European Review for Medical and Pharmacological Sciences 2019; 23: 8058-8067

Group housing with young mice relieves Alzheimer's disease behaviors in aging mice

Y.-L. ZHANG[1], J. ZHANG[1], H. LU[2], J. TANG[3], J.-L. LUO[3], H.-P. LONG[4], J.-C. LI[4], S.-L. SHI[1]

[1]Department of Neurology, The Second Affiliated Hospital of Guangxi Medical University, Nanning, China
[2]Department of Neurology, Southern Medical University, Guangzhou, China
[3]Department of Neurology, The Second Affiliated Hospital of Guangxi Medical University, Nanning, China
[4]Department of Neurology, Guangxi Medical University, Nanning, China

Abstract. – OBJECTIVE: Due to the increase in human life expectancy, a higher number of individuals are experiencing age-related cognitive impairments. Therefore, it is important to investigate the methods to tackle the effects of aging.

MATERIALS AND METHODS: The mice were divided into the following groups: the aging mice (male, 20 months) and young mice (male, 2 months) were pairing-housed together in the same cage and lasted for at least one month (Aging-Young). The following tests were performed for the mice in different groups: Open field test, Morris water maze (MWM) test, Contextual fear conditioning test, Novel Object Recognition (NOR) test, Pain threshold test, Olfactory habituation/dishabituation test, T-maze test, Electrophysiological recordings.

RESULTS: In this study, we housed aging and young mice together, and found that the paired housing for one-month improved the learning and memory of the aging mice. These mice exhibited better performance on the Morris water maze (MWM) test, a longer freezing duration in the contextual fear conditioning test, a higher alternation rate in the T-maze test, and an increased preference for novel objects in the novel object recognition (NOR) test. The paired housing with young mice also improved the impaired long-term potentiation (LTP) in aging mice.

CONCLUSIONS: Our results suggest that the paired housing with young mice has beneficial effects on learning and memory of aging mice. The manipulation of the systemic environment may, therefore, provide a new strategy for aging rejuvenation.

Key Words:
Aging, Cognitive deficits, Paired housing, Learning and memory, Long-term potentiation.

Introduction

Aging is a universal process that affects the global population. It is often accompanied by cognitive impairments and consequently can lead to the development of degenerative disorders in healthy individuals[1]. According to a report by the United Nations, the number of individuals worldwide aged 60 and above is predicted to more than double in the next 35 years, reaching almost 2.1 billion by 2050[2]. Aging is often accompanied by cognitive decline and dementia, which may then develop into Alzheimer's disease and other forms of neurodegenerative diseases. These diseases affect almost 50% of individuals aged 85 and older in the United States[3]. Furthermore, with the average age of the population gradually rising, this startling number will only continue to increase. Therefore, it is important to identify the methods to maintain cognitive integrity, which can protect against, or even counteract, this aging process in the aging population.

For a long time, the aging process has been considered irreversible[4]. However, over the past 30 years, this hypothesis has been challenged, and some investigators have provided powerful evidence that the aging process can be affected by several factors. These factors include the experiences that an organism has with its environment[5,6] and the genetic composition of the organism[7], indicating that the aging process is, in fact, not a definitive process but instead is open to manipulation. Furthermore, some studies[8,9] have shown that the exposure of an aged animal to young blood can improve the stem cell function in muscle, the liver[8], the spinal cord[10], and the brain[11],

Corresponding Author: Shengliang Shi, MD; e-mail: ssl_1964@163.com

and can also ameliorate the cardiac hypertrophy[12]. In addition, the young blood has been shown to counteract and reverse the pre-existing effects of brain aging at a molecular, structural, functional, and cognitive level[13]. From these findings, we can conclude that the systemic manipulations may be effective mediators in confronting aging. However, the rejuvenation of the aging brain using other methods remains a challenge in neuroscience research.

Therefore, this study aimed to investigate whether the paired housing of aging and young mice can improve the learning and memory ability of aging mice, demonstrating the rejuvenation of the aging brain.

Materials and Methods

Animals

This study was approved by the Animal Ethics Committee of Guangxi Medical University Animal Center. C57BL/6J male mice were housed in the standard laboratory cages at 24 ± 1°C. The mice were maintained on a 12-h light/dark cycle with lights on at 8:00 am and were feed with the standard food and water ad libitum. The behavioral testing was performed between 10:00 am and 5:00 pm. All procedures were conducted in accordance with the Chinese Council on the Animal Care Guidelines, and some efforts were made to minimize animal suffering and to reduce the number of animals used. The mice were normally housed (5 mice/group) before being divided into groups. The aging mice (male, 20 months) and young mice (male, 2 months) were housed together in the same cage for 1 month (Aging-Young). As controls, two male aging mice were housed in one cage (Aging-Aging), and two young male mice were housed in another cage (Young-Young) for the same period (Figures 1A and 1B).

