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Age-related deterioration of ability of acquisition in memory and learning in senescence accelerated mouse: SAM-P/8 as an animal model of disturbances in recent memory

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Memory, learning and behavior of senescence accelerated mouse (SAM-P/8) were investigated by using passive avoidance response, T-maze and open field and the findings were compared with those from senescence resistant mouse (SAM-R/1 control). SAM-P/8 mice showed a remarkable age-related deterioration in ability of memory and learning in passive avoidance response. This age-related memory and learning deficit was linked to a deterioration in the ability of acquisition and was not due to impairment in the ability of retention and hyperactivity, as observed in the open field. In the alternation T-maze tests, SAM-P/8 showed as high a rate of alternations as did the SAM-R/1 and in the T-maze avoidance tests, SAM-P/8 also showed as intact a memory ability as seen in the SAM-R/1, despite a memory deficit in the passive avoidance response. Thus, SAM-P/8 may prove to be a pertinent model for researching mechanisms related to the memory deficit seen in senile humans.

INTRODUCTION

Several pairs of AKR strain mice were donated to our laboratory by the Jackson Laboratory (Bar Harbor, ME), in 1968. We continued the sister–brother mating of these mice and became aware of the presence of certain litters in which most of the mice showed a moderate to severe loss of activity, hair loss and lack of hair glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis of the spine and a shortened life span, despite the relatively low incidence of thymic lymphoma. We selected and maintained 4 substrains with severe exhaustion as 'accelerated senescence prone' (P-series) and 3 substrains with a normal aging process as 'accelerated senescence resistant' (R-series), the former 4 series are -P/1, -P/2, -P/3 and -P/4 and the latter 3 series are -R/1, -R/2 and -R/3. Judging from findings in the survivors and for Gompertzian functions[6] together with the growth pattern in body weight, it seems that senescent manifestations in this model do not occur early in life, i.e., in the developmental stage, but do occur in an accelerated manner after the normal development, namely, the pattern of aging in this model seems to relate to an accelerated senescence rather than to a premature aging or senescence. Thus, the P-series was named 'Senescence Accelerated Mouse' (SAM)[21]. The strains of the P- and R-series have been designated SAM-P/1, -P/2, -P/3 and -P/4, and SAM-R/1, -R/2 and -R/3, respectively[9]. In addition to 'accelerated senescence' as a common characteristic in the SAM-P strains, there were several pathologic events closely associated with senescence, including systemic senile amyloidosis in SAM-P/1, -P/3 and -P/4 (refs. 7, 13, 20, 21), systemic secondary and senile amyloidosis in SAM-P/2 (refs. 7, 20, 21) and senile cataract in SAM-P/3 (ref. 10).

Recently, new strains, SAM-P/6 and SAM-P/8 have been separated from SAM-R/3 and SAM-P/2, respectively. SAM-P/6 has a characteristic pheno-

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type; severe senile osteoporosis[14] and SAM-P/8 shows a low incidence of senile amyloidosis, despite the accelerated senescence[15]. Further, it was reported that SAM-P/8 showed characteristic age-related learning and memory deficits in passive avoidance responses, determined under specific pathogen-free conditions[15].

We have now examined age-related changes in memory and behavior of SAM-P/8, under conventional conditions and using passive avoidance response, T-maze and open field tests. From the behavioral tests, we found that the remarkable deficit in memory of SAM-P/8 in the passive avoidance response was mainly due to a deterioration in the ability in acquisition and not to a deficit of ability in retention and hyperactivity observed in the open field. Despite the memory deficit in passive avoidance, the SAM-P/8 showed the same intact learning and memory in T-maze as did the SAM-R/1 controls. Different memory-related mechanisms may be operative between passive avoidance and T-maze.

MATERIALS AND METHODS

Tested mice and housing

Male SAM-P/8 mice and SAM-R/1 controls bred under conventional conditions, at the Chest Disease Research Institute, Kyoto University, were used at age of 1, 2, 4, 8, 9, 10, 11 and 12 months. All these mice were housed in group cages (average area of 0.01 m^2/mice) and had free access to pellet food (CLEA Japan) and tap water at a temperature of 24 ± 2 °C under a 12 h light–dark cycle with lights on 07.00 h. All the tests were performed between 13.00 and 19.00 h and the background noise was constant.

Tests at different ages were done using different groups of mice and a mouse used in one test was never used for other tests.

