Working Memory Deficit in Aged Rats in Delayed Nonmatching to Position Task and Effect of Physostigmine on Performance of Young and Aged Rats

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ABSTRACT — Young (5 month) and aged (23 month) male rats were tested in delayed nonmatching to position task using a T-maze, and their ability of working memory retention was assessed over interrun intervals ranging between 5 and 300 sec. There were no significant age differences in pretest performance at 0 sec interval, but significant memory loss was observed in aged rats when tested with the interrun intervals. Physostigmine (0.1 and 0.2 mg/kg) improved this age-related decline in working memory in a dose-dependent manner, whereas the treatment slightly but not significantly improved the performance of young rats. These results suggest that the central cholinergic system in aged rats was functionally deteriorated and that stimulation of the system could enhance working memory retention in aged rats.

It is well-established that aged rodents exhibit impaired performance on a variety of learning and memory tasks (1–5), but only limited information is available on working memory in aged rodents. To study this type of memory, an 8-arm radial maze task has been the most commonly used. Using the radial maze task, impaired performance of aged rats has been demonstrated mainly in acquisition of the task (5–7). As for the retention of working memory, it still remains unclear whether it is actually impaired in aged rats. Therefore, one of the purposes of the present study is to test the ability of working memory retention in aged rats. A few reports have focused on this point but conflicting results were obtained in these studies. Some investigators reported impaired working memory retention (8, 9), but others demonstrated preservation of the memory (10–12) in aged rats. In the present study, we employed delayed nonmatching to a position task, which is favorable for assessing the retention of spatial working memory (13). In addition, such delayed response tasks corresponded well to memory tasks in humans. A number of reports have proved these delayed response tasks to be appropriate and useful in studies of experimental amnesia in monkeys (14, 15) and memory dysfunction in human dementia (16). We also tested the effect of physostigmine (PHY) on the performance in this task to test the possibility that stimulation of the cholinergic system could ameliorate age-related decline in working memory.

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MATERIALS AND METHODS

Subjects
Eight male young rats and 10 male aged rats of the Fischer 344 strain, aged 5 and 23 months-old at the start of the experiment, respectively, were used. Three or four rats were housed in a cage with free access to water in an air-conditioned room and subjected to a 12:12 light-dark cycle. They were maintained on a restricted feeding schedule designed to keep their body weight at approximately 85% of the free-feeding level. One aged rat that showed signs of ill health during regular monitoring was eliminated from the data analysis.

Apparatus
The apparatus was a T-maze made of wood with a center stem and two side arms. The center stem was 72 cm long and 12 cm wide equipped with a guillotine door 20 cm apart from the end of the stem (start box). Each side arm was 60 cm long and 12 cm wide, and the entrance to each arm was equipped with a guillotine door. The side walls were 15 cm in height. The floor was made of black Plexiglas, and a black food cup, 3.5 cm in diameter, was placed at the end of each arm.

Pretraining
Prior to the experiments, each animal was handled 10 min daily over 10 days and was also given 5 days adaptation to the maze. On the first day of the adaptation period, two rats at a time were placed in the maze, where food pellets (45 mg, Bio-Serv) were scattered on the floor and in the food cups, for 30 min. From the second day, one rat was placed individually in the maze with food pellets only in the cups for 10 min. Following this adaptation period, all rats were trained to run to the goal boxes for over 5 days. The rat was placed at the start box; and after 5 sec, the guillotine door was raised. During this training period, one of the two guillotine doors at the side arms was lowered to allow the rat to enter the other arm for a food reward. Six trials, 3 for the right arm and 3 for the left one, were given in random sequences.

