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BRIEF COMMUNICATION

Age-Related Impairment in Learning but not Memory in SAMP8 Female Mice

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FLOOD, J. F., S. A. FARR, F. E. KAISER AND J. E. MORLEY. *Age-related impairment in learning but not memory in SAMP8 female mice.* PHARMACOL BIOCHEM BEHAV **50**(4) 661-664, 1995.—Four-month and 12-month-old SAM (Senescence Accelerated Mouse)-P8 and -R1 female mice, either intact or ovariectomized, were trained to avoid foot shock in a T-maze. Mice were trained until they made their first avoidance. Memory retention of this task was then tested 1 week later. The results indicated that P8, but not R1, female mice whether intact or ovariectomized, showed an age-related learning impairment. This impairment was more apparent in ovariectomized mice, as ovariectomy was associated with a significant reduction in the mean trials to make an avoidance in all groups except 12-month P8 females. Of greatest interest was the absence of any age-related impairment of retention in P8 females. In several previous studies, SAM-P8, but not R1, males showed an age-related impairment of learning and memory. These results indicate that age-related impairment of memory in P8 mice may be inherited in a sex-related manner, and suggests that the mechanisms involved in the development of impaired learning and memory are different.

Aging Aversive conditioning Female Inheritance Learning Memory Mice Progesterone

SAM (Senescence Accelerated Mouse) P8 mice show an age-related impairment of learning and memory across a variety of appetitive and aversive training tasks (1-4). This impairment begins at 6-8 months of age. The R1 strain, which is closely related genetically, does not show an early onset of impaired learning and memory. For example, using T-maze foot shock avoidance training, male P8 mice from 2 to 12 months of age show a progressive impairment of learning from 9 to 12 months of age and progressively impaired retention from 8 to 12 months of age. Yet, over the same range of ages and even up to 16 months of age R1 mice show no loss of learning and memory (2). Previous studies have indicated that this impairment cannot readily be explained in terms of decreased sensitivity to foot shock or decreased activity (1,3,4). Although research done on male mice of these strains suggests that they may be a useful model for age-related cognitive failure, no studies have been conducted on female mice of the strains. The purpose of this study was to determine if female mice show the same age-related impairment of learning and memory as the males on T-maze foot shock avoidance training.

METHODS

SAMP8/TaJf (P8) female mice 4 and 12 months of age and SAMR1/TaJf (R1) female mice were obtained from our breeding colony. The colony has been maintained as an inbred strain from siblings generously provided by Dr. T Takeda of Kyoto University, Japan, for 8 years under clean-room procedures (i.e., use of sterile gloves in handling mice, sterilized cages and bedding, restricted access to breeding area) but not a barrier free environment (1,2). Rooms were maintained on a 12 L : 12 D cycle, with lights on at 0600 h. Mice were

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trained and tested during the light cycle. All subjects were experimentally naive and virgin. We have previously reported that the median life expectancy was 17.2 months for our male P8 mice, compared to 21.0 months for the male R1 mice (2). Longevity data is not yet available on the female mice.

One month prior to training, P8 and R1 mice, 4 and 12 months of age, were ovariectomized with 15 mice per group. Mice were anesthetized with methoxyflurane (Metofane) until a toe pinch failed to elicit a reflex reaction. The hair on the abdomen was washed with Hibiclens and a 1/2″ incision was made in the abdomen. The skin and muscle were incised, the ovaries removed, and the wound sutured with silk. The mice were returned to clean cages containing autoclaved bedding. Surgery was performed under aseptic conditions.

Progesterone plasma values (ng/ml) were used to varify the success of the ovariectomy. In other work, we (Farr et al., unpublished observations) found that poor acquisition on the T-maze foot shock avoidance task was related to high levels of progesterone and not estrogen. Progesterone was determined by a nonextraction solid phase radioimmunoassay (Coat-A-Count Progesterone Kit, Diagnostic Products Corp., Los Angeles, CA). The lowest limit of detection for the assay is 0.03 ng/ml. No crossreactivity occurred with androstendiol, estradiol, medroxyprogesterone, pregnane, pregnenolone, or testosterone. There is 2.4% crossreactivity with 11-deoxycortisol, 2.0% with 20-α-dihydroprogesterone, 1.7% with 11-deoxycorticosterone, 0.4% with corticosterone, and 0.3% with 17-α-hydroxyprogesterone.