A single trial passive avoidance test

Passive avoidance tests were carried out in a dark room. A two compartment step-through passive avoidance apparatus was used. A front, illuminated chamber (8 × 8 cm; 25 cm height) was connected to a second, dark chamber (25 × 25 cm; 30 cm height) equipped with a grid floor, the two chambers being separated with a guillotine-type door (5 × 3 cm). After each mouse had been individually placed in the 'waiting box' (12 × 12 cm; 30 cm height) from its home cage for 1–2 h, it was put in the front chamber, the floor of which was brightly lit from above 40 cm by a 20 W bulb (750 lux at the floor). After a 10 s 'orientation period' the guillotine-type door was raised, allowing the mouse to freely explore the apparatus. Once the mouse entered the rear chamber on all 4 paws, the guillotine-type door was quickly closed and an AC 0.5 mA scrambled footshock (Shock generator scrambler BSG-104, Biomedica) was applied to the floor grid, for 3 s. The latency to entry into the dark chamber (i.e. latency before shock) was recorded. Following this single trial, the mouse was returned to its home cage. Retention of the passive avoidance response was examined by replacing the mouse in the front chamber 24 h after the training trial, and the latency to enter the dark chamber was measured. When the mouse failed to enter the dark chamber within 300 s, the test was terminated and a ceiling score of 300 s was assigned.

Acquisition test

The same passive avoidance apparatus and 'waiting box' as in a single trial passive avoidance test were used in the test. After being for 1–2 h in the 'waiting box', the mouse was put in the light chamber in the passive avoidance apparatus. When the mouse entered the dark chamber, a 1 s, 0.5 mA footshock was given, then the mouse was immediately removed from the dark chamber and returned to the 'waiting box'. One min later, the mouse was re-introduced to the light chamber. Acquisition of the avoidance response was judged successful if the mouse remained in the dark chamber for 300 s. The mouse entering the dark chamber within 300 s immediately received an identical shock (1 s, 0.5 mA) after closing the guillotine-type door, and the mouse was returned to the 'waiting box'. The trial was repeated until the mouse satisfied the acquisition criterion (300 s) and the number of the trials was recorded. When the mouse entered the dark chamber, despite 5 trials, the acquisition test was terminated and a ceiling score of 5 was assigned.

Retention test

The retention test was begun after the mouse had achieved a criterion (300 s) in the acquisition test. Three SAM-P/8 mice not reaching the criterion de-

spite the 5 trials in the acquisition test, were not given this test. The retention time (criterion of 300 s) in the light chamber was measured one day and one month after confirming the acquisition.

Shock sensitivity measurements

Shock sensitivity thresholds were determined using the flinch-jump threshold method[2,11]. An ascending series of footshocks with a 30 s interval presentation was administered stepwise (0.05 mA) from 0.05 to 0.7 mA to the mice and behavioral responses (flinch, jump and vocalization) were recorded at each intensity. Two kinds of shock durations (1 and 3 s) were adopted. Thresholds for flinch, jump (defined as a minimum of 2 paws leaving the grid) and vocalization (any auditory response) were defined as the lowest shock intensity eliciting each response.

Spontaneous alternation T-maze test

Testing of spontaneous alternation was conducted in a T-maze (stem: 30 × 6; arms: 24 × 6; height: 15 cm) made of gray plastic. A sliding door partitioned the first 8 cm of the stem as a 'start compartment'. The mice were placed in the maze and allowed to explore it for 5 min per day. There was no food or water in the T-maze. After 3 days of this pretraining, the spontaneous alternation test was carried out as follows. Each mouse was placed into the start compartment of the stem. Ten s later, the sliding door to the runway was raised. After entering one of the arms, the mice were gently removed and returned to the start compartment, and 10 s later a second trial was initiated. Directions of choice runs were recorded for each mouse and the rate of alternation was obtained. Such two trials were conducted daily for 3 consecutive days. The floor in the T-maze was illuminated with ceiling fluorescent lights (250 lux at the floor). The T-maze was cleaned between placement of each animal.