Open Feld Test

The open field test, conducted as described previously[14], was performed in a rectangular chamber (40 × 40 × 30 cm) made of gray polyvinyl chloride. The mice were gently placed at the center of the testing chamber for a 5-minute recording pe-

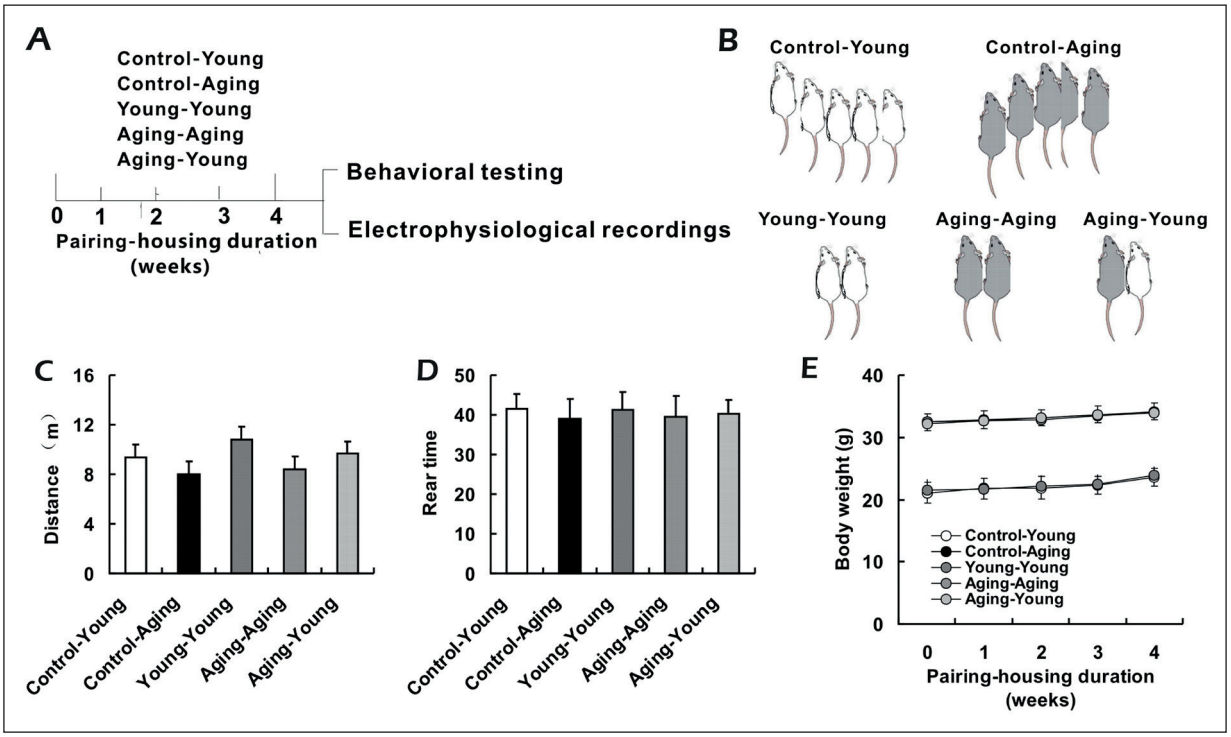

Figure 1. Paired housing with young mice did not affect locomotor activity or body weight. **A,** Schematic of the duration of the paired housing paradigm and experimental tests. **B,** Schematic of the paired housing conditions in every group. **C, D,** The paired housing treatment had no effect on locomotor activity in the open field test. **E,** Body weight is similar between the Aging-Aging and Aging-Young groups. n = 12/group. Data are presented as the mean ± SE. **C,** and **D,** = One-way ANOVA; **E,** = repeated measures two-way ANOVA.

riod, where their movement was monitored by an automated video tracking system. The digitized image of the path was then analyzed automatically using EthoVision 11.0 software.

Morris Water Maze Test

The Morris water maze (MWM) test was conducted as reported previously[15] with slight modifications. The training trials were conducted on day 1 to day 4, following group housing. For all trials and sessions, the platform (10 cm diameter, 1 cm below the water) was placed in the same position. We randomized the starting quadrant, and it was conducted in the same order for all animals. We recorded the time taken for the mice to reach the hidden platform and their swimming speed. Once they found the hidden platform, the animals could rest for 30 seconds on the platform between trials. However, if they failed to reach the platform in 120 seconds, the investigator guided the mouse to the platform. Each animal underwent this test on 4 consecutive days, comprising four trials (NE; NW; SE; SW) per day. On day 5, for the probe trial, we removed the platform, and the total distance travelled, time spent in the target quadrant, and the number of platform crossings were all monitored for 60 seconds. Two hours later, the platform was replaced, and the ability of the animals to locate it within 60 seconds was assessed.

Contextual Fear Conditioning Test

The contextual fear conditioning test was conducted as follows[14]: first, the mice were allowed to freely explore the apparatus for 3 minutes. During the training test, we placed the mice in conditioning chamber A, and the mice were then exposed to tone-footshock pairings (tone - 30 s, 80 dB; foot shock - 1 s, 0.4 mA), with an interval of 80 seconds. Twenty-four hours later, the mice were returned to chamber A to evaluate the contextual fear learning. Two hours later, we performed the cued fear conditioning in novel chamber B for 3 minutes. We monitored the freezing response of the mice during training and testing using Med Associates Video-Tracking and Scoring software.

Pain Threshold Test

The pain threshold test was performed as previously reported[16]. Each animal was put into chamber A and given 11 repeated shocks of various intensities (from 0.10 to 0.60 mA). These shocks lasted 1 second, and the intershock intervals lasted at least 2 minutes. When we observed the mouse curling up its feet, this was defined as a flinching event. When the mouse made an audible squeak, this was defined as vocalization, and when it propelled itself off the floor, it was defined as jumping.

Novel Object Recognition Test

The novel object recognition (NOR) test was conducted as previously described[15]. The NOR apparatus consisted of a rectangular chamber (33 × 33 × 20 cm) made of black polyvinyl chloride. Each mouse was habituated to the empty arena for 5 minutes the day before the familiarization session. During the familiarization session, two identical objects (towers of Lego bricks or Falcon tissue culture flasks filled with sand) were placed 5 cm away from the walls. The mice were allowed to explore each object freely until they reached 20 seconds of the total exploration time or when a 10 minutes period was over. Twenty-four hours later, one familiar object and one novel object were placed in the arena. Again, the experiment was stopped when there had been a 20-second exploration of both objects, and when a 10-minute period was over. The discrimination ratio was subsequently calculated to measure the mouse's recognition memory. This was calculated using the formula: time spent exploring novel object/ total exploring time.

