

Aging and exercise effects on motor learning and spatial memory

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Abstract

The aim of this experiment was to determine whether low impact forced exercise improved learning and memory in middle aged and older mice. The effects of 12 weeks of low impact, forced running on motor learning and spatial memory were compared in 32 C57BL/6 mice in three age groups with a mean age of 13, 17, and 25 months. Motor learning was assessed with the rotorod, and spatial memory was assessed with the Morris water maze (MWM). Mice were randomly assigned to the forced exercise or sedentary control group and were treated for 60 min/day (2 30-min sessions) for 12 weeks. Significant age differences were observed on the rotorod (15 and 25 RPM) with the 13-month-old mice outperforming 17- and 25-month-old mice. Age differences were also observed on the MWM, with 25-month mice impaired in latency to escape and distance covered. The effect of exercise on rotorod performance did not attain statistical significance, but the mean performance of runners was higher than that of controls. Exercise improved spatial memory retention in the MWM and led to significant improvement in acquisition distance. Low impact forced exercise did not have strong effects on motor learning as assessed by the rotorod. However, low impact forced exercise did improve retention of spatial memory in 13-month-old mice. Slight improvements in spatial memory were seen in the oldest runners, though this experiment suggested that the beneficial effect of exercise and plasticity in memory was generally limited to 13-month-old middle aged mice, with significant age-related differences in motor and spatial learning occurring in the 4-month period between 13 and 17 months.

Introduction

For decades the effects of enrichment on the brain have been documented.¹ The beneficial effects of aerobic exercise on the adult and aging brain have been demonstrated for almost as long as the effects of enrichment in young organisms.² Studies of cognitive bene-

fits of aerobic exercise in humans have also been documented for decades.^{3,4} Contemporary studies focusing on mechanism have demonstrated that higher levels of aerobic fitness in older humans are associated with larger hippocampal volume, which is associated with superior spatial memory.⁵

Given the significant implications of exercise for health and cognition, we aimed to refine the growing body of data on exercise effects on cognition at various points of the life span.⁶ Parallels between human and non-human species on the deleterious effects of inactivity in later life have been reported.⁷ To focus on the adult period of the life span of an organism, we tested mice ranging in age from 12 to 26 months. The goal was to extend knowledge about the relationship between physical exercise, aging and learning and memory.

Age differences in spatial memory^{7,8} and performance on the rotorod⁹ have been reported in a number of studies. In C57BL/6 mice, age deficits in spatial memory are seen at 24 months,^{8,10} 18 months,¹¹ 15 months⁷ and as early as 12 months.¹² Not all studies report age differences in mice on the MWM, as it has been reported that C57BL/6 mice (4, 12, and 18 months) showed significant age differences in latency to escape for the first session of the MWM but that those differences disappeared by session two.⁹ The same study reported age-related impairment in mice on the rotorod task. Other research confirms this age-related deficit in rotorod performance¹³ and suggests that deficits first appear around 12-13 months,¹⁴ though animals as young as 7 months can also show age-related impairment.^{15,16}

Forced exercise paradigms usually involve stress in an attempt to impair function,¹⁷⁻¹⁹ though a few studies have examined forced exercise without a stressful component^{20,21} and have generally reported improvements on tests of learning and memory. Older animals may be more susceptible to stressors,²² which could confound the ability to measure accurately age differences and exercise-induced improvements in learning and memory. Therefore, it is important to consider an exercise paradigm that does not impose a great deal of stress on the animals. Our goal was to develop a low impact paradigm that would improve spatial memory and motor learning at speeds that were not stressful for older mice.

The behavioral tasks used in the present study were measures of motor learning (rotorod) and spatial memory (MWM). Exercise has generally been associated with improved performance on these tasks, though there are considerable differences in the intensity and duration of exercise implemented in the literature. Six days of exposure to wheel running led to overall longer latencies to fall from the rotorod in rats aged 8-10 weeks

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compared to sedentary animals.²³ However, the rate of learning was comparable to that of sedentary animals. Other research has confirmed the beneficial effects of exercise on rotorod performance, including voluntary exercise in mice aged 3 months,²⁴ forced treadmill exercise in mice aged 3 months²⁵ and voluntary exercise in 5-month-old gerbils recovering from cranial irradiation.²⁶ Evidence is clear that exercise improves performance on the rotorod in young mice, but the effects on middle aged and older mice are less explored. Thouvarcq and colleagues¹⁴ reported that voluntary wheel running improved motor learning in mice aged 3 months but 12- and 20-month-old mice showed no significant improvement.

Improved spatial ability and performance on the MWM is also associated with exercise in young mice. As with the rotorod literature, there is considerable disagreement over the length of time of running necessary to see behavioral improvements. For the MWM, benefits have been reported in as short as one month¹¹ and up to five months of running wheel access.²⁷ High intensity treadmill running (25 m/min) can impair spatial learning abilities and MWM performance, whereas chronic, low intensity (8 m/min) running can facilitate spatial learning in rats aged 2-3

months.^{17,28} It has been reported that mice aged 19 months showed improvements in both acquisition and retention of spatial memory after six weeks of exercise, and acquisition was similar in 3- and 19-month-old mice.¹¹ These results suggest that the beneficial effects of exercise extend to older animals as well. However, studies comparing the effects of exercise on middle aged and older mice are few.