T-maze avoidance test

A gray plastic T-maze equipped with a grid floor was used. Alleys (stem and arms) were 30 × 8 × 27 cm and a choice area was 8 × 8 × 27 cm. Start and goal boxes were 12 × 12 × 27 cm. Vertical sliding doors were inserted between the start box and the stem and between the choice area and the arms. After 3 days of the same pretraining as was done in case of the spontaneous alternation test, the T-maze avoidance test[3], which consisted of a forced and choice run, under stay conditions[19] (the direction of choice run is the same as that of the forced run), was carried out as follows. Each mouse was placed into the start box. Ten s later, the sliding door to the stem was raised and an AC 0.5 mA scrambled shock was applied to the floor grids. On the first forced run, one of the maze arms was blocked by a sliding door and the mouse was forced to enter the other goal box which was free of footshock. After entering the goal box, the mouse was immediately and gently removed and returned to the start box for the choice run. Thirty s later, the choice run was initiated. The choice run was the same as the forced run, except that all the sliding doors were raised and the mouse was free to choose either goal box. The mouse could avoid the footshock only when it entered the same goal box as in the forced run (correct goal box). When the mouse entered the incorrect goal box, the footshock was continued until the mouse entered the correct goal box. Directions of the forced run followed an irregular but balanced schedule (Gellermann's series[4]), and half the number of mice examined was given one direction of the runs (r,r,l,l,l,r,r,l,r,l), the other half the opposite direction (l,l,r,r,r,l,l,r,l,r). The test was done once daily for 10 consecutive days and the percent of correct choices (percent correct) in the stay condition was recorded. T-maze (250 lux at the floor) was cleaned between placement of each mouse.

Open field

The open field box was 72 cm square with walls 50 cm high and was constructed of gray plastic. The floor was sectioned by thin black lines, divided into 64 squares of 9 × 9 cm each. The field was illuminated with ceiling fluorescent lights (250 lux at the floor). The mouse was placed in a certain one of the center 4 squares in the field and behavior was recorded by a trained observer, non-stop for 3 min. The time spent in the first square and the number of squares entered, on all 4 paws were recorded. The box was cleaned between placement of each mouse.

RESULTS

Shock sensitivity

Flinch, jump and vocalization thresholds in SAM-

P/8 and SAM-R/1 at various ages were evident for two kinds of shock durations (1 and 3 s) (Table Ia,b). These durations had no effect on the thresholds in SAM-R/1, while jump and vocalization thresholds in SAM-P/8 depended on the shock duration: the thresholds were lower for 3 s duration than for 1 s duration (at and over age 4 months for flinch and at all ages for vocalization). In both the SAM-P/8 and SAM-R/1, the flinch threshold differed little between the two groups. The jump threshold showed no difference between the two groups in case of a 3 s duration, however that for 1 s was higher in SAM-P/8 than in SAM-R/1 at and over age 4 months. Vocalization thresholds for both 1 and 3 s durations was higher in SAM-P/8 than in SAM-R/1, at all ages examined.

A single trial passive avoidance response

There was no significant difference in the latency before footshock between SAM-P/8 and SAM-R/1 except at age 2 and 10–12 months (Fig. 1a). After 3 s–0.5 mA footshock, most of SAM-R/1 controls at all ages tested reached the criterion (300 s), while the number of SAM-P/8 reaching the criterion decreased with advancing age ($U_{8,12} = 56$, $P < 0.05$ at age 8–9 months, $U_{8,16} = 56$, $P < 0.01$ at age 10–12 months) (Fig. 1b). Mean latency after the footshock in SAM-P/8 also decreased with advancing age. Thus, SAM-P/8 showed age-related deterioration in the passive avoidance performance.

Acquisition test

Acquisition trials needed to achieve the criterion (300 s) were fairly constant, independently of age in the SAM-R/1 controls (Fig. 2). The trials in SAM-P/8 increased with advancing age, and there was a significant difference in the trials between SAM-P/8 and SAM-R/1 at and over age 4 months ($U_{11,10} = 79$, $P < 0.05$ at 4 months, $U_{10,10} = 63$, $P < 0.01$ at 8–9 months, $U_{10,11} = 66$, $P < 0.01$ at 10–12 months). Thus, SAM-P/8 showed a remarkable impairment in ability of acquisition in the passive avoidance test.

TABLE I

Age-related changes in shock sensitivity in SAM-P/8 and SAM-R/1

Each value represents the mean threshold (mA, AC). Numbers of mice used are given in parentheses.