Olfactory Habituation/Dishabituation Test

The olfactory habituation/dishabituation test was performed in a chamber consisting of an open-top plastic box (12 × 12 × 26 cm) with a recessed odor port to provide odorant delivery at one side of this box. The mice were tested once a day in a single session lasting around 45 minutes, and 2-3 sessions were performed per animal. The animals were presented with three odorants three consecutive times for a duration of 120 seconds, followed by a 1-2-minute intertrial interval (ITI). To ensure the robust habituation to the test stimulus, each odorant was presented three times. The odorants used here were almond, banana, and the excreta of a different animal (for example, the C57Bl/6J or B6.129S6 mice) and were presented in the form of a 0.5% saturated vapor. We recorded the time spent sniffing the tip during each 2-minute odorant exposure period[17].

T-maze Test

The T-maze test was performed using a T-shaped elevated maze with a start arm (3×10 cm) and two-goal arms (30×10 cm)[18]. During

training, both goal arms were opened, and the mice were placed in the start arm and allowed to freely explore the maze for 20 minutes. Once the mice entered the start arm, we closed the choice door, restricting access to one of the goal arms. After one minute, the mice were removed from the maze. The retention test was then conducted five minutes later. Both goal arms were open during this test, and the mice were faced away from the choice point at the end of the start arm and allowed to freely explore for 5 minutes. The new arm preference of the mice was then calculated using the formula [(new arm)/(new and old).]

Electrophysiological Recordings

This study's protocol was based on the previous studies[14]. In brief, the mice were anesthetized with pentobarbital and subsequently decapitated, and their brains were quickly removed and placed in ice-cold oxygenated modified artificial cerebrospinal fluid (ACSF) containing 195 mM sucrose, 2 mM KCl, 0.2 mM $CaCl_2$, 12 mM $MgSO_4$, 1.3 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose. The medial prefrontal cortex (mPFC) slices (300 μm thickness) were prepared using a VT-1200S vibratome (Leica, Wetzlar, Germany) and subsequently transferred to a storage chamber containing normal ACSF (126 mM NaCl, 3.0 mM KCl, 1.25 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, 26 mM $NaHCO_3$, and 10 mM glucose) for a 30 minute recovery period at 34°C and subsequently stored at room temperature (25 ± 1°C) for an additional 2-8 hours. We measured the initial slope of field excitatory postsynaptic potentials (fEPSPs) as the strength of the synaptic transmission. The long-term potentiation (LTP) was induced by one train of electrical stimulation delivered at 100 Hz. The level of LTP was then determined at 30-60 minutes following this tetanic stimulation.

Statistical Analysis

In the experimental data, the *t*-test was used to compare the means of two independent samples, and a One-way analysis of variance (ANOVA) followed by Post Hoc Test (Least Significant Difference) was used to compare the means of multiple groups of samples using the Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA). The number of experimental animals is indicated by "n.". Unless stated, the mean values shown in the text and figures are expressed as the mean ± standard error of the mean (SEM). $p < 0.05$ was considered statistically significant. GraphPad Prism 6.0 software (La Jolla, CA, USA) was used to draw the graph.

Results

Behavioral Test Results

Open Field Test

Firstly, we examined the locomotor activity of each group and found that the paired housing with young mice for one month had no effect on the total path length ($F_{4,55} = 2.154$; $p = 0.532$; Figure 1C) or rearing time ($F_{4,55} = 2.432$; $p = 0.713$; Figure 1D). The body weight was also not different in aging mice housed with young mice compared to those housed with aging mice ($t = 3.542$, $p = 0.465$; Figure 1E).

Morris Water Maze Test

The MWM test was used to examine hippocampal learning and memory[19]. The control mice (Control-Young and Young-Young) reached the hidden platform in a faster time during training. The aging mice (Control-Aging and Aging-Aging) took significantly longer to reach the platform ($F_{4,55} = 1.458$; $p = 0.006$; Figure 2A), verifying the fact that these elder mice (20 months of age) exhibited deficits in learning and memory[1]. Compared to the Aging-Aging mice, the Aging-Young mice took significantly less time to find the hidden platform ($F_{4,55} = 1.458$; $p = 0.006$; Figure 2A). In the probe test, compared to the Young-Young mice, the Aging-Aging mice spent significantly less time in the target quadrant ($F_{4,55} = 2.918$; $p = 0.005$; Figure 2B), swam a reduced distance in the target quadrant ($F_{4,55} = 2.018$; $p = 0.006$; Figure 2C), and demonstrated a significantly reduced number of platform crossings ($F_{4,55} = 1.148$; $p = 0.007$; Figure 2D). Overall, the paired housing with young mice was, therefore, seen to rescue the memory deficits in elder mice (Figures 2B-2D). To validate our findings, we tested the sensory responses during the MWM. We found no significant difference in the average swimming speed ($F_{4,55} = 0.866$; $p = 0.462$; Figure 2E) or escape latency ($F_{4,55} = 0.675$; $p = 0.682$; Figure 2F) among the groups during the probe trial where the platform was removed. These results clearly show that the paired housing with young mice improved learning and memory performance in aging mice.