Mice were trained to avoid receiving foot shock in a T-maze. The apparatus, training, and testing procedures have been previously described (2). The maze consisted of a black plastic start alley with a start box at one end and two goal boxes at the other. A stainless steel rod floor ran throughout the maze. The start box was separated from the start alley by a plastic guillotine door that prevented the mouse from moving down the alley until the training started. The intertrial interval was 45 s with a doorbell-type buzzer (65 dB) as the conditioned stimulus to warn the mice of impending foot shock of 0.35 mA.

A training trial started when a mouse was placed into the start box. The guillotine door was raised, and the buzzer sounded simultaneously. After 5 s, foot shock was applied. The goal box first entered on the first trial was designated as incorrect. The foot shock continued until the mouse entered the other goal box, which on all subsequent trials was designated correct for the particular mouse. At the end of each trial, the mouse was removed from the goal box and returned to its home cage. A new trial began by placing the mouse in the start box, sounding the buzzer, and raising the guillotine door. Footshock was applied 5 s later if the mouse did not leave the start box or if they did not enter the correct goal box. All mice were trained until they first succeeded in avoiding the foot shock (avoidance response). The measure of acquisition (i.e., learning) was trials to first avoidance response, i.e., reaching the correct arm in less than 5 s. This criterion was used because, in previous studies (1,2), it resulted in good retention in mice not showing impaired long-term retention and training to a higher criterion might mask impaired long-term retention. This criterion results in good retention by both young and old male mice 1 h after training (2).

Retention was tested 1 week later by continuing to train each mouse until it made five avoidances in six consecutive trials. Trials to first avoidance response and trials to make five avoidance responses in six trials were used as measures of retention.

Experimental Design

The experimental design is that of a three-way ANOVA with factors for strain (P8 or R1), age (4 or 12 months old), and operation (intact or ovariectomized). Eight groups of 15 female mice were used. Separate three-way ANOVAs were run on trials to make a first avoidance response during training as well as on trials to make a first avoidance or five avoidances in six trials on the retention test. Significant differences among group means were determined by Tukey's *t*-test. The results are expressed in terms of means and standard errors of the means.

RESULTS

Progesterone Levels

A three-way ANOVA (age, strain, operation) indicated that only the main effect of operation yielded a significant effect, $F(1, 112) = 111.67$, $p < 0.001$, because of significantly lower progesterone levels in all ovariectomized mice, regardless of age or strain ($p < 0.01$ for all comparisons). The interaction of age and operation was the only interaction showing significance, $F(1, 112) = 8.35$, $p < 0.005$. This interaction was significant, because progesterone values across intact mice decreased as a function of age while they did not decrease with age across ovariectomized mice. The progesterone levels across ovariectomized mice were uniformly low (0.76 ± 0.25 to 1.40 ± 0.38 ng/ml plasma). Most of the effect in this interaction was due to a sizable decrease in progesterone levels in 12-month-old P8 mice relative to 12-month-old R1 or 4-month P8 mice. The progesterone levels of 12-month-old P8 mice were significantly lower than 4-month-old P8 mice ($t = 5.12$, $p < 0.05$), but not significantly lower than 12-month R1 mice ($t = 1.82$). The mean plasma levels of progesterone are given in Table 1.

Effects on Acquisition

A three-way ANOVA run on acquisition data (trials to first avoidance) indicated that the main effects age, $F(1, 112) = 51.49$, strain, $F = 36.67$, and operation, $F = 70.51$, were significant at $p < 0.001$. The interaction of age by strain was significant, $F(1, 112) = 28.55$, $p < 0.001$, as was the three-way interaction, $F(1, 112) = 9.64$, $p < 0.005$. The interactions of strain by operation and age by operation were not

TABLE 1

PLASMA PROGESTERONE LEVELS (ng/ml) AS A FUNCTION OF STRAIN, AGE, AND OPERATION

Strain	Age	Intact	Ovariectomized
R1	4 month	4.8 + 0.6	0.8 + 0.1
	12 month	4.1 + 0.4	1.1 + 0.1
P8	4 month	5.5 + 0.8	1.1 + 0.1
	12 month	3.3 + 0.5*	1.4 + 0.1

The table gives the mean plasma progesterone (ng/ml) and standard error of the mean. Only the main effect of operation had a significant effect on progesterone. The progesterone levels of the ovariectomized mice were all significantly lower than those in the corresponding intact mice at $p < 0.01$, by Tukey's *t*-test. The * indicates the progesterone level of the 12-month intact P8 mice was significantly lower than that found in the intact 4 month old P8 mice at $p < 0.01$.