Age (months)	*Mean threshold*				
	Subject	*Flinch*	*Jump*	*Vocalization*	
(a) 1 s shock duration					
1	SAM-R/1	0.10	0.25	0.30	(8)
	SAM-P/8	0.10	0.23	0.42*	(8)
2	SAM-R/1	0.12	0.24	0.36	(8)
	SAM-P/8	0.11	0.27	0.67**	(15)
4	SAM-R/1	0.13	0.27	0.34	(10)
	SAM-P/8	0.14	0.38*	0.70**	(16)
8–9	SAM-R/1	0.13	0.24	0.32	(10)
	SAM-P/8	0.16	0.36*	0.69**	(10)
10–12	SAM-R/1	0.13	0.27	0.33	(11)
	SAM-P/8	0.18	0.34*	0.72**	(11)
(b) 3 s shock duration					
1	SAM-R/1	0.10	0.23	0.28	(8)
	SAM-P/8	0.10	0.25	0.35	(8)
2	SAM-R/1	0.11	0.28	0.33	(8)
	SAM-P/8	0.13	0.29	0.47*	(8)
4	SAM-R/1	0.12	0.26	0.30	(8)
	SAM-P/8	0.13	0.32	0.44*	(8)
8–9	SAM-R/1	0.12	0.26	0.30	(10)
	SAM-P/8	0.16*	0.33	0.45*	(12)
10–12	SAM-R/1	0.14	0.27	0.34	(10)
	SAM-P/8	0.16	0.33	0.45*	(10)

*$P < 0.05$, **$P < 0.01$ (Mann–Whitney U-test) for SAM-R/1.

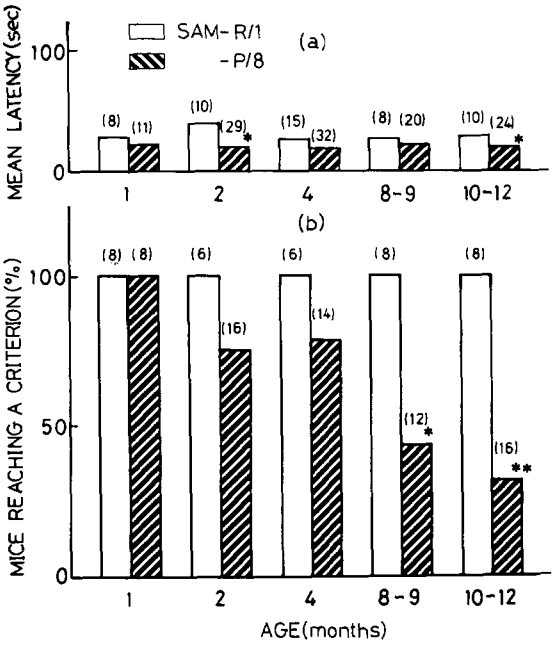

Fig. 1. Age-related changes in a single trial passive avoidance response in SAM-P/8 and SAM-R/1. a: mean latency before footshock. b: numbers of mice reaching a criterion (300 s) after footshock. $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney U-test) for SAM-R/1 controls.

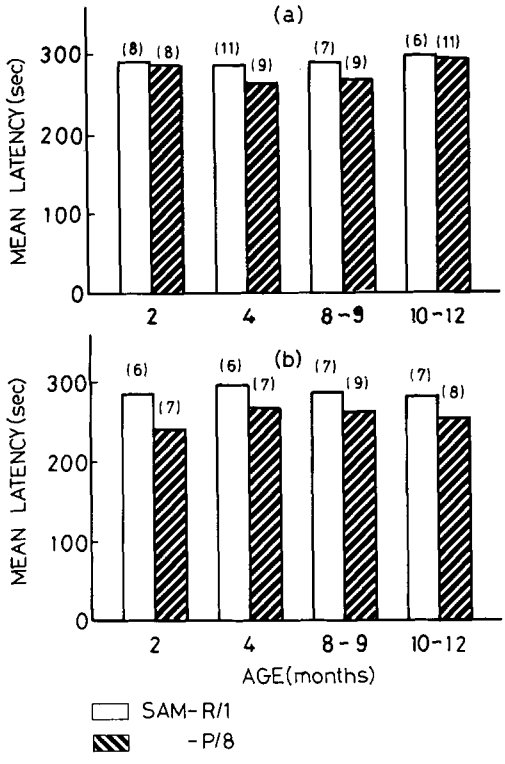

Fig. 3. Age-related changes in ability of retention in SAM-P/8 and SAM-R/1. The retention test was carried out (a) one day and (b) one month after the mice had satisfied the acquisition criterion.