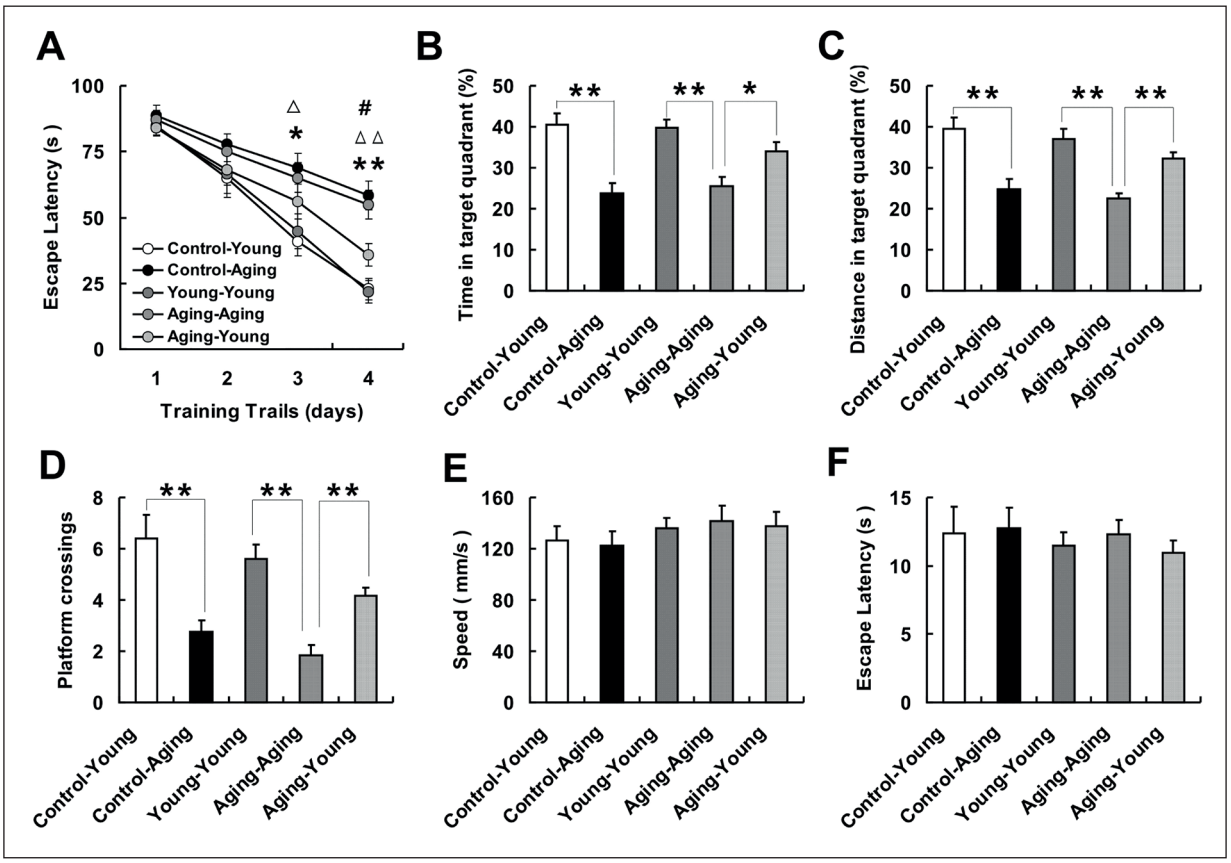

Figure 2. Paired housing with young mice improved the learning and memory ability of aging mice in the MWM test. The aging mice that shared housing with young mice spent a shorter time searching for the platform (**A**) *indicates the differences between Control-Young and Control-Aging groups, Δ indicates the differences between Young-Young and Aging-Aging groups, #indicates the differences between Aging-Aging and Aging-Young groups. We spent more time in the target quadrant (**B**), swam a greater distance in the target quadrant (**C**), and crossed the target quadrant a greater number of times (**D**), compared to aging mice that shared housing with other aging mice. However, paired housing did not affect swimming speed (**E**) or escape latency (**F**) after the platform was removed in the MWM. n = 12/group. The data are presented as the mean ± SE. *p<0.05, **p<0.01; **A,** = repeated measures Two-way ANOVA; **B, C, D, E,** and **F,** = One-way ANOVA.

Contextual Fear Conditioning Test

In the contextual fear conditioning test, we observed a reduction in freezing the duration in the Control-Aging and Aging-Aging mice compared to the Control-Young and Young-Young mice. The paired housing with young mice for one mouth was shown to significantly increase the freezing duration in aging mice ($F_{4,55}$ = 4.468; p= 0.004; Figure 3A). Twenty-four hours after fear conditioning, aging mice exhibited 45-50% reduced freezing durations compared to young mice. In the Aging-Young group, the paired housing was shown to relieve this behavior deficit. Freezing durations were 66.63 ± 5.23, 35.97 ± 5.79, 64.18 ± 6.24, 33.01 ± 6.68, 52.47 ± 4.94, respectively ($F_{4,55}$ = 2.908; p = 0.005; Figure 3B). To validate our findings, we tested the sensory responses during this behavior. We found that there was no significant difference in the pain threshold (as an index of pain sensitivity; $F_{4,55}$ = 2.024; p = 0.367; Figure 3C) or pre-tone freezing behaviors (as an index of baseline startle response; $F_{4,55}$ = 3.567; p = 0.352; Figure 3B). These results also indicate that the paired housing with young mice had a beneficial effect on learning and memory in aging mice.

