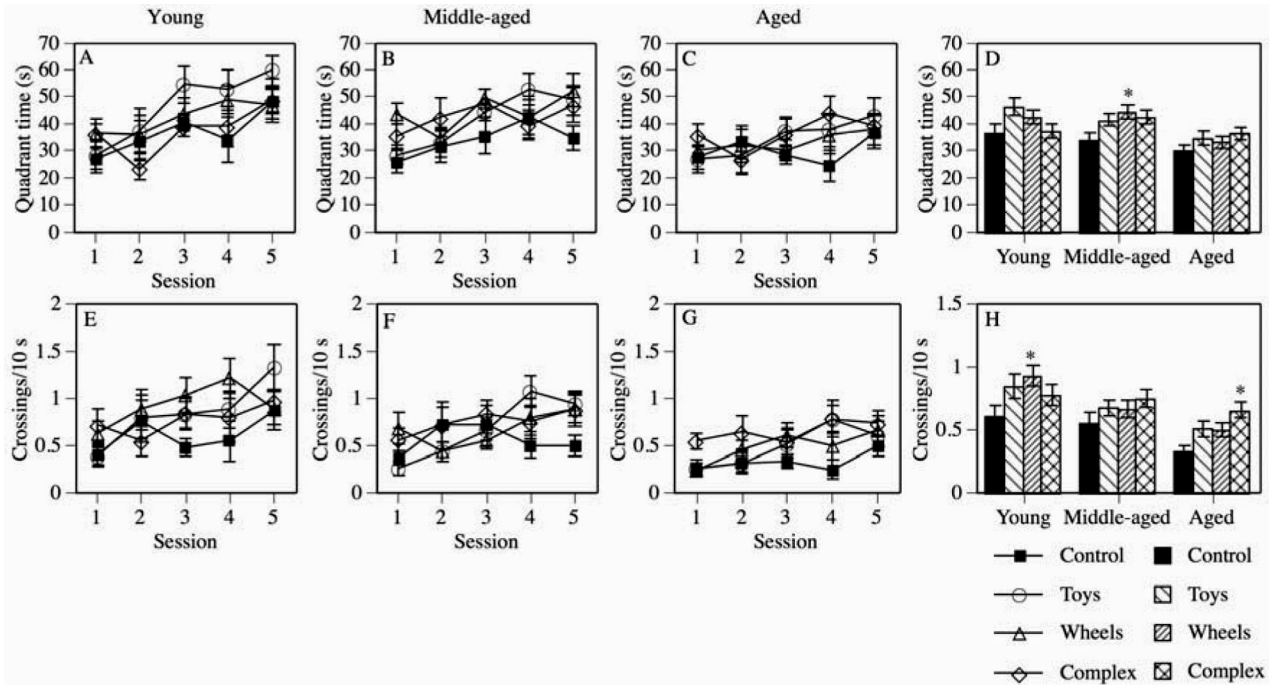


Figure 3. Performance in the spatial probe trials as illustrated for young (A and E), middle-aged (B and F), and aged (C and G) mice. Each point represents the mean \pm SEM for each group during one test session. The bars in D and H are means (\pm SEM) of all five sessions and illustrate differences among enrichment treatment groups of the same age in quadrant time and platform crossings, respectively. * = significantly different from same-age controls, + = significantly different from same-age toy group, \pm = significantly different from same-age wheel group ($p < .05$).

effects for all three measures ($ps > .05$, Figures 4D, 4H, and 4L). Significant main effects of session for swim time, swim distance, and swim speed suggested that performance improved over the course of training, $F_s(2, 88) = 28.11\text{--}60.94$, $ps < .001$ (Figures 4A, 4E, and 4I). A significant Session \times Enrichment interaction for swim speed was due to the fact that the toy and control groups increased swim speed during training, $F(6, 88) = 2.94$, $p < .05$. No other interactions were significant.