The present study included C57BL/6 mice in three age groups (mean age of 13, 17, and 25 months) that were assigned to either a forced exercise (runners) or sedentary (controls) condition in which they were handled. After 12 weeks of exercise training or control handling, mice were tested behaviorally on the rotorod and MWM. Two main variables were tested: age-related deficits and exercise-induced improvements and their combined effects on motor learning and spatial memory. Based on previous studies, we hypothesized that 13-month-old mice would outperform 17- and 25-month-old mice, that runners would outperform the sedentary controls, and that forced exercise would benefit the oldest mice as well as 17- and 13-month-old mice.

Materials and Methods

Subjects

Thirty-six C57BL/6 mice from Jackson Laboratory (Bar Harbor, Maine) were exercised for 12 weeks and tested behaviorally. A total of 32 mice were included (22 male) in all analyses, whereas four statistical outliers were not included. After 12 weeks of exercise, as well as behavioral testing, mice ranged in age from 12.1 to 26.0 months ($M=17.61$ mo, $SD=4.70$ mo) and were classified into one of three age groups: 13 months ($n=10$, $M=12.84$ mo, $SD=0.26$ mo), 17 months ($n=14$, $M=16.84$ mo, $SD=0.22$ mo) or 25 months ($n=8$, $M=24.92$ mo, $SD=0.29$ mo). Mice were randomly assigned to either a runner ($n=18$) or sedentary control ($n=14$) condition. Mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at Temple University. The colony room was temperature- and humidity-controlled with a 12 hr light/dark schedule. Mice were housed in groups of 2 to 4 in polycarbonate cages with ad libitum access to food and water. This research was approved by Temple University's Institutional Animal Care and Use Committee (IACUC).

Exercise

A Forced Walking Wheel System (Lafayette Instrument Co., Model 80801) with individual running wheels was used to exercise up to 20 mice each session. Mice randomly assigned to

the runner condition were trained in two (morning and afternoon) 30-minute sessions for 5 days/week. Exercise continued for a total of 12 weeks (Figure 1). During each session, mice were required to walk/run at a speed of 3.16 m/min. Mice that clung to the running wheel were gently prodded with small soft paint brushes to insure that they were continually moving during the session. This gentle prodding occurred infrequently throughout training. Sedentary controls were taken from their cages and handled for the duration of each session of exercise training. After 12 weeks, all animals were behaviorally tested on the rotorod and MWM.

Rotorod

A four-lane motorized rotorod (San Diego Instruments, Inc., San Diego, CA) was used for testing locomotor coordination in mice. Each rod was 3 cm in diameter and 11 cm long, and maintained at 46 cm above the foam-covered base. An electronically controlled motor maintained the rod speed with the speed indicated on the front panel of the control chassis (with a resolution of 0.1 seconds). Seven closely spaced photo beams detected the falling of the subject in each lane. The time to fall was shown on each lane's timer display.

Rotorod training took place over three days with one session per day. Each session consisted of eight trials, four at each speed (15 and 25 RPM). There was a five-minute inter-trial interval and 30 minutes between speed changes. On each day, mice were placed on the rotorod in the direction opposite of the rotation of the rod. To avoid a fall, subjects were required to move forward in a coordinated manner. Based on previous work in our lab⁹ that reported age differences in the C57BL/6 strain, trials were discontinued if the subject did not fall after 80 seconds. Latency to fall at each speed was recorded.

Morris water maze

The training apparatus was a circular pool, 100 cm diameter and 60 cm deep. The pool was located in a laboratory room containing camera and computer equipment, a portable partition to reduce the viewing area, and various visual cues. The interior of the pool was painted black. The water was maintained at a temperature aversive enough to motivate escape but not be dangerous (between 20 and 26°C), and the depth was 16 cm. The hidden platform was an 11 cm square clear Plexiglas platform positioned 1 cm below the surface of the water. The same platform was used for the visible platform, and it was marked by a black flag (10 by 7 cm) suspended 15 cm above it on a wooden stick.

Training in the MWM took place over five days. Around the test room in close proximity to the pool were multiple cues such as artificial plants and flowers and graphic prints. Computer and camera equipment used to record the session were also visible to the mouse. Each trial was initiated by placing the animal in the water at the edge of the pool in a quadrant either opposite or adjacent to the quadrant containing the platform. The start locations were varied among the three quadrants not containing the platform; with three different start locations being used in each block of four trials. The platform remained in the same location on every trial during the hidden platform task and varied across the four quadrants in the visible platform task. Each trial lasted 120 seconds or until the subject located the platform. Subjects that did not find the platform were guided to it, placed on it, and given a latency score of 120 seconds. Whether the platform was located or not, each mouse was required to spend 15 seconds on the platform at the end of each trial. Between blocks of four trials the mice were placed in individually heated, extra-absorbent paper towel lined plas-

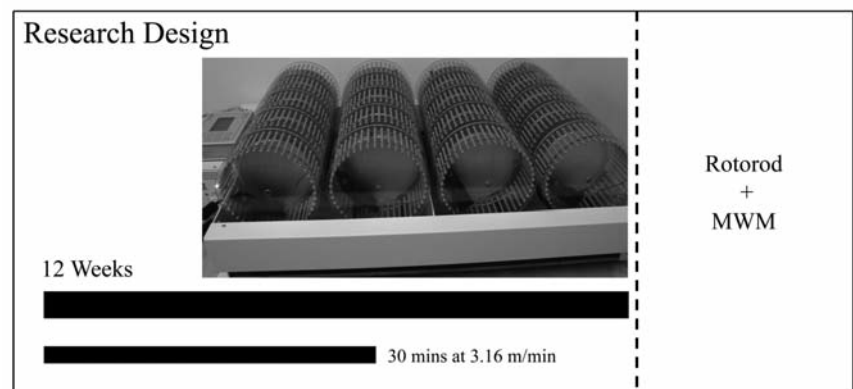


Figure 1. Research design. Training included two 30-minute exercise sessions at a speed of 3.16 m/min five days a week for a total of 12 weeks. At the end of the exercise training phase, all animals were behaviorally tested on the rotorod and Morris water maze .

tic holding cages for at least 30 minutes. Two commonly reported MWM measures, time (latency to escape) and distance traveled to reach the platform were recorded for each trial and averaged for each block of 4 trials during acquisition.