T-Maze and NOR Test

T-maze and NOR are additional animal behavioral tests used to assess learning and memory[18,20]. In the T-maze test, we found that the Control-Aging and Aging-Aging mice had a lower alternation rate than the Control-Young and Young-Young mice. The results of the Aging-Young mice demonstrated that the paired housing rescued

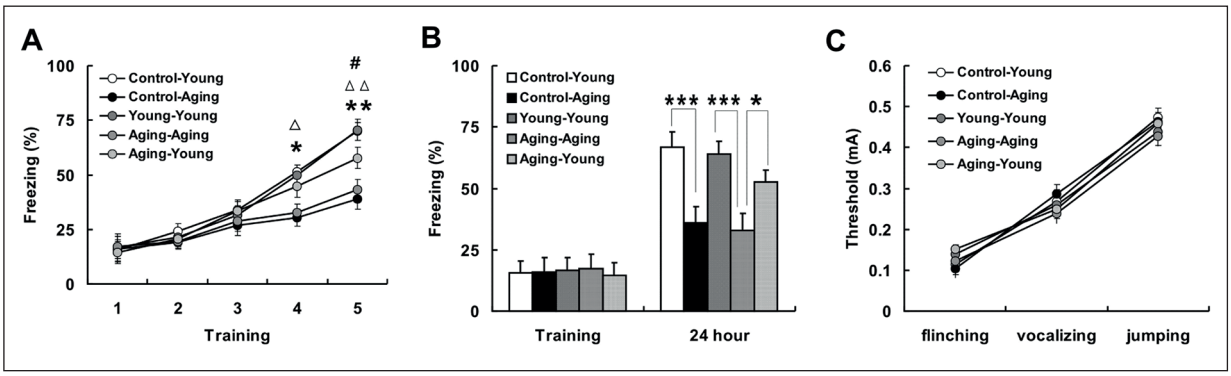

Figure 3. Paired housing with young mice improved learning and memory ability in aging mice in the contextual fear conditioning test. **A,** This paired housing treatment with young mice also had a beneficial effect on the learning process in the training test in aging mice. **B,** The paired housing with young mice increased the freezing duration in aging mice 24 hours after training. **C,** There was no significant difference in pain threshold observed. n = 12/group. The data are presented as the mean ± SE. *p<0.05, **p<0.01, ***p<0.001; A and C = repeated measures two-way ANOVA; B = One-way ANOVA.

these behavioral deficits. The alternation rates were 76.83 ± 4.93, 48.94 ± 3.93, 78.76 ± 4.31, 51.63 ± 5.34, 70.10 ± 7.13, respectively ($F_{4,55}$ = 3.543; p=0.012; Figure 4). To validate our findings, we tested the sensory responses during this behavior and found no significant difference in the olfactory responses ($F_{4,55}$ = 24.328; p = 0.753; Figure 5A). In the NOR test, while the Aging-Aging mice exhibited a lack of preference, the Aging-Young group exhibited a preference to the novel object over the familiar object: 75.86 ± 2.35, 49.02 ± 2.13, 73.76 ± 4.30, 50.00 ± 3.35, 66.10 ± 4.13, respectively ($F_{4,55}$ = 4.678; p = 0.026; Figure 5B).

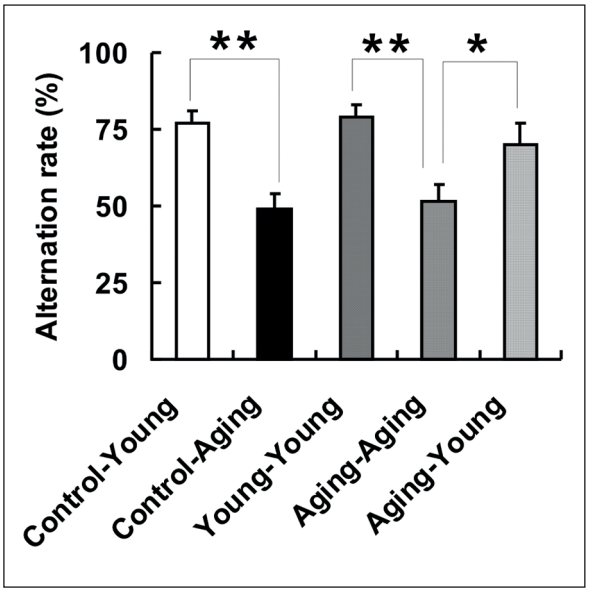

Figure 4. Paired housing with young mice improved the memory ability in aging mice in the T-maze test. Aging mice housed with young mice exhibited higher alternation rates than aging mice housed with aging mice. n = 12/group. The data are presented as the mean ± SE. *p<0.05, **p<0.01, One-way ANOVA.

Electrophysiological Results

LTP induction

Hippocampal LTP is thought to be the cellular mechanism underlying learning and memory[21]. We compared LTP induction in mice groups and found a significant difference between all groups (Figures 6A, 6B). In the HFS-induced LTP, the slope of fEPSPs was 155.50 ± 3.70, 110.50 ± 2.74, 153.59 ± 1.71, 102.58 ± 2.54, 134.20 ± 3.10, respectively ($F_{4,25}$ = 5.258; p < 0.0001; Figure 6C). Compared to the young mice, LTP was unable to be induced in the slices obtained from the aging mice. Therefore, these results show that the paired housing with young mice can rescue LTP impairment in aging mice.

