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Age-related defects in sensorimotor activity, spatial learning and memory in C57BL/6 mice

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Abstract

Impaired locomotor activity and spatial memory are common features in the natural aging process, and aging is an important risk factor for neurodegenerative disease and postoperative cognitive dysfunction. To characterize age-related changes in psychomotor performance we assessed sensorimotor activity, spatial learning and memory in C57BL/6 mice using the Rotarod, foot fault and Barnes maze tests. Old mice exhibit significant deficits in locomotor activity and spatial memory relative to young mice, but improve with training. These tests will be useful to assess outcome in neurodegenerative disease and postoperative cognitive dysfunction models performed in aged mice.

Keywords

C57BL/6 mice; Postoperative cognitive dysfunction; aging; behavior; sensorimotor; spatial learning; memory

Sensorimotor deficits, and impaired spatial learning and memory are the most common consequences of the natural aging process¹ in the central nervous system in the absence of overt neurodegenerative disease.^{2–4} To assess these deficits, behavioural tests provide critical measures of the state of cognitive and locomotor systems, and are widely employed to evaluate outcome in animal models of aging, neurodegeneration, and postoperative cognitive dysfunction. As the hippocampus is important for memory acquisition and learning, assessment of hippocampal function is particularly important for studies in aged animals. Although age is a widely recognized risk factor for stroke and other neurodegenerative diseases, as well as for post-operative cognitive dysfunction,⁵ to date the majority of studies have been conducted in young adult rodents. This includes the common use of the Rotarod test and foot fault in rodent models of stroke.^{6–7} Although these tests have been used in animal models of brain injury,^{7–8} they have not been well studied in normal aging animals.

The Barnes Maze (BM) has been used to assess spatial learning in rodents.^{9–10} Experimental studies on cognitive aging in rats have revealed age-associated memory impairments that suggest a deterioration of hippocampal function.¹¹ While several behavioral studies have been performed in aged rats^{12–13}, there is a paucity of information regarding age-dependent deficits in cognition and locomotor activity in mice. To determine which of these tests could be used

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successfully in normal aging animals and therefore could be used in future studies of postoperative cognitive dysfunction or neurodegeneration we characterized the effect of aging on locomotor and cognitive performance in C57BL/6 mice.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice aged 2 (n = 12) and 18 months (n = 20) old were obtained from the National Institute on Aging (NIA) aged rodent colonies. Young animals were housed in groups of four, and old ones in individual cages, in a light- and temperature controlled environment, 12h light/12h dark schedule and food and water *ad libitum*. All experiments using animals were performed in accordance with a protocol approved by the Stanford University animal care and use committee and in keeping with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Behavioral testing

Motor coordination and learning were assessed with the Rotarod. Training on the Rotarod (San Diego Instruments) began with a constant low speed rotation (5 rpm) for 2 min on the first day of training. On all subsequent days each mouse was placed on the rod with rotation speed increasing from 5 to 10 rpm over 5 min. The time the mouse was able to stay on the rotating rod before falling was determined up to a maximum duration of 300 s. Each mouse was tested three times a day, 3 days/week for 3 weeks.

For the foot fault test, mice walk across a horizontal ladder. If a paw falls or slips between the bars it is recorded as a foot fault. In order to motivate animals to step only on the ladder and not fall through, they received one shock at trial 2 when they commit a foot fault. The total number of foot faults committed while crossing the ladder once was recorded for each trial.

For the Barnes maze, trials were recorded with a CCD camera connected to a desktop HP computer and analysed by Ethovision Pro v. 3.1 software (Noldus Information Technology). At the beginning of each trial, the mouse was placed in the same dark start chamber, and 1 min after turning on six bright lights, the start chamber was lifted and the mouse was free to explore the maze. Brightness was 8100 lux measured at the center of the arena (LX-102 Light Meter, Lutron, Taipei, Taiwan). Mice were trained 3 trials per day for 6 consecutive days. The following parameters were recorded: time the mouse took to escape into the box, total distance moved, mean velocity, and mean number of errors.

Statistical Analysis

All results were analyzed to determine the effect of aging alone, training alone, or the interaction between these two variables by repeated measures two-way ANOVA. The *t*-test was used when only two groups and one condition were compared, as in the case of body weight, with $p < 0.05$ considered to be significant. In most cases the standard deviations were comparable between young and old and between timepoints, so the *p* values determined by 2 way ANOVA should be reasonably reliable.

RESULTS

Body weight with Age

Changes in body weight are a common feature of aging. The young mice (age 2-3 months) weighed 26.08 ± 0.56 g, while the 18 months old mice weighed 35.47 ± 0.57 g, significantly different ($p < 0.0001$).