Hidden platform training

Each subject was given three blocks of four trials each (12 trials/day/mouse) for three consecutive days of training. Mice were returned to the holding cage between blocks. On the fourth training day, the subjects were given a probe trial, in which the platform was removed from the pool. After swimming for the entire length of the trial (120 seconds) the mouse was removed from the pool and returned to its holding cage. The training trials and the probe trial were videotaped and recorded using the SMART (Spontaneous Motor Activity Recording and Tracking) program manufactured by Panlab (Barcelona, Spain). The probe trial was analyzed to measure the amount of time spent in each quadrant and the number of crossings made over the platform location in the trained quadrant and the equivalent area in the untrained quadrants.

Visible platform training

On the fifth day of training, all mice were given the visible platform task. A flag attached to the platform made it visible. Training was the same as in the hidden platform version except the location of the platform and the start position were varied across trials. The latency and distance to escape were recorded.

Statistical analyses

All statistical analyses were carried out using the SPSS (Version 17) statistical software package. The main analysis used in this experiment was the repeated measures analysis of variance (ANOVA) to compare the effects of age and exercise on dependent measures from the rotorod and MWM. Significance was set at $P < .05$. When appropriate, post-hoc analyses were conducted using the Tukey Honestly Significant Difference (HSD) test incorporating adjusted critical values. Some cell sizes were small; however, there were no major violations of ANOVA assumptions. Thus, the repeated measures ANOVA was used to investigate effects of age and exercise as well as their interactions. When violations of sphericity occurred, analyses including adjusted degrees of freedom using the Greenhouse-Geisser correction were examined and reported if results were altered.

Results

The major aim of this experiment was to determine whether low impact forced exercise

improved motor learning and spatial memory in middle aged and older mice. Analyses compared performance on rotorod and MWM in 13-, 17- and 25-month-old mice that had been exercised for 12 weeks to mice of the same age that had been handled during the exercise period. To address the small sample sizes in this study, the data were examined for outliers before the main analyses were conducted. Four animals (one 13-month control and 3 17-month controls) were identified as statistical outliers in that mean behavioral performance was at least 1.5 times greater than the inter-quartile range. In one instance, performance was more than 3 times the inter-quartile range. These animals were excluded from all analyses and are not represented in figures.

Rotorod

Rotorod data were analyzed using a 2 (Condition, runners vs. controls) x 3 (Age) x 3 (Training Session) repeated measures analysis of variance (ANOVA) with latency to fall from the beam in seconds as the dependent

variable. Separate ANOVAs were conducted for the two rotation speeds, 15 and 25 RPM. For the 15 RPM data, results showed main effects of Training Session, $F(2, 52) = 71.81$, $P < .001$, $\eta^2 = .73$, and Age, $F(2, 26) = 6.79$, $P < .05$, $\eta^2 = .52$ (Figure 2A). Tukey post hoc tests of the significant effect of Training Session provided evidence that learning occurred in all age groups, as latency to fall increased across all three Training Sessions ($P < .001$). Post hoc tests examining the main effect of Age suggested that overall, the 13-month group had a longer latency to fall than the 17- ($P = .05$) and 25-month ($P < .05$) groups. There was no significant effect of Exercise Condition, and none of the interaction effects were significant, though runners did outperform the sedentary controls across all three ages (Figure 2B, C, D). A 2 (Condition, runners vs. controls) x 3 (Age) x 3 (Training Session) repeated measures ANOVA examining the 25 RPM data revealed main effects of Training Session, $F(2, 52) = 28.13$, $P < .001$, $\eta^2 = .52$, and Age, $F(2, 26) = 14.69$, $P < .001$, $\eta^2 = .53$ as well as an interaction between Training Session and Age, $F(4,$