Middle-aged groups. The main effects of enrichment were significant for swim time and swim speed, $F_s(3, 52) = 3.79$ and 4.65, respectively, $ps < .05$ (Figures 4B, 4D, 4J, and 4L). Post hoc tests indicated that both the toy and complex groups had shorter swim times and faster swim speeds relative to those of the wheel group ($ps < .05$, Figures 4D and 4L). The toy group also swam faster than controls ($p < .05$). Performance in middle-aged mice improved over training, as suggested by significant session effects for swim time, swim distance, and swim speed, $F_s(2, 104) = 39.34\text{--}127.12$, $ps < .05$ (Figures 4B, 4F, and 4J). There was a significant Session \times Enrichment interaction for swim speed, indicating that the toy group increased swim speed most rapidly over the course of training, $F(6, 104) = 5.80$, $p < .05$. No other interactions were significant.

Aged group. The main effect of enrichment was not significant for any cued task measure, indicating that all aged groups performed similarly on this task ($ps > .05$, Figures 4D, 4H, and 4L). Significant session effects for all measures demonstrated that cued task performance improved over training, $F_s(2, 104) = 44.27\text{--}$

74.65, $ps < .001$ (Figures 4C, 4G, and 4K). Aged toy and complex groups decreased swim distance most over training, as suggested by a significant Session \times Enrichment interaction for swim distance, $F(6, 104) = 2.40$, $p < .05$. There was also a significant Session \times Enrichment interaction for swim speed, $F(6, 104) = 3.41$, $p < .05$, driven by the fact that the control and toy groups increased swim speed most throughout training and that the wheel group swam faster than controls early in training. No other interactions were significant.

Discussion

The present study is the first to report that single enrichment factors differentially improve spatial memory in young, middle-aged, and aged mice. In young females, performance in the spatial water maze was enhanced only by exercise. In middle-aged females, exercise alone and complex enrichment were similarly effective in improving spatial task performance, whereas all enrichment treatments in aged females improved some aspect of spatial performance.

Exercise alone significantly improved spatial swim time and increased platform crossings in young females. However, exercise also increased swim speed, which might indicate that differences in swim time and platform crossings were due to increased swim speeds. Nevertheless, the fact that exercise also tended to improve other cognitive measures such as swim distance (Figure 2H) and quadrant time (Figure 3D) but had no effect on cued performance