Rotarod Performance changes with Age

Both young and old mice improved performance over subsequent trials as evidenced by the increased latency to fall from the Rotarod (Fig. 1A). We also found interesting differences in motor coordination and motor learning between young and old. Significant main effects of age ($F = 166.46$, $DF=1$, $p < 0.0001$) and training ($F = 34.07$, $DF=8$, $p < 0.0001$) were observed. On the other hand, there was no interaction between age and training ($F = 1.40$, $DF=8$, $p = 0.19$). This reflects that old mice start from a worse baseline, but improve with training similar to young mice. Old mice have impaired motor performance on Rotarod compared to young subjects, both at the start of training and at the end of training.

Foot fault Performance changes with Age

Foot fault is a useful and sensitive test to evaluate locomotor activity and coordination. An effect of aging was observed in foot fault performance (Fig. 1B, $F = 73.99$, $DF=1$, $p < 0.0001$). As with Rotarod, both groups of animals improved significantly with training ($F = 7.30$, $DF=2$, $p = 0.0012$). A two-way ANOVA analysis showed significant interaction between age and training, demonstrating that both performance and learning differ between young and old on foot fault ($F = 3.43$, $DF=2$, $p = 0.03$), in contrast to Rotarod.

Barnes Maze performance with Age

On Barnes Maze a decrease in latency (Fig. 2A) was observed for each group of mice, indicating that they learned the task over the 6-day training period. However, we observed that old animals spent more time in the arena than did young mice. Two-way ANOVA showed no interaction between age and training response. On the other hand, each factor alone, age ($F = 3.87$, $DF=1$, $p = 0.05$) and training ($F = 6.34$, $DF=5$, $p < 0.0001$) affects the performance on Barnes Maze.

The distance moved (cm) from the center to enter the escape box was analyzed in the same way as for latency. Two-way ANOVA showed that although age did not affect the distance moved (Fig. 2B, $F = 0.02$, $DF=1$, $p = 0.88$), training significantly improved performance in both young and old animals ($F = 9.23$, $DF=5$, $p < 0.0001$); interaction between these two variables was not significant ($F = 1.09$, $DF=5$, $p = 0.36$).

The mean velocity during Barnes maze performance (Fig. 2C) is affected by age ($F = 16.19$, $DF=1$, $p < 0.0001$), with an increasing value over the period for both groups. Overtraining does not influence velocity in either group ($F = 2.10$, $DF=5$, $p = 0.06$), neither is there an interaction between velocity and age ($F = 0.92$, $DF=5$, $p = 0.46$). The total number of errors decreased during training in both groups, indicating that mice of both ages learned to use spatial orientation and cues as reference points. However, as shown in Fig. 2D, old mice committed fewer errors than did young animals. Training also decreased the number of errors ($F = 5.81$, $DF=5$, $p < 0.0001$), however the interaction between age and training was not significant ($F = 0.44$, $DF=5$, $p = 0.81$), indicating that while young and old start at different baselines, they improve similarly with training when scored in this manner.

During Barnes maze, some mice, especially young subjects, lost interest in entering the escape box, even though they learned the association between the spatial room cues and the escape location. Lack of motivation is likely since right after finding the hole without entering it, they start exploring the maze. Another relevant observation is that some mice stay in the escape box area, look inside, but do not enter, an observation also reported by others.¹⁴ Due to these observations a previous report¹⁵ proposed a different analysis based on calculating latency, path length, and number of errors until the first encounter of the escape hole, called primary latency, primary path length, and primary errors, respectively.

We analyzed these additional parameters using two-way ANOVA. Aging differences for primary latency (Fig. 3A, $F = 16.66$, $DF=1$, $p < 0.0001$), primary path length (Fig. 3B, $F = 4.52$, $DF=1$, $p = 0.03$) and primary errors (Fig. 3C, $F = 41.20$, $DF=5$, $p < 0.0001$) to reach the escape box were observed. As observed in Fig. 3, old mice needed more time, travelled a longer distance, and committed more errors than did young animals before first encountering the escape box. Training significantly decreased the primary time to reach the escape hole ($F = 16.54$, $DF=5$, $p < 0.0001$), the distance travelled to reach the escape hole area ($F = 13.55$, $DF=5$, $p < 0.0001$), and mean number of errors ($F = 19.16$, $DF=5$, $p < 0.0001$). There was a significant interaction between age and training in primary latency ($F = 3.87$, $DF=5$, $p = 0.002$), primary path length ($F = 6.67$, $DF=5$, $p = 0.0001$) and mean primary errors ($F = 6.94$, $DF=5$, $p = 0.0001$). Thus when the more sensitive assessments of times and distances to the first approach to the escape box are analyzed they reveal impaired learning as well as impaired initial ability in the old mice.