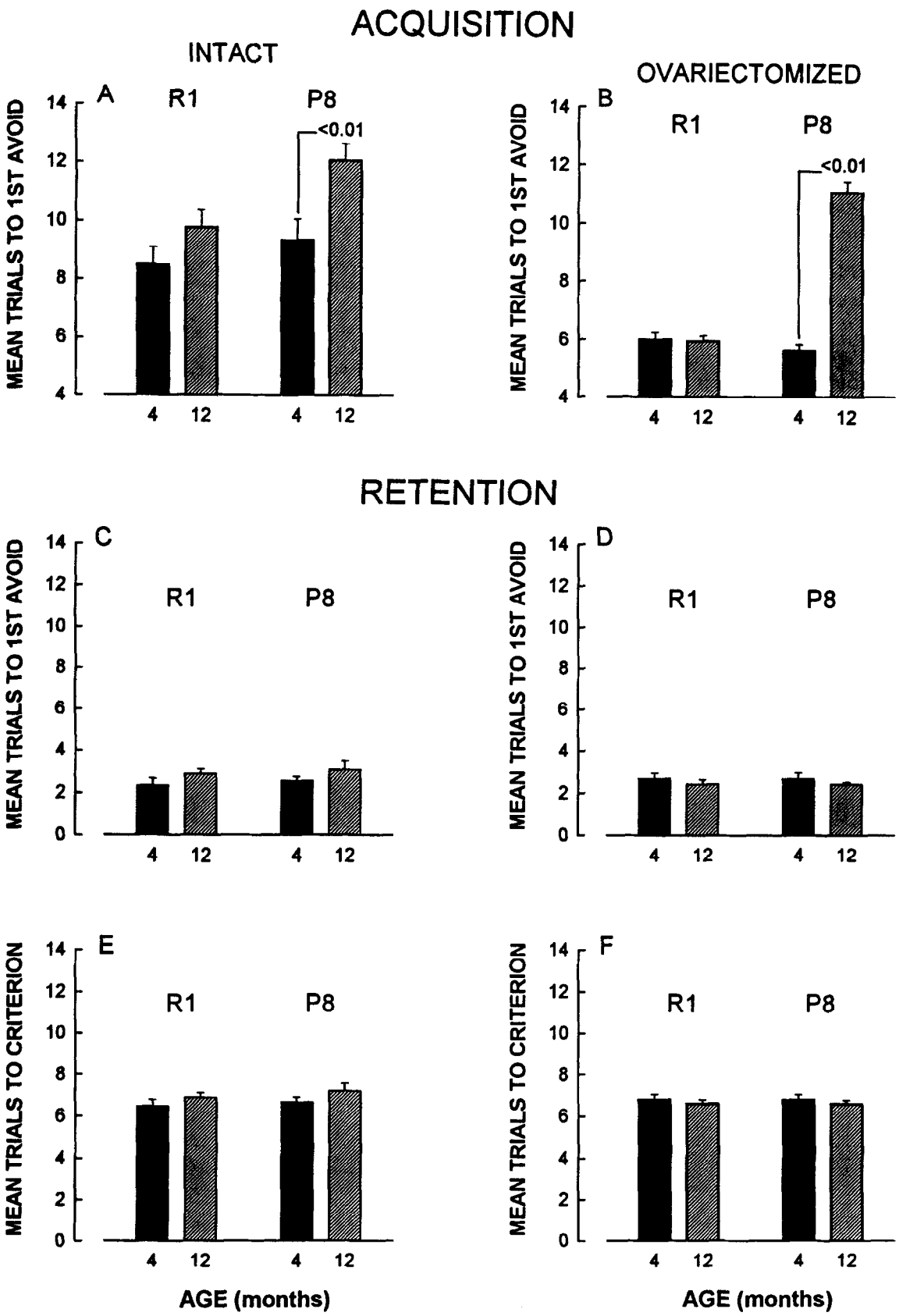

FIG. 1. The effects of age, strain, and operation on acquisition (A,B) and retention of foot shock avoidance training to trials to first avoidance (C,D) or trials to make five avoidances in six trials (E,F). The error bar represent the standard error of the mean.

