Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 3, pp. 637–640, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00179-4

Effect of Chronic Piracetam on Age-Related Changes of Cross-Maze Exploration in Mice

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SALIMOV, R., N. SALIMOVA, L. SHVETS AND N. SHVETS. *Effect of chronic piracetam on age-related changes of cross-maze exploration in mice.* PHARMACOL BIOCHEM BEHAV **52**(3) 637–640, 1995.—Normal aging is known to deteriorate memory, spatial orientation, and perceptual recognition. Experiment 1 examined behavioral manifestations of aging by using a cross-maze exploration test in 2-, 6-, and 10-month-old hybrid mice (CBA × C57BL). A decrease in explorative patrolling and an increase in arm reentries, a latency to start and a total time of exploration were found in 10-month-old mice. In Experiment 2, administration of the cognition enhancer piracetam (2-oxo-1-pirrolidone acetamide) (400 mg/kg, IP, once a day for 10 days) enhanced arm patrolling and decreased reentries in 10-month-old mice to the level displayed by the 2-month-old animals. The results suggest that the cross-maze test may be useful for a preliminary screening of antisenescent drugs.

Exploration Aging Piracetam Mice

IN RECENT YEARS, there have seen increasing efforts to understand the neurobiological mechanisms of normal aging and to search for drugs capable of improving behavioral deficiencies induced by senescence. Normal aging in humans is characterized by deterioration of learning, memory, spatial orientation (6,21), and emotional perceptual recognition (18, 23). Animal studies also show that learning and memory losses are the most robust behavioral markers of senescence in rodents that develop during the second year of life (1,10,31). In 22- to 24-month-old rodents, the conventional radial maze learning test reveals a decline in performance and a elevation of arm reentry, which are considered to be measures for spatial mapping and working memory errors (3,4,11,17,31).

Patrolling strategies observed in the residential cross-maze has also been considered to be a manifestation of cognitive mapping (2). Recently, a cross-maze explorative test capable of evaluating arm patrolling and reentry behaviors was proposed for rodents (24–26). A correlation between the cross-maze patrolling and arm reentry measures on the one hand, and Krushinsky behavioral extrapolation (13) on the other hand was found in 2-month-old mice of CBA and C57BL strains (25). Similar to the decline of spatial mapping and increase of arm reentries induced by NMDA-receptor antagonists in the radial maze (5,32), the cross-maze test reveals the deterioration of patrolling and elevation of arm reentries after neurotoxic injury of glutamate sensitive neurons (26). In young animals, the cross-maze patrolling behavior was improved by the cognition enhancing drugs piracetam (24) and (+)-UH 232 (12).

Our pilot study with 2- and 10-month-old hybrid mice (CBA × C57BL) revealed age-related changes in the cross-maze patrolling and reentry behaviors. Therefore, the first objective of the present study (Experiment 1) was to evaluate age-dependent changes of the cross-maze exploration in 2-, 6-, and 10-month-old mice. Becaue a nootropic drug piracetam is known to enhance memory and learning in both middle-aged (14,15,24) and aged (30) rodents, the second objective (Experiment 2) was to examine the effects of piracetam on the behavioral pattern of aging displayed by 10-month-old mice.

METHOD

Animals

Naive male mice produced by crossing CBA with C57BL strains (F1 generation) were purchased by Svetljie Gorji experimental animal supplier (Moscow region) and lived in cages (36 × 24 × 10 cm, six animals per cage) located in a room maintained at 22° to 24°C, approximately 60% humidity. The light was on from 0600 to 1800 h. Animals had free access to pellets of a Standard Dry Protein mouse diet and tap water.

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Procedure

In Experiment 1, 75 naive young, middle-aged, and aged mice (2-, 6-, and 10-month-old respectively, each n = 25) were used as subjects the for cross-maze test.

In Experiment 2, 50 naive 10-month-old mice were randomly divided into two groups (each n = 25); one group was chronically administered with piracetam while the second group was given vehicle. Fresh 4% piracetam (Polfa, Poland) solution (w/v) was prepared in physiological saline, 1 h before injections. Each mouse received an intraperitoneal injection of the drug (400 mg/kg) or placebo solution once a day (10 ml of liquid per kg body weight, between 0900 and 1000 h) for 10 consecutive days. Physiological saline was used as a vehicle. The cross-maze exploration test was performed on the first posttreatment day.