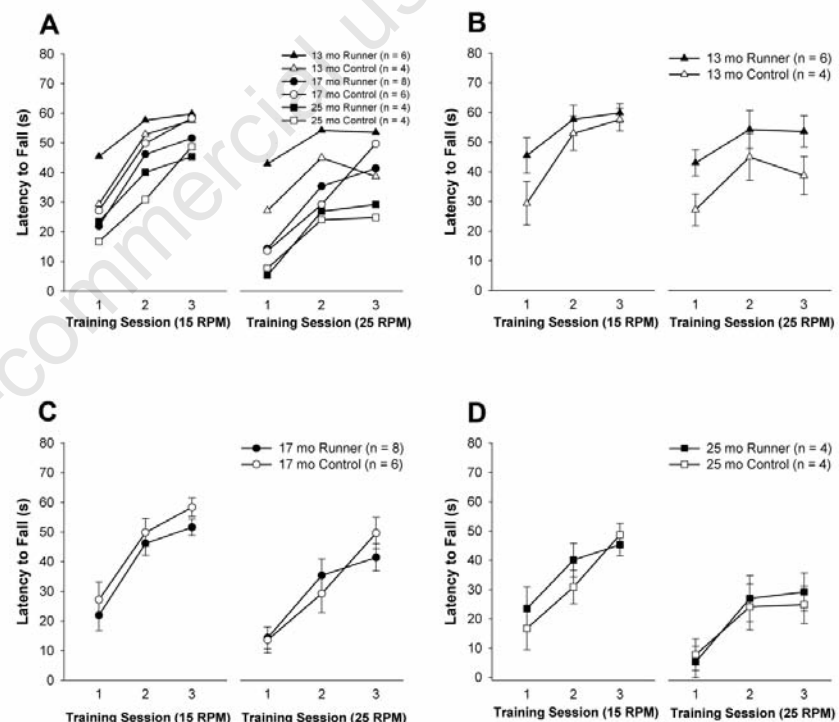


Figure 2. Rotorod performance in three ages of mice by exercise condition. (A) Animals aged 13, 17 and 25 months were assigned to either a runner or sedentary control condition and tested at 15 or 25 revolutions per minute (RPM) over three days. Learning is expressed by all age groups at both speeds, though older mice showed impairment at both 15 and 25 RPM. Overall, the 13-month group had a significantly longer latency to fall than the 17- ($P = .05$) and 25-month ($P < .05$) groups at 15 RPM. Age differences were evident at 25 RPM (13 vs 17 $P < .01$; 13 vs 25 $P < .001$; 17 vs 25 $P < .05$) as well. Runners outperformed sedentary controls, but differences did not achieve statistical significance. (B) Rotorod performance in 13-month runners and sedentary controls. Runners generally outperformed controls at both 15 and 25 RPM speeds. (C) In 17 month mice there was no significant effect of exercise. (D) In mice aged 25 months, runners generally outperformed controls at 15 RPM and to a lesser extent at 25 RPM speeds. Values are mean \pm SEM.

52)=2.66, $P<.05$, $\eta^2=.20$ (Figure 2A). Learning occurred in all three groups, as latency to fall increased from Training Session 1 to 2 ($P<.001$), but did not significantly increase from Training Session 2 to 3 according to Tukey post hoc tests. To examine the main effect of Age, post hoc tests determined that 13-month mice significantly outperformed 17-month mice ($P<.01$) and 25-month mice ($P<.001$) and 17-month mice outperformed 25-month mice ($P<.05$) across all three Training Sessions. To examine the Training Session x Age interaction, post hoc tests determined that 13-month mice outperformed 25-month mice in Training Session 1 ($P<.05$), 2 ($P<.01$) and 3 ($P<.01$). The effect of Exercise Condition was not statistically significant, but runners outperformed controls (Figure 2B,C,D).

Morris water maze

During the hidden platform training, mice were placed in a pool filled with aversively cool water and required to learn the location of a partially submerged escape platform using contextual cues surrounding the edge of the pool. One dependent measure of learning was latency to escape the water by climbing onto the platform. Data were analyzed using a 2 (Condition, runners vs. controls) x 3 (Age) x 9 (Training Session) repeated measures ANOVA. There were slight violations of sphericity, though using Greenhouse-Geisser adjusted degrees of freedom did not alter statistical results. Results showed a significant three-way interaction between Training Session, Age and Exercise Condition, $F(16, 208) = 2.153$, $P<.01$, $\eta^2=.17$ (Figure 3A). This interaction resulted from the learning patterns of the 13-month and 25-month groups. For the 13-month mice, the control and runners performed approximately equally well. Around Training Session 4, the runners began to outperform the controls. The pattern was reversed for the 25-month mice. Runners outperformed controls until Training Session 6, when performances switched.

Significant main effects of Training Session, $F(8, 208)=14.09$, $P<.001$, $\eta^2=.54$, and Age, $F(2, 26)=4.50$, $P<.05$, $\eta^2=.35$, were found. All mice showed evidence of learning, as latency to escape the pool decreased across Training Sessions. A Tukey post hoc test examining the main effect of age indicated that the 25-month group had a significantly longer latency to escape than the 17-month group ($P<.05$). The effect of Exercise Condition was marginally significant, $F(1, 26) = 3.88$, $P = .06$, $\eta^2=.15$. As one of the hypotheses of this study was that exercise would benefit mice of all ages similarly, planned comparison between runners and controls within each age group were conducted. Within the 13- and 25-, but not 17-month age groups, runners tended to outperform controls (Figure 3B,C,D). In 13-month animals, a

Tukey post hoc test determined that runners significantly outperformed controls in Training Session 7 ($P<.05$) and 9 ($P<.05$). No significant effect of exercise was found in the 17-month group. The oldest runners outperformed sedentary controls in Training Sessions 6 ($P=.082$) and 8 ($P=.067$) and were significantly better in Training Session 4 ($P<.01$).