Retention test

Most of SAM-P/8 and SAM-R/1 reached the retention criterion (300 s) both 1 day and 1 month after the mice had achieved the acquisition criterion (Fig. 3a,b) and there was no statistically significant difference between the two groups. Thus, memory retention in SAM-P/8 was all but completely intact.

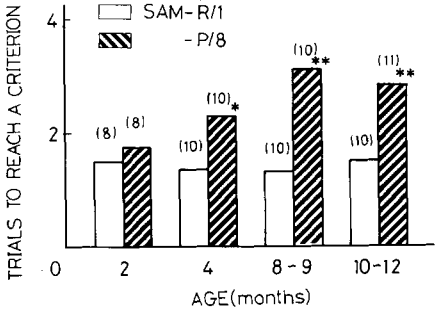

Fig. 2. Age-related changes in ability of acquisition in SAM-P/8 and SAM-R/1. An ability of acquisition was represented as number of trials to achieve a criterion (300 s). $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney U-test) for SAM-R/1 controls.

Spontaneous alternation T-maze test

Rate of alternation on two trials was examined for SAM-P/8 and SAM-R/1 at ages 2 and 8 months (Table II). The rate for 3 consecutive days was between 80 and 100% in both strains, and with no statistically significant difference.

TABLE II

Spontaneous alternation in T-maze in SAM-P/8 and SAM-R/1

Each value represents percent (%). Numbers of mice used are given in parentheses.

Age (months)	*Rate of alternation*				
	Subject	*Day 1*	*Day 2*	*Day 3*	*Average*
2	SAM-R/1	80	100	90	90 (10)
	SAM-P/8	80	90	90	87 (10)
8	SAM-R/1	90	80	90	90 (10)
	SAM-P/8	80	100	80	87 (10)

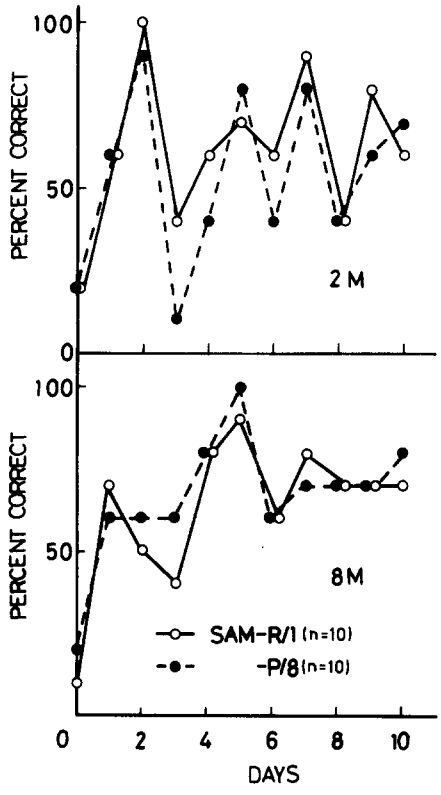

Fig. 4. T-maze avoidance performances in SAM-P/8 and SAM-R/1. The test was done once daily for 10 consecutive days.

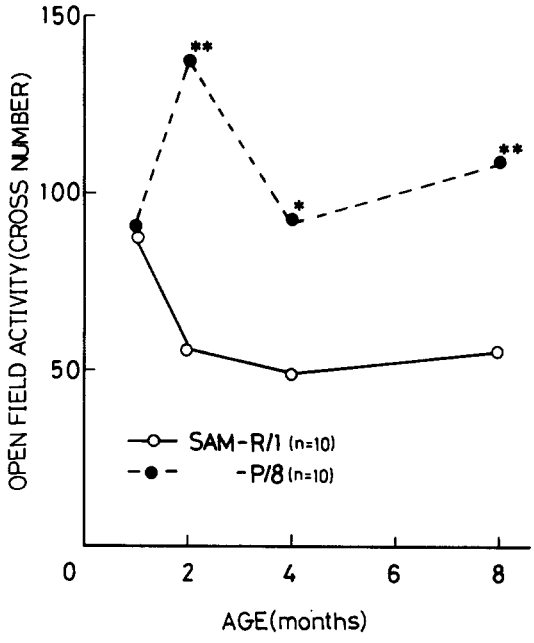

Fig. 5. Open field performances in SAM-P/8 and SAM-R/1. The number of squares entered on all four paws were recorded, non-stop for 3 min. $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney U-test) for SAM-R/1 controls.