DISCUSSION

The results presented provide a detailed analysis of motor learning and performance and spatial memory in 2 and 18 months old mice. We observed a statistically significant dependence of the performance of the 18 months old mice on time/training, which suggests that these tests may be effectively used in mouse neurodegeneration or postoperative cognitive dysfunction models that use old animals. The performance of 18 months old B6 mice showed reduced motor skills, with diminished performance on the Rotarod, and impaired motor coordination on the foot fault test compared to 2 months old mice. Our first observation is that performance at the start of training in both Rotarod and foot fault is different between the groups. Old animals spent less time on the rod on the first day of training, demonstrating decreased balance and motor skills compared to young mice. In foot fault performance old mice committed more errors, and this value decreased with training. Our results are consistent with a previous report showing an age-related impairment in the Rotarod task.¹⁶

Over the training period both young and old mice learned the Barnes Maze task, and our results show that young mice spent less time and committed fewer errors to reach the escape box for the first time than did old animals. Consistent with some previous studies, we found a significant age-related defect in spatial memory on Barnes Maze performance¹⁷, and training was able to decrease this deficit. The finding of an age-related spatial memory deficit in this study is consistent with previous findings in C57BL/6 mice on Morris water maze⁸ and T-maze,¹⁸ and other strains of mice,¹⁰ as well as with previous studies in rats.^{12,19} Although the old mice acquire the spatial task more slowly than young mice, they improved with training and reached a plateau comparable to the performance of young mice by day 4 in most parameters of the Barnes Maze.

While some studies have focused on aging-related effects on behavior in senescence-accelerated mice,^{3,20} and other transgenic mice,²¹ it is important to note that this study was conducted with normal aging mice. The Morris water maze has been the most commonly used task to assess hippocampal dependent cognition in different models of aging.²²⁻²⁴ However, there are several advantages of dry mazes over the water maze for mice in that they do not like to swim, get chilled easily, and especially in elderly mouse subjects may not perform the task, due to fatigability or lack of motivation. Recently a few studies have assessed learning and cognitive impairment in old mice using Barnes maze,²⁵⁻²⁶ however only middle-aged or transgenic animals were used rather than normal aging subjects.

Rotarod and foot fault are widely used in animal models of stroke,^{7, 27-29} however BM is used much less frequently, yet it is useful as it involves both motor and spatial memory components. To date the Barnes maze has not been used to assess cognitive function in animal models of

postoperative cognitive dysfunction. Since memory impairment is a key feature of postoperative cognitive dysfunction, and age is an important risk factor,⁵ Barnes maze performance should be useful in models of postoperative cognitive dysfunction that employ aged mice. In the present study we show that all three of these behavioral tasks are useful in normal aging animals and are thus suitable for both normal aging-related studies and assessment of loss of function and recovery in models of neurodegeneration.

A number of factors must be taken into consideration when analysing the results of the present study. First, the older mice are heavier and this may affect their motor performance and contribute to the differences observed.³⁰ Second, young and old animals originated from the same breeding colonies (NIA aged rodent colony) to minimize differences due to rearing and housing conditions, however, the young mice were group housed, while the older mice were single housed. Mice may be individually housed because of experimental considerations including minimizing aggression; however, studies have shown that individual housing can result in physiological, neurochemical, and behavioural changes in animals.³¹⁻³² The magnitude of the effects of individual housing is influenced by the strain, sex, and age of the animals when they are individually housed, with less difference observed in C57BL/6 mice.³³ Thus, both age and housing likely contribute to the observed differences. Since C57BL/6 mice showed less change in behavior with single housing than other strains, and since there is a large body of data on decreased behavioral performance with age, it is likely that the difference in age contributes to the differences observed here. However, in the current study we cannot separate the contributions of age and housing.

This study was limited to male mice because the majority of stroke studies are conducted in male mice. It will however, be important to perform similar studies in female mice. Males have also often been used in behavioral studies to eliminate the difficulty of assessing behaviour at matched times during the oestrous cycle in females. Furthermore, the spatial perception and memory of males have been reported to be superior to those of female mice.³⁴ Another consideration is the age of mice tested. Here we studied 18 months old mice, which represent a suitable age for studying age-related cognitive decline.¹⁷ Future studies should be performed to more fully assess the time course of progressive decline using animals of several different ages and both sexes.

In conclusion, we show a difference in motor coordination and spatial memory acquisition between C57BL/6 mice of 2 and 18 months of age. The older animals are able to learn Rotarod, foot fault and BM, so these tests may be effectively used to assess function in neurodegeneration and postoperative cognitive dysfunction models in aged mice.

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Figure 1.

Age-related changes in sensorimotor performance. (A) Effect of aging on the average time to fall off the Rotarod. Day 1 represents baseline performance, maximum time is 300 seconds.

(B) Age-related performance on Foot Fault test. Data are expressed as Mean $\pm$ S.E.M.

Figure 2.

Effect of aging on Barnes Maze performance. (A) Mean Latency (s) to enter the escape box. (B) Total Distance travelled (cm) to get into the escape box. (C) Mean Velocity (cm/s) during performance. (D) Mean Number of Errors. Data are expressed as Mean $\pm$ S.E.M.

Figure 3.

Effect of aging on the primary Barnes Maze parameters. (A) Primary Latency (s) to approach the escape box. (B) Primary Path Length (cm) to reach the escape box. (C) Mean Primary Errors to first approach the target box in the maze. All mice improved their performance; however young mice had shorter primary latency and fewer primary errors than did aged mice. Data are expressed as Mean $\pm$ S.E.M.