Age differences were also apparent when using the dependent measure of the distance traveled before finding the platform. Data were analyzed using a 2 (Condition, runners vs. controls) x 3 (Age) x 9 (Training Session) repeated measures ANOVA. There were slight violations of sphericity, though using Greenhouse-Geisser adjusted degrees of freedom did not alter statistical results. Results showed a significant three-way interaction between Training Session, Age and Exercise Condition, $F(16, 208)=2.07$, $P<.05$, $\eta^2=.16$ (Figure 4A). There was also a significant main effect of Training Session, $F(8, 208)=14.36$, $P<.01$, $\eta^2=.55$, and interaction between Training Session and Age, $F(16, 208)=2.04$,

$P<.05$, $\eta^2=.16$. All mice showed evidence of learning, as distance covered decreased across Training Sessions. Tukey post hoc tests examining the Training Session x Age interaction revealed that 25-month mice covered more distance than 17-month mice in Training Sessions 5 ($P=.081$), 6 ($P<.05$), and 7 ($P<.05$).

The main effects of Age, $F(2, 26)=3.69$, $P<.05$, $\eta^2=.28$, and Exercise Condition, $F(1, 26)=5.32$, $P<.05$, $\eta^2=.20$, were significant. Post hoc tests examining these main effects determined that overall, 25-month mice covered more distance than 17-month mice ($P<.05$) and controls covered more distance than runners ($P<.05$). As with the latency data, planned comparison between runners and controls within each age group were conducted. Within each age group, Tukey HSD post hoc tests revealed similar results to the latency analysis: 13-month runners covered less distance than controls (Figure 4B) in Training Sessions 7 ($P<.01$) and 9 ($P<.05$), 17-month runners showed no benefits of exercise (Figure 4C), and 25-month runners traveled less distance in Training Sessions 4 ($P<.01$), 6

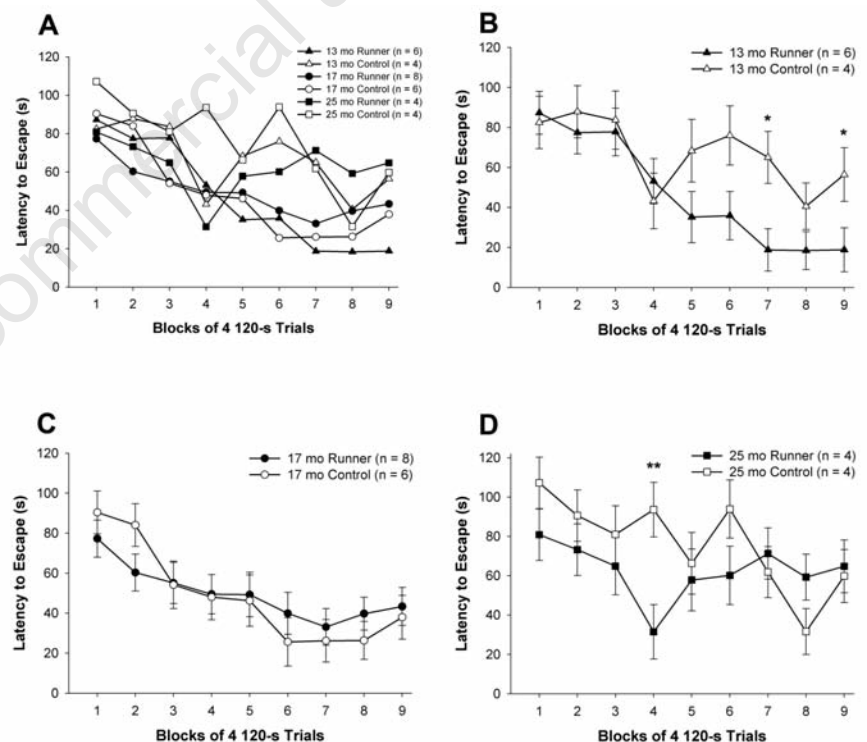


Figure 3. MWM acquisition in three ages of mice by exercise condition: Latency to platform. (A) Latency to escape the MWM in animals aged 13, 17 and 25 months that were assigned to either a runner or sedentary control condition is shown. Learning is expressed by all groups, though 25-month mice were impaired relative to 17-month mice ($P<.05$). No effects of exercise are reported. (B) MWM acquisition latency in 13-month runners and sedentary controls. Runners took less time to find the hidden platform in Training Sessions 7 ($P<.05$) and 9 ($P<.05$). (C) In 17-month mice there was no significant effect of exercise. (D) In mice aged 25 months runners outperformed controls in Training Sessions 4 ($P<.01$), 6 ($P=.082$) and 8 ($P=.067$). Values are mean \pm SEM. * $P<.05$; ** $P<.01$.

($P < .05$), and 8 ($P = .082$) than control mice (Figure 4D).

After hidden platform training, the platform was removed and mice were placed in the pool for one trial as a measure of retention of place learning. The first dependent measure was the number of times the mouse crossed over the area where the location of the platform was learned. A second dependent variable was the amount of time spent swimming in each quadrant. It was expected that the mice would cross the area of the former location of the platform more and spend more time swimming in the quadrant where the platform had been located compared to the other quadrants. Both dependent variables were investigated with 2 (Condition, runners vs. controls) \times 3 (Age) \times 4 (Quadrant) repeated measures ANOVAs. For the number of platform crossings, results showed a significant main effect of Quadrant, $F(3, 78) = 4.00$, $P < .05$, $\eta^2 = .15$ and the effect of Exercise Condition approached significance, $F(1, 26) = 3.29$, $P = .081$, $\eta^2 = .13$ (Figure 5). Overall, mice crossed the location of the platform in the trained quadrant significantly more than the other quadrants, with the exception of one adjacent quadrant. As the performance in the trained quadrant is the strongest index of spatial memory retention, we planned to examine age and exercise effects only in this quadrant. In the trained quadrant, runners crossed the former platform location significantly more than the sedentary controls, $F(1, 30) = 4.02$, $P < .05$, $\eta^2 = .13$. To explore the hypothesis that exercise would benefit mice of all ages similarly, planned contrast analyses were conducted to examine the effect of exercise within each age group. Tukey post hoc tests indicated that runners showed significantly improved retention of spatial memory in the 13-month age group ($P < .05$) only.