T-maze avoidance

Percentage correct in the T-maze avoidance under the stay condition was very low in SAM-P/8 and SAM-R/1 when the footshock was not given (i.e. day 0) (Fig. 4) as was expected from the result in the alternation test. In the first trial (i.e. 1st day) both SAM-P/8 and SAM-R/1 at age 2 and 8 months chose the correct arm at a level of about 60%, despite the stay condition[19]. Though the (percent correct) tended to decrease when the direction of the forced run was altered, it finally reached a constant seventy. There was no significant difference in the T-maze avoidance response between SAM-P/8 and SAM-R/1.

TABLE III

The time spent in the first square of the open field in SAM-P/8 and SAM-R/1

Each value represents time (s). Numbers of mice used are given in parentheses.

Subject	*Age*			
	1 Month	*2 Months*	*4 Months*	*8 Months*
SAM-R/1	5.3 (11)	10.8 (11)	6.3 (12)	6.8 (11)
SAM-P/8	8.3 (12)	4.1 (12)	9.3 (12)	5.3 (12)

Open field

There was no significant difference in the time spent in the first square between SAM-P/8 and SAM-R/1 (Table III). However, regarding the number of squares entered, the number in SAM-P/8 was larger than that in SAM-R/1 at and over age 2 months ($U_{11,12} = 71$, $P < 0.01$ at 2 months, $U_{12,12} = 120$, $P < 0.05$ at 4 months, $U_{11,12} = 72$, $P < 0.01$ at 8 months) (Fig. 5). Thus, SAM-P/8 showed high exploratory behavior and/or hyperactivity in the open field.

DISCUSSION

The SAM-P/8 strain bred under conventional conditions shows age-associated deficits in ability of memory in passive avoidance response seen in the SAM-P/8/Ta bred under SPF conditions[15]. However, the thresholds for flinch and jump tended to be

higher in SAM-P/8 raised under the conventional conditions. This difference seems to be related to differences between single and group housings and not to differences between conventional and SPF conditions, because the single-housed animals showed a high reactivity compared to findings in the group housed animals[5]. Regarding the thresholds of SAM-P/8 and SAM-R/1 raised under conventional conditions, the flinch threshold hardly differed between the two groups, but the vocalization threshold was significantly higher in SAM-P/8 than in SAM-R/1, a finding which suggests that the high vocalization threshold is related to disturbances in a path from sensory mechanism to motor function for vocalization and/or in motor function itself.

The results in acquisition and retention tests clarified that the deficits in memory of SAM-P/8 observed in the single passive avoidance tests were mainly due to a deterioration in the ability in acquisition and not due to a deficit in the ability in retention. Though the SAM-P/8 showed a high exploratory and/or hyperactivity in the open field, this hyperactivity does not seem to be related to a deterioration in the passive avoidance performance, because most of the SAM-P/8 which had once achieved the acquisition criterion never entered the dark component in the passive avoidance apparatus, despite their hyperactivity. Thus, it is concluded that the deterioration of learning and memory in the SAM-P/8 strain is caused by deficits in ability in acquisition and not by deficits in ability in retention and disturbance of the behavior.

On the other hand, there was no significant difference in spontaneous alternation and T-maze avoidance tests between SAM-P/8 and SAM-R/1. In the alternation T-maze test, SAM-P/8 and SAM-R/1 showed a high rate of alternation, as seen in rats and the other strains of mice[1,12]. In the T-maze avoidance response, neither the SAM-P/8 or SAM-R/1 reached (percentage correct) 100%. The percentage (70%) in this test seems reasonable, as percent of correct responses decreased with an increase in delay between the forced run and choice run. The percentage was expected to be 70–80 for the delay (30 sec) in this experiment, as deduced from the result of Stanton et. al.[19]. The T-maze tests reflect working and reference memories[8,17,18] and are closely related to septal and/or hippocampal damage[1,3,16,19]. Therefore, the almost intact T-maze performance of the SAM-P/8 strain suggests that SAM-P/8 has little or no impairment of working and reference memories and little or no septal and/or hippocampal damage, at least up to age 8 months.

Thus, the SAM-P/8 mice show a spontaneously occurring age-related memory and learning deficits and may prove to be a valuable model for researching mechanisms related to memory and behavior. The SAM-P/8 may give new insights regarding ability in acquisition.

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