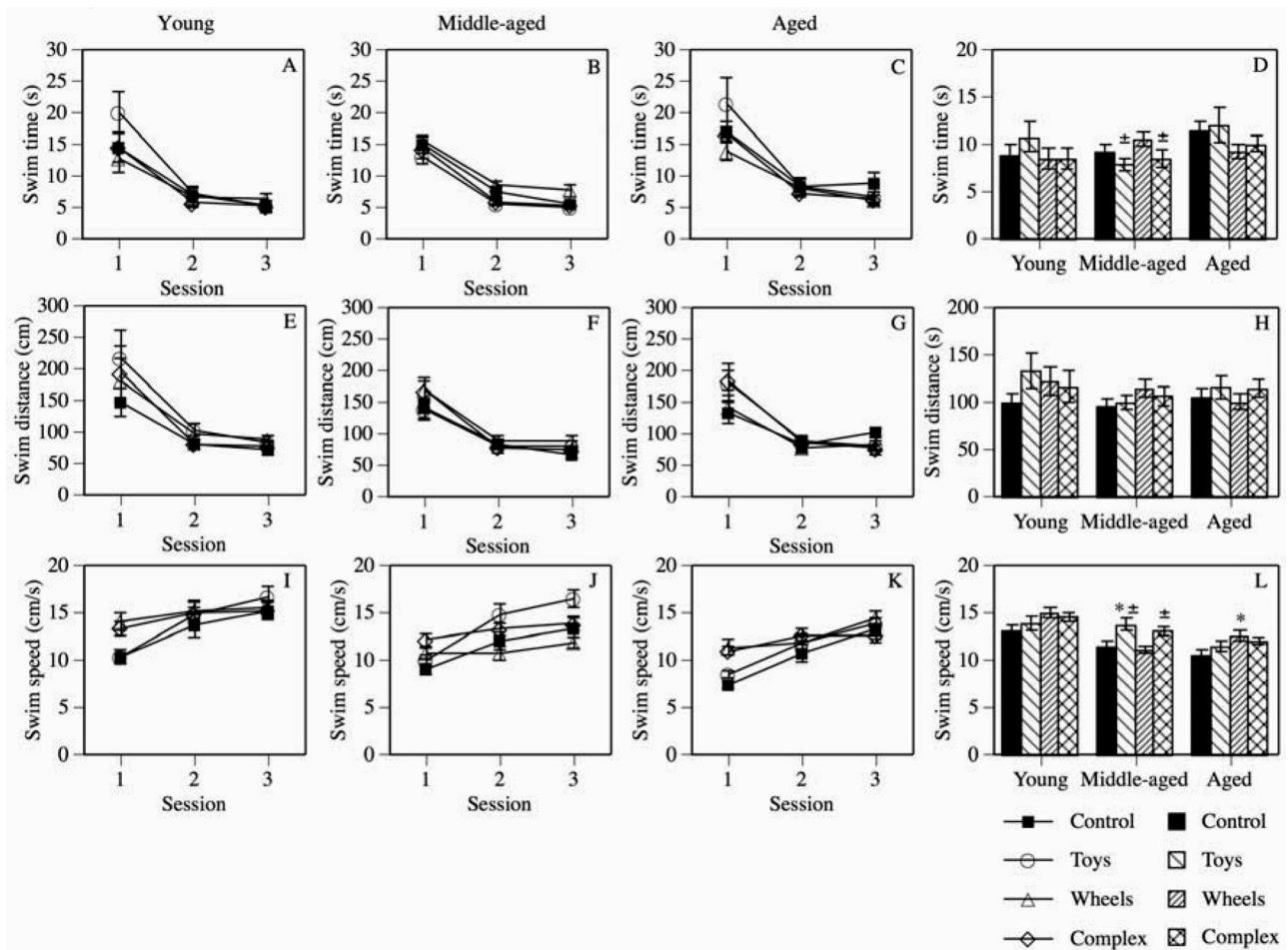


Figure 4. Performance in the cued water maze task as illustrated for young (A, E, D), middle-aged (B, F, J), and aged (C, G, K) female mice. Each point represents the mean \pm SEM for each group during one test session. Significant differences between same-aged enrichment treatment groups in swim time, swim distance, and swim speed are illustrated in Panels D, H, and L, respectively. Each bar represents the mean \pm SEM for each group for all three sessions. * = significantly different from same-age controls, + = significantly different from same-age toy group, \pm = significantly different from same-age wheel group ($p < .05$).

suggests that the beneficial effects of exercise on swim time reflect an improvement in memory for the platform location rather than an overall increase in swim speed. Specifically, the fact that exercise significantly increased platform crossings but not quadrant time suggests that exercise enhanced memory for the exact platform location more than knowledge of the general platform location. This finding is consistent with previous studies from other labs in which exercise improved spatial water maze performance in young male and female mice (Fordyce & Wehner, 1993; van Praag et al., 1999). These data are also consistent with a previous study from our lab that demonstrated enhanced working memory in young female mice that received exercise alone, but not in those that received cognitive stimulation alone or acrobatic (i.e., obstacle course) training (Lambert et al., 2005). Together, these data indicate that spatial memory in young female mice benefits more from exercise than from cognitive stimulation or complex enrichment. Indeed, compared with complex enrichment, exercise in young female mice also enhances extinction to a conditioned tone response (Pietropaolo, Feldon, Alleva, Cirulli, & Yee, 2006).