An analysis of the time spent swimming showed a significant main effect of Quadrant, $F(3, 78) = 9.30$, $P < .001$, $\eta^2 = .36$. Mice spent significantly more time swimming in the trained quadrant than the other quadrants, with the exception of one untrained adjacent quadrant. No significant effects of Exercise Condition, Age or interactions were found.

For visible platform training, a flag was attached to the platform to mark its location. A 2 (Condition, runners vs. controls) \times 3 (Age) \times 3 (Training Session) repeated measures ANOVA with latency to escape as the dependent variable revealed no significant main effects of Training Session, Age or Exercise Condition. The interaction between Age and Training Session was significant, $F(2, 52) = 2.92$, $P < .05$, $\eta^2 = .22$. A Tukey post-hoc test determined that the mice aged 25 months performed significantly better in Training Session 2 compared to Training Session 1 ($P < .05$) but did not significantly improve from Training Session 2 to 3. The other age groups showed no significant change in performance across the three training sessions.

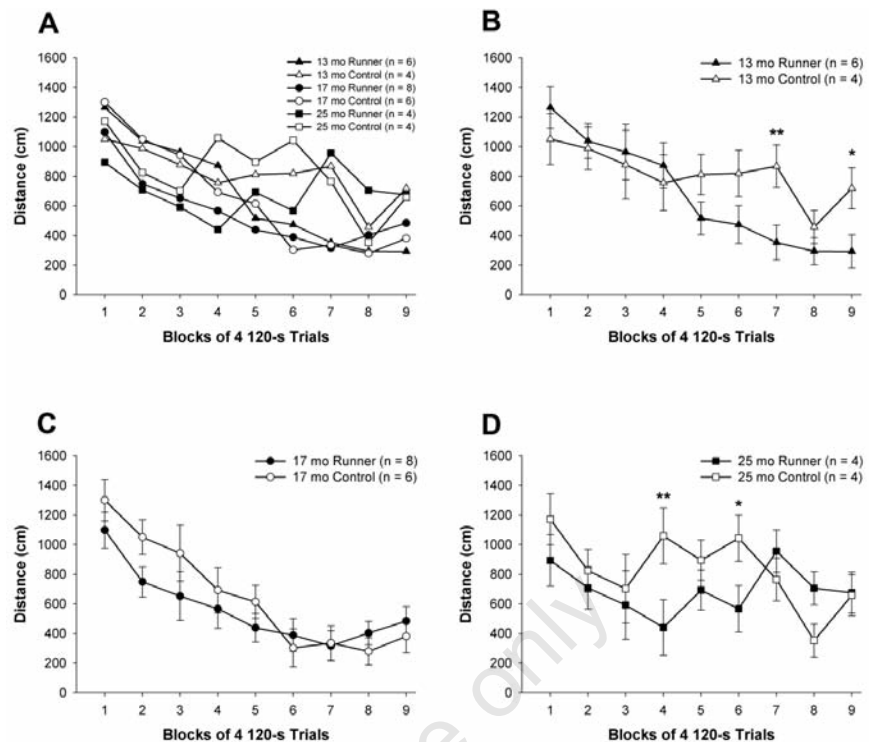


Figure 4. MWM acquisition in three ages of mice by exercise condition: distance to platform. (A) Distance traveled before escaping the MWM in mice aged 13, 17 and 25 months that were assigned to either a runner or sedentary control condition is shown. Learning is expressed by all groups, though rates of learning differed across the three age groups. Overall, 25-month mice covered more distance than 17-month mice ($P < .05$). A significant effect of exercise is reported, as runners covered less distance than controls ($P < .05$). (B) MWM acquisition distance in 13-month runners and controls. Runners covered less distance in Training Sessions 7 ($P < .01$) and 9 ($P < .05$). (C) In 17-month mice there was no significant effect of exercise. (D) In mice aged 25 months runners outperformed controls in Training Sessions 4 ($P < .01$), 6 ($P < .05$), and 8 ($P = .082$). Values are mean \pm SEM. * $P < .05$; ** $P < .01$.

Results

Results provided evidence of age-related impairment at both speeds of the rotodisc as well as in the MWM. Middle-aged mice (13 months) tended to outperform the older mice (17 and 25 months) at both 15 and 25 RPM speeds, whereas deficits in spatial memory were seen in the 25-month (latency and distance) mice in the MWM. Low impact forced exercise at a speed of 3.16 m/min yielded no statistically significant differences in motor learning, though retention and, to a lesser extent, acquisition of spatial memory were improved. Runners were found to have improved retention of spatial memory by crossing the former location of the hidden platform more often in the trained quadrant than their sedentary controls and improved acquisition by covering less distance in acquisition. In addition, runners tended to outperform controls at both speeds of the rotodisc across all three testing days, though these differences did not achieve statistical significance.