Cross-Maze Test

The cross-maze was located in an isolated general-purpose laboratory room (diffuse artificial light, 18° to 20°C temperature, background noise 63 dB[A]). It was mounted on wooden pedestal (40-cm high) located in the center of room. The maze was made of five transparent acrylic cubes (numbered 1, 2, 3, 4) with 15-cm sides covered with a translucent lid; 7 × 7 cm doorways permitted the mouse to visit the arms via a central box. Masking 70 dB[A] white noise was employed.

The mouse was put into the central box until 13 free visits (trials) to the arms had been made, with all four paws inside the compartment being the criterion for a visit. The sequence and timing of arms visited were recorded by an observer directly into a personal computer. Subsequent computer analysis was used to reveal several independent behavioral measures (for details see (24,26)):

1. Length of first episode of maze patrolling—condition when each arm had been visited at least once. For instance, if the arm-entering sequence for the 13 trials was 1241413344321, then the length of first patrolling is 7 because the mice finally entered arm number 3 and completed the initial exploration on trial 7.
2. Patrolling strategy—condition when three consecutive arm transitions match any of six patterns: right(R)-right-right, left(L)-left-left, right-forward(F)-left, left-forward-right, forward-right-forward, forward-left-forward. Each pattern is equivalent to patrolling completed after four visits without reentries. In this example, the arm transition sequence is LFLRLFBLBRRR. (B is a symbol of a return to the arm.) One can see that the sequence contains only one strategy (RRR) that appears on the last three transitions.
3. Immediate reentry to the arm just previously visited (letter B labels this event in the second example).
4. Stereotyped walks—scored if mouse visits two arms in an alternating manner. In the first example, there is only one episode of stereotyped walk (the 4141 pattern starting from the third trial).
5. Number of the right and left directed turns during arm-to-arm transitions.
6. Latency to start exploration—time before the first arm entry.
7. Total time in the maze until 13 visits have been made.

The cross-maze test was conducted between 1000 and 1400 h.

Statistical analysis was performed with the CSS package. The statistical methods used were the analysis of variances (ANOVA), t-test for independent measures, and Mann-Whitney and Fisher exact probability tests.

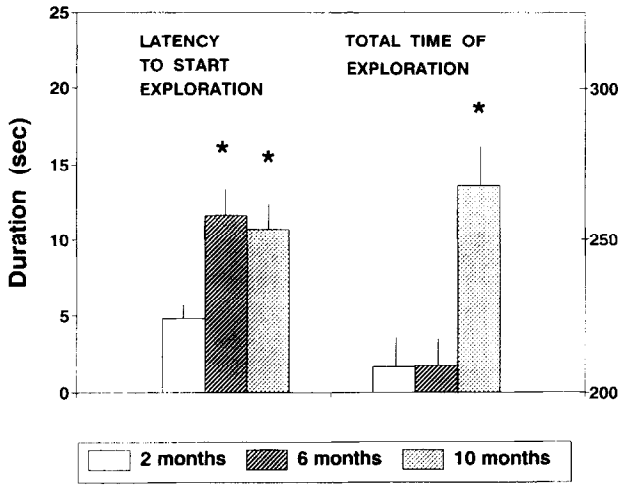

FIG. 1. Latency [seconds (sec) as mean ± SE] to start (time before first arm entry, primary ordinate, on the left) and total time of exploration (secondary ordinate, on the right) in young, adult, and aged mice (2-, 6-, and 10-month-old, respectively). *Marks a significant difference between young and one of the other mice groups.