Nevertheless, it is interesting that complex enrichment did not improve performance in any spatial measure of the Morris water maze in young females. These results differ from those previously reported in enriched male and female rats (Leggio et al., 2005; Nilsson et al., 1999; Williams et al., 2001) and female mice (Williams et al., 2001) tested on the same task. There are several possible reasons for this discrepancy. For example, controls in the present study were socially housed, whereas controls in Nilsson et al. (1999) were individually housed. The effects of complex enrichment on memory and neural morphology in young rodents are typically more robust when compared with effects in isolated controls rather than in social controls (Hellemans, Bengel, & Olmstead, 2004; Rosenzweig et al., 1978), so housing conditions likely contributed to the discrepancy. Also, the present study enriched 3-month-old mice for 4 weeks before testing, whereas previous studies enriched younger animals (Leggio et al., 2005) and for longer amounts of time (Leggio et al., 2005; Nilsson et al., 1999; Williams et al., 2001). The longer duration of complex enrichment in previous studies may also have resulted in greater enrichment-

induced enhancements in water maze performance. Nevertheless, the fact that complex enrichment in the present study did not improve memory in young mice is surprising given that mice that received complex enrichment also received exercise. However, the number of running wheels available to complex-enriched mice was three times less than that available to exercise-enriched mice, so there may have been an insufficient number of running wheels available in the complex environment for exercise to improve spatial maze performance in young mice.

In middle-aged mice, both exercise alone and complex enrichment increased spatial swim time and swim distance (Figures 2B and 2F), and exercise also increased quadrant time (Figure 3D). In contrast, cognitive stimulation alone had no beneficial effect on performance in the spatial task. These data suggest that exercise is the most critical aspect of environmental enrichment for the purpose of improving spatial memory in middle-aged female mice. The fact that exercise alone improved quadrant time, but not platform crossings, suggests that exercise primarily improved general knowledge for the platform location rather than for the exact platform location. A previous study in middle-aged male mice also reported that voluntary exercise for 2 hrs/day improved passive avoidance (Samorajski et al., 1985). Further, complex enrichment has been shown to enhance spatial memory in the Morris water maze in middle-aged male (Frick et al., 2003) and female (Frick et al., 2003; Kempermann et al., 1998) mice, as well as in middle-aged female rats (Pham et al., 1999), and our present data indicate that exercise is the key contributor to this effect. However, compared with exercise alone, both cognitive stimulation and complex enrichment increased swim speed in the spatial task and affected both swim time and swim speed in the cued task. These data suggest that cognitive stimulation, either alone or in combination with exercise, may have generally improved task performance (e.g., sensory and/or motor abilities), whereas exercise more specifically improved spatial memory. If so, it is unclear how cognitive stimulation would have affected general task performance independent of memory. This will need to be addressed in future studies. Nevertheless, the data support a role for exercise and cognitive stimulation in improving task performance.

In aged mice, all three enrichment treatments improved spatial swim time and complex enrichment also increased platform crossings. Because all groups spent similar amounts of time in the quadrant containing the collapsed platform, a significant increase in platform crossings indicated that complex enrichment enhanced memory for the exact platform location. Swim speed was affected by exercise and complex enrichment in the spatial task, but only by exercise in the cued task. As such, this may indicate that wheel- and complex enrichment-induced improvements in spatial task performance were due to faster swim speeds. However, the fact that complex enrichment also improved the more challenging platform crossings measure suggests that this treatment was specifically beneficial to spatial memory in aged mice. We have previously reported that 3 hr/day of a less sophisticated complex enrichment treatment in aged female mice improved spatial memory in the Morris water maze (Frick & Fernandez, 2003). The present study extends these findings by showing that 24 hr/day of complex enrichment can also improve spatial water maze performance in aged females and that cognitive stimulation alone and exercise alone can also enhance some aspect of spatial task performance in aged females.