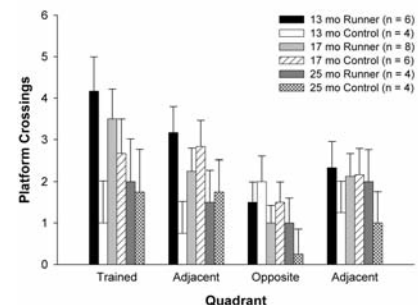


Figure 5. MWM probe performance in three ages of mice. The number of platform crossings in trained and untrained quadrants of mice aged 13, 17 and 25 months assigned to either a runner or sedentary control condition is shown. All mice crossed the area in the trained platform quadrant more times than the same area in untrained adjacent and opposite quadrants. Within the trained quadrant, runners crossed the location of the platform significantly more than the sedentary controls ($P < .05$). In mice aged 13 months this difference was statistically significant ($P < .05$). Values are mean \pm SEM. * $P < .05$.

Discussion

Mice aged 13, 17 and 25 months were either exercised in a forced running wheel apparatus or handled while the experimental animals were running. All mice were behaviorally tested on rotorod and MWM. The aim was to determine whether middle aged and older mice showed improvement in learning and memory as a consequence of forced exercise. Our first hypothesis, that deficits in performance of the behavioral tasks would be seen with age was supported. Middle-aged mice outperformed older mice on the rotorod and the MWM. The oldest mice had the longest latency to escape and covered the most distance in the MWM. A second hypothesis was that the runners would outperform the sedentary controls. The data only partially supported this hypothesis. Runners tended to outperform their sedentary controls on both rotorod and MWM, but the differences did not attain statistical significance on the rotorod. Forced exercise improved retention of spatial memory at a level that achieved statistical significance, and acquisition was improved in a few training sessions in 13- and 25-month mice. The third hypothesis that older mice would benefit from low impact forced exercise similarly to middle-aged mice was not supported, as runners showed some improvement on both the rotorod and the MWM, but these differences occurred only in the 13-month and 25-month mice. Cell sizes were generally small, so conclusions may be limited; however, effect sizes suggested that age differences were robust. Conclusions regarding exercise effects are more restricted as effect sizes were generally small.

Age differences

Results supported previous research suggesting age-related performance deficits on the rotorod.^{9,14} The 13-month mice outperformed the two older groups (17 and 25 months) at both 15 and 25 RPM speeds and 17-month mice outperformed 25-month mice at the 25 RPM speed. Age differences are likely supported by an age-associated decrease in cerebellar Purkinje neurons.^{29,30} There was no interaction between exercise and age, which suggests that the impact of the forced exercise was the same for all age groups. Significant age effects were reported in both latency to escape and distance covered in the acquisition portion of the MWM. The oldest mice showed impairment in both latency and distance. Age deficits in the MWM may be supported by reduced functional efficiency of the hippocampus,³¹ though the loss of Purkinje neurons in the cerebellum is likely involved as well.³²

Forced exercise and behavioral tests

Forced exercise has been associated, at varying speeds and intensities, with improvements in performance on both the rotorod in mice aged 3 months²⁰ and the MWM in rats aged 2-3 months.¹⁷ The present data show that runners outperformed the sedentary controls in each age group at both 15 and 25 RPM speeds on the rotorod, with the exception of the 17 month group. This result is in line with previous research; however, our results did not attain statistical significance. The absence of a statistically significant effect of exercise may be due to methodological issues including running speeds, training time, and differences between treadmill and wheel running. Fordyce and Wehner²⁰ incorporated a treadmill that included a metal shock grid to encourage running, as well as a faster training speed (12 m/min) and shorter training duration (8 weeks). Blustein and colleagues¹⁷ also used a treadmill operating at speeds much higher (25 m/min) than the speeds in the present study.

Another explanation for the present results is that improved rotorod performance may depend on changes in gait or motor strategy. Buitrago and colleagues²³ reported that enhanced physical fitness due to wheel running improved overall performance but did not alter the rate of learning; a change in motor strategy accounted for improvements. The differential impact of motor learning and physical exercise on rotorod performance is supported by differences in cerebellar plasticity. It has been reported that motor skill learning increases the number of synapses per Purkinje cell, whereas exercise leads to an increase in blood vessel density and no discernable increase in synapse number relative to inactive controls.³³⁻³⁵ The mice in the present study may have had increased blood vessel density but no increase in synapse number, which could account for the absence of a clear effect of exercise on motor learning.

Our results demonstrated that retention of spatial memory and, to a lesser extent, acquisition of spatial memory were improved after low impact forced exercise. Runners crossed the former location of the platform in the trained quadrant more often than controls when the platform was removed and covered less distance than controls in acquisition. These results are consistent with previous reports that both forced and voluntary exercise can improve acquisition and retention of spatial memory,^{28,36-38} though not all studies report exercise-induced benefits in spatial memory.³⁹ However, van Praag and colleagues¹¹ reported that mice with access to running wheels showed improved acquisition of spatial memory but no improvement in retention compared to controls. In the present study acquisition was significantly improved in terms of dis-

tance traveled, and only 13- and 25-month but not 17-month mice showed the effect. Aside from differences in the MWM protocol, discrepancies between the present data and previous research could be attributed to the exercise program implemented. The training paradigm used by van Praag and colleagues¹¹ involved voluntary running in wheels, which generally allows the animal to run a significantly longer distance, including 20,000-40,000 revolutions in a single day⁴⁰ and an average of 2.21 km/day.⁴¹ The mice in the present study ran approximately 190 m/day, which is less than the distance reported for 19-month-old mice (3.9 km/day) in van Praag and colleagues,¹¹ and may account for the absence of an exercise-induced improvement in spatial memory acquisition.