Pre-Synaptic Mechanism

We, then, investigated paired-pulse facilitation (PPF), which is accepted as an efficient model to test the changes within the presynaptic terminal[22,23]. We found that, while the aging mice exhibited a decrease in PPF compared to the young mice, paired housing with young mice can rescue this decreased PPF, suggesting that the paired housing is able to induce the presynaptic effects ($F_{4,25}$ = 4.964; p = 0.031; Figure 7A). We, then,

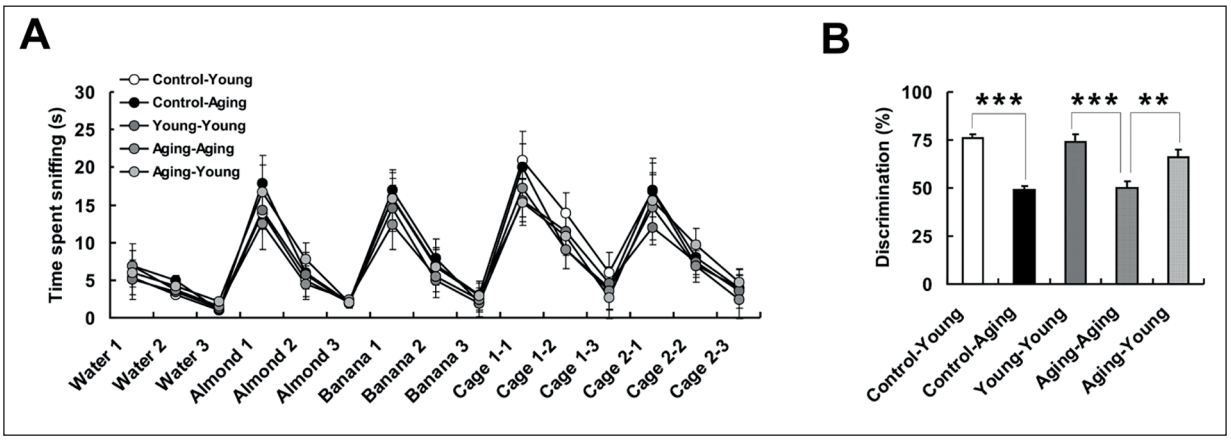

Figure 5. Paired housing with young mice improved the memory ability in aging mice in the NOR test. **A,** There were no significant differences in the olfactory responses. **B,** Aging mice housed with young mice had a greater preference to the novel object than those housed with aging mice. n = 12/group. The data are presented as the mean ± SE. **p<0.01, ***p<0.001; A = repeated measures Two-way ANOVA; B = One-way ANOVA.

performed the complete input-output (I-O) curves and observed no detectable changes in the basal synaptic transmission ($F_{4,25}$ = 2.347; p = 0.451; Figure 7B), showing that the paired housing with young mice did not affect basal synaptic neurotransmission in aging mice.

Discussion

We discovered several major in our study. Firstly, paired housing with young mice for one-month improved learning and memory performance in aging mice, as assessed by the escape latency in the MWM test, freezing duration in the contextual fear conditioning test, alternation rate in the T-maze test, and preference in the NOR test. Secondly, the paired housing with young mice also rescued impaired LTP induction in aging mice and demonstrated an effect on presynaptic neurotransmission. Altogether, our results suggest that the paired housing with young mice may have a beneficial effect on learning and memory abilities in aging mice.

It is universally understood that aging is an inevitable process, and the cognitive decline that accompanies this decline is emerging as one of the greatest health threats of the twenty-first

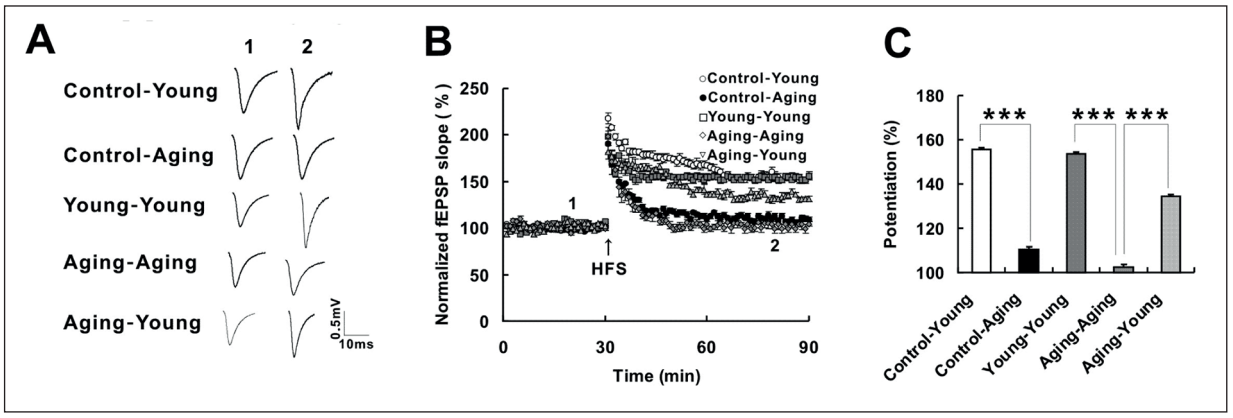

Figure 6. Paired housing with young mice facilitated LTP induction. **A,** Average traces obtained before (1) and 60 min after (2) tetanic stimulation. Scale bars = 0.5 mV, 10 ms. **B,** Normalized fEPSP slope plotted for each group. The arrow indicates 1*HFS (100 Hz, 1s) tetanus stimulation. **C,** Histogram showing the average percentage of potentiation after tetanus *vs.* baseline (100%) for each group (recorded 30-60 min after tetanus). n = 6/group. The data are presented as the mean ± SE. ***p<0.001; One-way ANOVA.