Because the ovaries of mice in the present study were intact, young and some middle-aged mice (approximately 20%; Frick et al., 2000) experienced fluctuating levels of endogenous hormones during the 8 days of spatial and cued water maze testing. The estrous cycle was not monitored in the present study because the stress of daily vaginal lavage may have interfered with the effect of enrichment. However, it is unlikely that hormone fluctuations contributed to the observed enrichment effects because the results of studies examining the effects of the estrous cycle on spatial water maze performance have been inconsistent (Berry, McMahhan, & Gallagher, 1997; Frick & Berger-Sweeney, 2001; Frye, 1995; S. G. Warren & Juraska, 1997) and suggest that any effects of the cycle on spatial memory are subtle at best. Effects of high levels of estrogen (improvement in Frick & Berger-Sweeney, 2001; impairment in S. G. Warren & Juraska, 1997) have been seen only in 1-day water maze tasks. Therefore, the long (5 days of spatial and 3 days of cued) water maze testing protocol employed here would have mitigated any effects of ovarian hormones on task performance. Further, the effects of enrichment on memory reported in the literature are far more robust than those of the estrous cycle. As such, it is unlikely that endogenous hormone fluctuations in the present study had any influence on the enrichment treatments used in this study.

The mechanisms by which enrichment enhances cognitive function remain unknown. Complex enrichment has been shown to increase neocortical neuron size (Diamond, 1967), cortical thickness (Diamond, Krech, & Rosenzweig, 1964), and size of the synaptic contact area (West & Greenough, 1972). In the hippocampus, enrichment produces a number of changes such as increased neurogenesis (Kempermann, Kuhn, & Gage, 1997; Segovia et al., 2006), synaptophysin levels (Frick & Fernandez, 2003), and long-term potentiation (Duffy et al., 2001). Enrichment can also increase hippocampal CREB immunoreactivity (Williams et al., 2001), thus potentially enhancing memory through activation of second messenger pathways. In a previous report from our laboratory, cognitive stimulation alone and voluntary exercise alone increased synaptophysin levels in the frontoparietal cortex and hippocampus of young female mice (Lambert et al., 2005). However, another study reported increases in dendritic volume and length of CA1 pyramidal neurons in complex-enriched mice, but not in mice that received voluntary exercise alone (Faherty, Kerley, & Smeyne, 2003). Nevertheless, voluntary exercise in aged mice has been shown to increase synaptophysin and GAP-43 expression in the hippocampus (Chen et al., 1998). On the basis of the present results, future studies should investigate the enrichment-induced neurobiological changes associated with enhanced memory in single enrichment paradigms. On one hand, the fact that exercise alone was sufficient to enhance spatial water maze performance in young mice may indicate that vascular changes in the brain are the underlying cause of enrichment-enhanced memory in young mice. Indeed, exercise alone has been shown to increase brain blood perfusion and capillary growth in young adult rats (Swain et al., 2003). On the other hand, because exercise and cognitive stimulation both enhanced spatial task performance in aged mice, either vascular or dendritic changes may result in memory improvements in aged mice, and studies have reported enhanced hippocampal neurogenesis in complex-enriched middle-aged mice (Kempermann et al., 1998) and aged rats (Segovia et al., 2006). Future studies in young, middle-aged,

and aged rodents should address whether the various treatments used here produce vascular and dendritic changes that parallel the observed behavioral results (i.e., vascular changes only in young vs. both vascular and dendritic changes in aged).

In conclusion, results from the present study suggest that single enrichment variables produce different effects on spatial memory in young, middle-aged, and aged mice. In young mice, only exercise alone improved performance in the spatial water maze, whereas both exercise alone and complex enrichment improved water maze performance in middle-aged mice. In aged mice, all enrichment treatments improved spatial task performance. As such, the type of enrichment should be carefully considered when planning studies in multiple age groups and when investigating the neural basis of enrichment. The results from aged mice demonstrate that although cognitive stimulation and exercise alone can be sufficient to enhance spatial memory in senescence, the combination of these elements can improve more aspects of spatial task performance. If applicable to older humans, these data may suggest that enrichment initiated at any age can significantly improve memory function. The data also suggest that either cognitive stimulation or exercise alone can be beneficial in middle and old age, and that the combination of both cognitive stimulation and exercise offers the most robust and widespread benefits for memory function.

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Received December 31, 2006

Revision received March 26, 2007

Accepted March 27, 2007 ■