One limitation of the forced exercise paradigm is that increasing the intensity or duration of exercise to better approximate voluntary running distance leads to elevation in circulating stress hormones,^{42,43} which disrupts hippocampal function and impairs learning and memory.^{44,45} One goal of this study was to develop a forced exercise protocol that was not stressful, especially for the older mice tested. Based on both a predictive equation⁴⁶ and other published data,^{47,48} it can be estimated that animals in the present study were exercised at approximately 57% of maximal aerobic capacity. These rates suggest that aerobic requirements in the present paradigm are significantly less demanding and stressful than other forced exercise paradigms, though direct physiological data were not obtained. Given that both the effects of exercise and aerobic requirements were small in the current study, increases in speed and duration may lead to larger exercise effects while avoiding increases in stress.

Morris water maze, hippocampal neurogenesis, angiogenesis and age

One mechanism suggested to underlie improvements spatial memory after exercise is an increase in neurogenesis in the hippocampus.^{11,42,49} Age-related differences in neurogenesis have been reported,^{11,50} including reduced levels of neurogenesis in some regions of the dentate gyrus between 2 and 18 months of age in C57BL/6 mice.⁵¹ Changes included a decrease in proliferation but not survival of new neurons. No decline was reported between 18 and 24 months of age, though other studies have reported progressive decreases in hippocampal neurogenesis through 24 months.⁵⁰ Physical exercise can reverse the decline in neurogenesis in older mice to levels comparable to sedentary younger animals.¹¹ It is possible that the mice aged 13 months were still able to benefit from forced exercise due to higher levels of neurogenesis, although 25-month mice in our study did show some improvements in spa-

tial memory.

Another potential mechanism to support improvements in spatial memory is an increase in angiogenesis in the hippocampus.^{52,53} It has been reported that angiogenesis, and not neurogenesis, is critical for acquisition learning in rats.⁵⁴ Increased vascular supply to the brain may facilitate synaptic plasticity and enhance learning. Similar to neurogenesis, there are reductions in angiogenesis in older rodents⁵⁵ and both voluntary¹¹ and forced physical exercise⁵⁶ can enhance angiogenesis. In addition, improvements in angiogenesis in response to physical exercise vary depending on the age of the animal. van Praag and colleagues¹¹ reported increases in blood vessel size in young (3 months) but not old (19 months) C57BL/6 mice in response to voluntary exercise. The differential impact of forced exercise in this study may be supported by variations in the angiogenic response to exercise in mice aged 13, 17 and 25 months. Conclusions regarding mechanism remain speculative, though, as no measures of neurogenesis or angiogenesis were included in the current study.

Forced exercise benefits in younger and older mice

Results from both the rotarod and MWM suggested that the benefits of forced exercise were not similar at all ages of animals tested. Both 13-month and 25-month old runners showed a numerical advantage in motor learning, with the larger gains seen in the younger mice. Some limited improvements in latency to escape and distance covered in spatial memory acquisition were also reported in these two age groups. Enhancement of spatial memory retention was restricted to the youngest animals, whereas the 17-month runners showed no real improvements on either behavioral test. In previous studies and in our ongoing work, we have observed that some 17- to 18-month mice perform more poorly and have numerically fewer Purkinje neurons than many of the 24-month mice.^{30,57} This observation has led us to consider that selection factors may be operating. Not all 17-month mice will survive to 25-months, and we may be including in our samples of 17-month mice that are approaching death. Mice that survive to be tested at 25 months may have individual attributes that contribute to better performance and greater longevity. Baseline differences in motor learning and spatial memory were not assessed, so the present study does not include any longitudinal data regarding performance that could be used to identify successfully aging mice.

Results reported here on age differences in the effect of exercise are comparable to previous studies in the literature that suggest a differential impact of exercise depending on the

age of the organism that is tested. In an examination of different means to ameliorate learning and memory deficits⁷ it was reported that in order to reliably improve spatial memory performance in older (15 and 21 month) C57BL/6 mice, both exercise and other aspects of enrichment (toys and a combination of running wheels and toys) were necessary. For young mice (3 month), exercise alone improved spatial memory performance. Our results add to this literature suggesting that exercise alone is not sufficient to improve motor learning or spatial memory in 17-month C57BL/6 mice and leads to limited improvements in 25-month animals. Social interactions due to group housing may have contributed to the modest improvements in spatial memory and cannot be ruled out; however, larger improvements in 17- and 25-month mice would have been expected based on the environmental enrichment literature.

Conclusions

Examination of age-related differences, forced exercise-induced improvements, and the combined effects of age and exercise on motor learning and spatial memory indicated different results for motor learning and spatial memory. Overall, age was a more significant effect than exercise. For motor learning as assessed by the rotarod task, age was the most significant effect, with 13-month-old mice outperforming 17- and 25-month-old mice. Significant age differences in latency to escape and distance covered in MWM acquisition were also reported. In contrast, forced exercise had no significant effect on motor learning, but it improved spatial memory at a statistically significant level. Normal aging altered the beneficial impact of forced exercise, as spatial memory showed statistically significant improvements in runners over sedentary controls, primarily in 13-month-old mice. Sample sizes were small; however, effect sizes suggested that age deficits in motor learning were robust. Exercise effects on spatial learning were generally smaller, which may be attributed to sample size or exercise parameters. Increasing the running speed may enhance the modest improvements in spatial learning reported in this study.

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