significant. Figure 1A and B shows the mean trials to make an avoidance response for intact and ovariectomized mice. Among both the intact and ovariectomized groups, the main effect of age and strain occurred because 12-month-old P8 mice took more trials to make their first avoidance than did 4-month-old mice of the same strain. The difference in mean trials to first avoidance between 4- and 12-month-old R1 mice was not significant. The main effect of operation was significant because it resulted in a reduction in the mean trials to first avoidance for all groups of mice except the 12-month-old P8 mice.

Effects on Retention

While strain, age, and operation had significant effects on acquisition, Fig. 1C–F shows that none of these factors had a major effect on the 1-week retention test. The three-way ANOVA run on trials to first avoidance indicated that only the interaction of age by operation was significant ($p < 0.05$). This occurred because 12-month ovariectomized mice of both strains required slightly fewer trials to reach criterion than intact mice of the same age and strain. However, none of the means of ovariectomized and intact mice were significantly different (Fig. 1C and D). The three-way ANOVA run on trials to make five avoidance responses in six trials yielded similar results, with none of the main effects or interactions having significant effects on retention test performance (Fig. 1E and F).

DISCUSSION

Overall, ovariectomized young, but not old, SAM mice required fewer trials to make their first avoidance response during acquisition than intact mice. We have obtained similar results with CD-1 intact and ovariectomized mice using the same task and training procedures (Farr et al., unpublished observations). Thus, the effect is not unique to the SAM strains. CD-1 ovariectomized mice with progesterone implants, but not estrogen implants, had impaired acquisition similar to that of intact mice, suggesting that impaired acquisition of this task in intact females is associated with phases of the estrous cycle when progesterone is higher than estrogen. In contrast to the other groups of SAM mice, ovariectomy did not improve acquisition of 12-month-old P8 mice, suggesting that impaired learning was not related to progesterone in intact 12-month-old P8 mice.

The acquisition of ovariectomized P8 and R1 female mice is similar to that of male mice of the same ages and strains trained on the same task using the same procedures (2). P8 males and females, 12 months of age, both show impaired acquisition relative to 4-month-old P8 mice or 4- or 12-month-old mice of the R1 strain. Retention across all groups of female mice regardless of age, strain, or operation was good. Based on previous studies (2), 4-month-old male R1 and P8 mice and 12-month-old R1 mice had good retention test scores. The 12-month-old P8 male mice had impaired retention. Thus, while male and female mice 12 months of age both show impaired acquisition of foot shock avoidance learning, only the males show impaired retention. The results indicate that P8 males and females show similar inheritance or expression of age-related impairment of learning, but differ with respect to retention. This suggests that the mechanisms responsible for impaired learning and memory retention may differ. The results indicate that the age-related deficit in memory retention in male P8 mice may involve sex-related inheritance.

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REFERENCES

1. Flood, J. F.; Morley, J. E. Early onset of age-related impairment of aversive and appetitive learning in the SAM-P/8 mouse. J. Gerontol. Biol. Sci. 47:B52–B59; 1992.
2. Flood, J. F.; Morley, J. E. Age-related changes in foot shock avoidance acquisition and retention in Senescence accelerated mouse (SAM). Neurobiol. Aging 14:153–157; 1993.
3. Miyamoto, J; Kiyota, Y.; Yamazaki, N.; Nagaoka, A.; Matsuo, T.; Nagawa, Y.; Takeda, T. Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol Behav. 38:399–409; 1986.
4. Yagi, H.; Katoh, S.; Akiguchi, I.; Takeda, T. 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. Brain Res. 474:86–93; 1988.