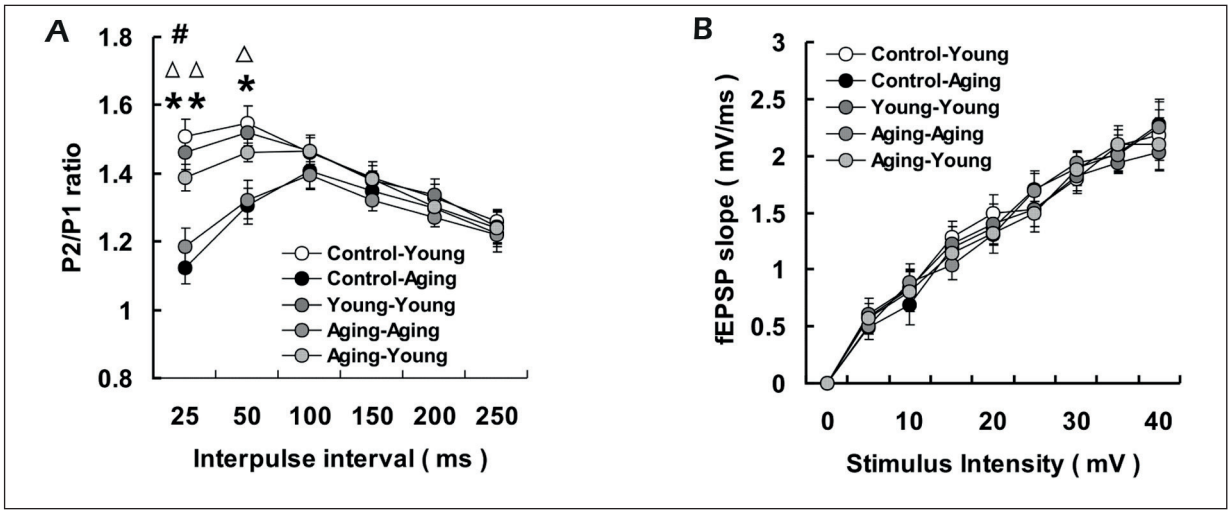

Figure 7. A presynaptic mechanism was shown to be involved in the paired housing modulated LTP induction. **A,** At 25 inter-stimulus interval PPF was increased in aging mice after paired housing with young mice. * indicates the differences between Control-Young and Control-Aging groups, Δ indicates the differences between Young-Young and Aging-Aging groups, # indicates the differences between Aging-Aging and Aging-Young groups. **B,** Paired housing treatment caused no changes in the I-O curves. n = 6/group. The data are presented as the mean ± SE. *p<0.05; **p<0.01; repeated measures Two-way ANOVA.

century[1,2,4]. Like other mammals, humans are susceptible to age-related cognitive decline, for example, Alzheimer's disease and other forms of neurodegenerative disease[4]. Therefore, exploring the mechanisms underlying the aging process is an important topic within neuroscience research. Fortunately, we have obtained a greater understanding of the molecular mechanisms of aging[24-26]. It has been discovered that the rate of aging is, in fact, not inevitable but rather is open to modification[27]. Similarly, the cognitive decline associated with mammalian brain aging also seems to be variable and potentially open to modification[13].

Based on the above findings, one potential approach to confront the disadvantageous effects of aging is to manipulate the individual target genes which have been implicated in the aging process[28]. However, it is a challenge to target the genes in this manner. The manipulation of the systemic environment may, therefore, provide other means to rejuvenate aging[29,30]. Indeed, reports into systemic manipulations, such as caloric restriction[31], exercise[32], and changes to blood composition by heterochronic parabiosis[33], young plasma administration, or exposure of aged mice to young blood[13], have already demonstrated a potential reversal of aging, both in peripheral tissues and the CNS. Specifically, these aforementioned studies have verified that the systemic manipulations can relieve impairments in the cognitive functions and synaptic plasticity in the aged CNS.

To date, the above evidence of rejuvenated aging has been attributed to the changes in the systemic environment. However, it should be noted that currently, the effects of young blood have not been fully comprehended[34]. Therefore, it is important that future studies continue to explore the rejuvenating effects of the systemic environment. In our study, we found that the paired housing with young mice for one month improved the learning and memory ability of the aging mice across several behavioral tests. To our knowledge, these results directly indicate for the first time that the group housing with young mice has a beneficial effect on learning and memory in aging mice. Previous studies[35,36] have also reported that group housing can have other physiological consequences, for example, the changes in the group temperature, oxygen consumption, and respiration rate. Group housing can also affect competitive interactions, social behavior, and influence growth through complex physiological interactions[37-41]. Some clinical studies have also indicated that housing is a well-recognized social determinant of health, which is closely linked with social, economic, and geographic factors. Poor housing environments can affect health through a myriad of direct and indirect pathways. Therefore, our investigations could provide a new strategy to prevent the observed decline in learning and memory in aging.

LTP is thought to be the cellular and molecular mechanism underlying learning and memory[21]. Bishop et al[3] have indicated that in aging mice, learning and memory ability and LTP are disturbed when compared to adult mice. Indeed, from our results, we found that aging mice exhibited impaired LTP. Meanwhile, paired housing with young mice was seen to rescue this LTP impairment (Figure 6). Furthermore, the alterations in presynaptic neurotransmission were found to play some role in the observed paired housing mediated LTP induction (Figure 7).

Conclusions

This study revealed the functional role of the paired housing with young mice in the learning and memory ability of aging mice. This may provide a new strategy for aging rejuvenation through manipulation of the systemic environment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This study was supported by the National Natural Science Foundation Funded Project (201581460183). The authors declare no competing financial interests in relation to the work described.