Discrete trial alternation training
Each trial involved one forced run and one choice run. The forced run was carried out as described in the pretraining procedure. One food pellet was placed in the cup of one arm that was not blocked. Immediately after the forced run, the rat was placed again at the start box for a subsequent choice run. At this time, no guillotine door was lowered, and the rat was allowed to choose between two arms. The rat was judged to have made a choice when all four paws entered an arm, where-upon the guillotine door was lowered. When the rat entered the arm opposite to the one rewarded in the forced run, a correct response was recorded, and the animal was further rewarded with 4 pellets. If it chose an incorrect arm, the rat was confined in the arm for approximately 10 sec. Six trials daily were carried out for each rat. The number of right-correct and left-correct trials were equal in each day, but their sequences were randomized. The intertrial intervals (ITI) were approximately 3 min. Criterion for acquisition of this task was that a rat did not make more than one incorrect response during each session for 5 consecutive days.

Delayed nonmatching to position task
After the alternation training, delayed alternation was tested. A delay interval of 5, 30, 90, 150 or 300 sec was introduced between the forced and choice run (interrun interval: IRI). During the IRI, the rats were placed in the home cage. Six daily trials, which consisted of two 5-sec IRI trials and one other IRI trial in a randomized sequence, were carried out over 10 days. The total number of right-correct and left-correct trials were equal for each IRI trial. Data obtained during this period were collected and analyzed.

Drug treatment
Before starting the drug test, saline was injected intraperitoneally prior to the test for 3 days to avoid the interference by the injection.
Thereafter, the effect of physostigmine salicylate (Merck; PHY) on the performance in this delayed alternation task was examined. PHY or saline was injected intraperitoneally 15 min before the test. In the drug test, an IRI of 5, 90 or 300 sec was introduced between the forced and choice run. Six daily trials for a rat were carried out, in which each IRI trial was conducted twice, one right-correct and one left-correct in a randomized sequence. Each rat was tested under three conditions of drug administration: no drug (saline only), 0.1 mg/kg PHY, and 0.2 mg/kg PHY. The tests were conducted once every 3rd day. The test under each drug condition was repeated 3 times to obtain 18 trials.

**Statistics**

Repeated measure analyses of variance (ANOVA) were used for evaluating the significance of main and interaction effects produced by age, IRI and drugs on the percent correct responses and followed by the post hoc Newman-Keuls test. Effect of age on goal latency and the number of days required to fulfill criterion was analyzed by Student’s t-test and the Mann-Whitney U-test, respectively.

**RESULTS**

**Spontaneous alternation in alternation training**

After 5 days training, all rats of the two groups could reach goal boxes within 10 sec. The mean latency to reach goal at the last training session in young and aged rats were $3.3 \pm 0.4$ (mean ± S.E., $n = 8$) and $4.2 \pm 0.2$ sec ($n = 9$) ($P > 0.05$), respectively. The acquisition curves of alternation behavior with 0 sec delay interval are shown in Fig. 1. Aged rats as well as young rats exhibited spontaneous alternation rates of more than 85 percent throughout the training period. Furthermore, the number of days required to fulfill criterion in the young group, $5.3 \pm 0.3$ days, did not differ from that in the aged group, $6.7 \pm 1.1$ days. According to the analysis of variance, there were no significant differences in the percentage of correct responses in terms of session, $F(5,90) = 1.77$, $P > 0.05$ and age, $F(1,90) = 3.39$, $P > 0.05$.

**Delayed nonmatching to position**

The percentage correct responses at individual IRIs are shown in Fig. 2. The percentage of correct responses of both young and aged rats declined as IRI increased. The aged rats showed a lower choice accuracy across
each delay interval in comparison with young rats. Two factors, age and delay time, significantly affected the retention of memory in this test: $F(1,75) = 6.88$, $P > 0.02$ and $F(4,75) = 12.16$, $P < 0.0001$, respectively, but there was no significant interaction between these two factors, $F(4,75) = 0.60$, $P > 0.05$. Specific Newman-Keuls comparison revealed that old rats were impaired in comparison to young rats at 5 and 90 sec delays.

**Effect of PHY on performance of young rats**

As shown in Fig. 3 (top panel), PHY (0.1 and 0.2 mg/kg, i.p.) did not change the percentage of correct responses of young rats, $F(2,63) = 2.46$, $P = 0.09$ (Fig. 3). The percentage of correct responses was significantly decreased with delay, $F(2,63) = 12.4$, $P < 0.0001$, but there was no significant interaction between PHY treatment and delay interval, $F(4,63) = 2.17$, $P > 0.05$ (Fig. 3).