RESULTS

The results from Experiment 1 are shown in Figs. 1 and 2. An one-way ANOVA yielded a significant effect of age on the latency to start cross-maze exploration [$F(2, 72) = 7.021$, $p = 0.002$] and the total time in the maze [$F(2, 72) = 9.492$, $p < 0.001$]. The 2-month-old group displayed shorter time before the first visit relative to the 6- or 10-month-old mice

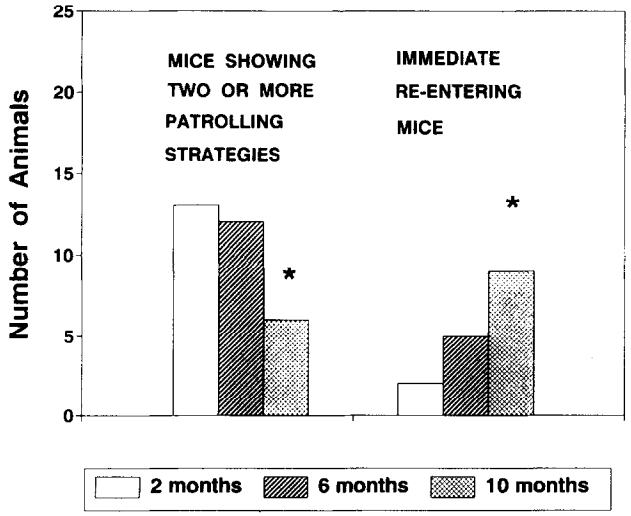

FIG. 2. Number of individuals (of total 25) showing at least two patrolling strategy patterns (left) and at least once entered the same arm two times in a row (immediate re-entering) (right) in young, adult, and aged mice. *Marks a significant difference between young and one of the other mice groups.

[$t(48) = 3.357$, $p = 0.001$; $t(48) = 3.224$, $p = 0.003$]. The 10-month-old mice exhibited greater total time to complete the test relative to the 2- or 6-month-old animals [$t(48) = 3.394$, $p = 0.002$; $t(48) = 3.811$, $p = 0.001$] (Fig. 1). The two younger groups did not differ from each other on this measure.

An one-way ANOVA did not show significant effect of age on the remaining cross-maze measures. The Mann-Whitney test, however, revealed a significant difference between the 2- and 10-month-old mice on the measures of number of strategies ($Z = 1.999, p = 0.045$) and number of reentries made ($Z = 2.212, p = 0.027$). Because the frequency distribution for the latter two variables deviated substantially from the normal (χ^2 (1) = 24.63, $p < 0.001$ and χ^2 (1) = 49.93, $p < 0.001$, respectively), the proportion of individuals showing at least two patrolling strategies and those who reentered the same arm at least once (25,26) was used for further analysis (Fig. 2). The 10-month-old group contained significantly fewer individuals showing more than one patrolling strategy (Fisher exact $p = 0.04$). This group contained a greater number of the reentering mice (Fisher exact $p = 0.019$). The 6-month-old mice showed an intermediate result and did not differ from the 2- or 10-month-old ones on these measures.

The results from Experiment 2 are given in Figs. 3 and 4. The behavioral pattern displayed by vehicle-treated mice in Experiment 2 did not differ significantly from those shown by nontreated individuals of the same age in Experiment 1.

An one-way ANOVA showed no significant effect of the treatment on the latency to start exploration or on the total time in maze (Fig. 3). The Mann-Whitney test revealed a significant effect of piracetam treatment on the number of strategies and arm reentries displayed by the 10-month-old mice ($Z = 1.97$, $p = 0.049$ and $Z = 1.94$, $p = 0.052$, respectively). In the piracetam-treated group, there was a significantly greater number of the mice showing at least two patrolling strategies and fewer individuals making the arm reentries compared to the control mice (Fisher exact $p = 0.021$ and $p = 0.009$) (Fig. 4).