References

1) Hedden T, Gabrieli JD. Insights into the ageing mind: a view from cognitive neuroscience. Nat Rev Neurosci 2004; 5: 87-96.
2) Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003; 60: 1119-1122.
3) Bishop NA, Lu T, Yankner BA. Neural mechanisms of ageing and cognitive decline. Nature 2010; 464: 529-535.
4) Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013; 153: 1194-1217.
5) Alcedo J, Kenyon C. Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron 2004; 41: 45-55.
6) Apfeld J, Kenyon C. Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature 1999; 402: 804-809.
7) Kenyon CJ. The genetics of ageing. Nature 2010; 464: 504-512.
8) Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005; 433: 760-764.
9) Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007; 317: 807-810.
10) Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJ. Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell 2012; 10: 96-103.
11) Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS, Couillard-Despres S, Aigner L, Li G, Peskind ER, Kaye JA, Quinn JF, Galasko DR, Xie XS, Rando TA, Wyss-Coray T. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 2011; 477: 90-94.
12) Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A, Psychogios N, Gerszten RE, Hartigan AJ, Kim MJ, Serwold T, Wagers AJ, Lee RT. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 2013; 153: 828-839.
13) Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, Smith LK, Bieri G, Lin K, Berdnik D, Wabl R, Udeochu J, Wheatley EG, Zou B, Simmons DA, Xie XS, Longo FM, Wyss-Coray T. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med 2014; 20: 659-663.
14) Liu JH, You QL, Wei MD, Wang Q, Luo ZY, Lin S, Huang L, Li SJ, Li XW, Gao TM. Social isolation during adolescence strengthens retention of fear memories and facilitates induction of late-phase long-term potentiation. Mol Neurobiol 2015; 52: 1421-1429.
15) Tian J, Geng F, Gao F, Chen YH, Liu JH, Wu JL, Lan YJ, Zeng YN, Li XW, Yang JM, Gao TM. Down-regulation of neuregulin1/ErbB4 signaling in the hippocampus is critical for learning and memory. Mol Neurobiol 2017; 54: 3976-3987.
16) Chen YH, Lan YJ, Zhang SR, Li WP, Luo ZY, Lin S, Zhuang JP, Li XW, Li SJ, Yang JM, Gao TM. ErbB4 signaling in the prelimbic cortex regulates fear expression. Transl Psychiatry 2017; 7: e1168.
17) Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci 2009; Chapter 8: Unit 8.24.
18) Deacon RM, Rawlins JN. T-maze alternation in the rodent. Nat Protoc 2006; 1: 7-12.
19) Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1: 848-858.
20) Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc 2006; 1: 1306-1311.

21) Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31-39.

22) Schulz PE, Cook EP, Johnston D. Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. J Neurosci 1994; 14: 5325-5337.

23) Schulz PE, Cook EP, Johnston D. Using paired-pulse facilitation to probe the mechanisms for long-term potentiation (LTP). J Physiol Paris 1995; 89: 3-9.

24) Yates D. Brain ageing: blood-derived rejuvenation. Nat Rev Neurosci 2014; 15: 352-353.

25) Bowers WJ, Breakefield XO, Sena-Esteves M. Genetic therapy for the nervous system. Hum Mol Genet 2011; 20: R28-R41.

26) Mazzoccoli G, Tevy MF, Borghesan M, Delle VM, Vinciguerra M. Caloric restriction and aging stem cells: the stick and the carrot? Exp Gerontol 2014; 50: 137-148.

27) Edwards LM, Holloway CJ, Murray AJ, Knight NS, Carter EE, Kemp GJ, Thompson CH, Tyler DJ, Neubauer S, Robbins PA, Clarke K. Endurance exercise training blunts the deleterious effect of high-fat feeding on whole body efficiency. Am J Physiol Regul Integr Comp Physiol 2011; 301: R320-R326.

28) Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 2014; 344: 630-634.

29) Laviano A. Young blood. N Engl J Med 2014; 371: 573-575.

30) Shelton DS, Meyer PM, Ocasio KM. Environmental structure and energetic consequences in groups of young mice. Physiol Behav 2017; 177: 155-160.

31) Harshaw C, Alberts JR. Group and individual regulation of physiology and behavior: a behavioral, thermographic, and acoustic study of mouse development. Physiol Behav 2012; 106: 670-682.

32) Mazerolle DF, Hobson KA. Physiological ramifications of habitat selection in territorial male ovenbirds: consequences of landscape fragmentation. Oecologia 2002; 130: 356-363.

33) Mathot KJ, Dingemanse NJ. Energetics and behavior: unrequited needs and new directions. Trends Ecol Evol 2015; 30: 199-206.

34) Ockenfels A, Schapiro I, Gartner W. Rhodopsins carrying modified chromophores--the 'making of', structural modelling and their light-induced reactivity. Photochem Photobiol Sci 2016; 15: 297-308.

35) Marras S, Killen SS, Lindstrom J, McKenzie DJ, Steffensen JF, Domenici P. Fish swimming in schools save energy regardless of their spatial position. Behav Ecol Sociobiol 2015; 69: 219-226.

36) Liao JC. A review of fish swimming mechanics and behaviour in altered flows. Philos Trans R Soc Lond B Biol Sci 2007; 362: 1973-1993.

37) Baker MG, Howden-Chapman P. Time to invest in better housing for New Zealand children. N Z Med J 2012; 125: 6-10.

38) La Grutta S, Indinnimeo L, di Coste A, Ferrante G, Landi M, Pelosi U, Rusconi F. Environmental risk factors and lung diseases in children: from guidelines to health effects. Early Hum Dev 2013; 89 Suppl 3: S59-S62.

39) Hamilton I, Milner J, Chalabi Z, Das P, Jones B, Shrubsole C, Davies M, Wilkinson P. Health effects of home energy efficiency interventions in England: a modelling study. BMJ Open 2015; 5: e007298.

40) Baker E, Lester LH, Bentley R, Beer A. Poor housing quality: prevalence and health effects. J Prev Interv Community 2016; 44: 219-232.

41) Morrison JH, Baxter MG. The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat Rev Neurosci 2012; 13: 240-250.