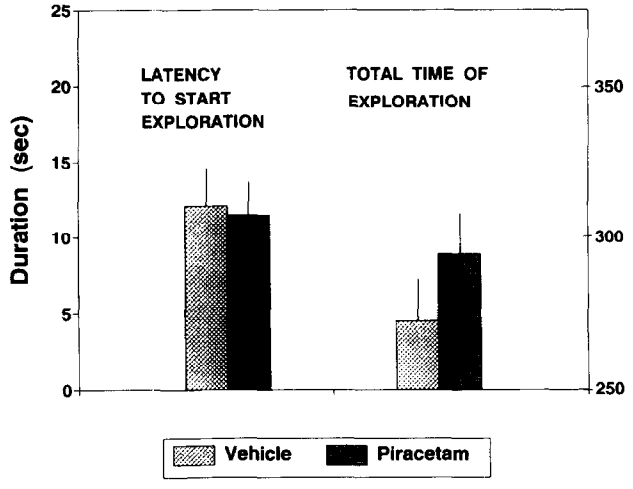

FIG. 3. Latency [seconds (sec) as mean ± SE] to start (time before first arm entry, primary ordinate, on the left) and total time of exploration (secondary ordinate, on the right) in after 10 days of vehicle or piracetam (400 mg/kg) administration in 10-month-old mice. *Marks significant differences between the groups.

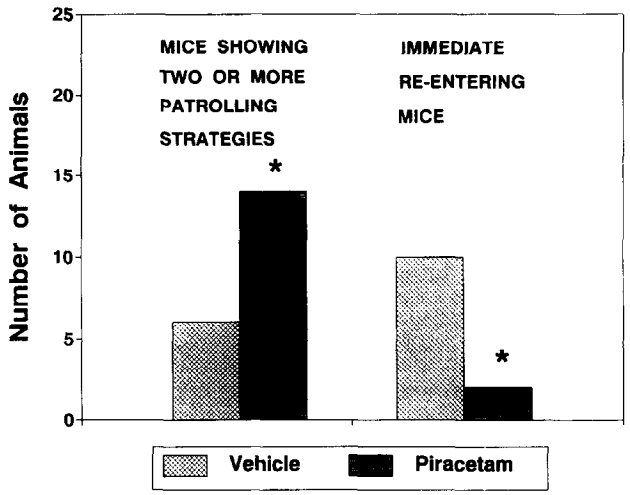

FIG. 4. Number of individuals (of total 25) showing at least two patrolling strategies (left) and at least once displayed arm immediate re-entering (right) after 10 days of vehicle or piracetam (400 mg/kg) administration in 10-month-old mice. *Marks significant differences between the groups.

DISCUSSION

The results of the present study give convincing evidence for age-related changes in the ratio of individuals with patrolling strategies and those with arm reentries. There was also a clear age-dependent increase in the latency before the first arm visit and of the total time in the maze. The first of the two measures is known to reflect an interference between fear and curiosity about novel environment and has been proposed as a behavioral assay for evaluating anxiolytic agents (29). Therefore, increased neophobia in the old mice group is suggested. The elevation of the total time in the maze can be attributed to distinct factors such as curiosity, fear, habituation, body weight, etc. (7,8,19,22,24).

Although changes in patrolling and arm reentry observed in the 10-month-old mice are analogous to the decline in radial maze performance and one-trial learning in rodents during the second year of life (3,4,11,17,30,31), additional research is necessary to determine if there is a relationship between them. An alternative explanation of the augmentation of the ratio of individuals showing reentry behavior in the old group may be based on probable accelerated habituation (2,7) or on the putative increase of neophobia taking place in old animals.

The behavioral pattern of aging demonstrated by naive 10-month-old mice in Experiment 1 was replicated by vehicle-treated animals in Experiment 2. Piracetam recovered patrolling and arm reentry behaviors at the level shown by the younger animals but did not change the latency to start and time to complete exploration. The results obtained agree with the well-known cognition enhancing property of piracetam reported in humans and animals whereas the drug has no anxiolytic effect (6,9,14-16,20,21,27,28). Therefore, the selective recovery by piracetam of the cross-maze patrolling and reentry behaviors in the old mice suggests that these changes can not likely be explained via age-related neophobia. The amelioration of the arm reentries taking place after piracetam administration does not agree with the suggestion that the old mice involve more individuals making reentries since it shows rather additive interaction with the habituation of exploratory behavior (19,22).

The results of the present study show that the cross-maze explorative test may be a useful tool for the preliminary screening of antisenescent drugs.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. W. J. McBride at Indiana University for his assistance with the English in the manuscript.

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