**Effect of PHY on performance of aged rats**

The effect of PHY on the performance of aged rats is shown in Fig. 3 (bottom panel). At this stage, the aged rats were about 24 months-old. According to analysis of each of the saline control values of young and aged rats from Fig. 3 (top and bottom panel), the percentages of correct responses of aged rats were still lower than those of young rats across the 5, 90 and 300 sec delays, $F(1,45) = 7.49$, $P < 0.01$, indicating that this memory retention deficit in the aged rats was not improved by training. There was also a significant delay effect, $F(2,45) = 10.04$, $P < 0.0003$, but no significant age $\times$ delay interaction, $F(2,45) = 0.21$, $P > 0.05$.

As shown in Fig. 3 (bottom panel), the performance of aged rats was significantly improved by PHY in a dose-dependent manner, $F(2,72) = 3.71$, $P < 0.03$ (Fig. 3). Increase in delay interval significantly affected the memory retention, $F(2,72) = 10.8$, $P < 0.0001$, but there was no significant interaction between PHY treatment and delay interval in the aged rats, $F(4,72) = 0.36$, $P > 0.05$.

**DISCUSSION**

The present results clearly demonstrated deterioration in the working memory of aged rats. The delayed nonmatching to position task that we employed requires two types of memory. One is the memory of the alternation (win-shift) "rule" (reference memory) and the other is recall of the most recently visited arm (working memory). However, young rats are known to prefer win-shift strategy to win-stay when searching for food (17). Moreover, the alternation behavior on the T-maze is characteristic of rats (spontaneous alternation behavior) (18). Therefore, the choice of arms by the rat might be dependent only on the working memory component. In the present study, this nature of alternation
was shown to be preserved in the aged rats (Fig. 1). This finding indicates that the memory deficit observed in the present experiment (Fig. 2) does not reflect impairment in reference memory. On the other hand, in research on memory using aged animals, factors other than memory, such as motor and/or sensory dysfunction or motivational change, often interfere with correct analysis of the data. However, this does not seem to be the case in our experiments, because the mean latency to reach goal in aged rats was not significantly different from that in young rats. These results indicate a deficit in the working memory in aged rats.

The present finding that aged rats exhibited impaired working memory retention is essentially consistent with other reports (8, 9). However, Willig et al. (10) and Aggleton et al. (12) found no significant age decline in the working memory using a comparable T-maze task. The reason for the discrepancy remains to be made clear. It might be due to differences in experimental conditions, such as age or strain of rats.

In the present results, the aged rats exhibited significantly lower choice accuracy at shorter (5 and 90 sec) rather than longer delay intervals (Fig. 2). However according to 2-way analysis of variance, the correct response curves of young and aged rats were essentially parallel across all 5 delay intervals. Dunnet et al. (9) have reported, using a 2-lever operant chamber for delayed nonmatching to position task, that aged rats showed a progressively greater impaired performance as the delays lengthened. On the other hand, Ordy et al. (8) have reported that aged Long-Evans rats exhibited parallel decrease in choice accuracy at 10, 90 and 180 sec delays compared with young and middle aged groups. We employed the wide ranged delay intervals (5 to 300 sec) on the assumption that memory impairment in aged rats should be greater with extended delay intervals. However the delay-performance curve of aged rats shifted downwardly in parallel. This result, a parallel downward shift of the curve, is similar to that of young rats treated with scopolamine in our preliminary experiment (data not shown) and in other reports using similar delayed response tasks (19–21).

Ibotenic acid lesion of the nucleus basalis and/or medial septum, which include a number of cholinergic neurons projecting to the cortex and hippocampus, respectively, has been reported to produce a decrease in choice accuracy using this T-maze alternation task (19, 22–24). A similar memory decline was observed in rats that received an intracerebroventricular injection of AF64A, a cholinergic neurotoxin (25). These facts indicate that central cholinergic dysfunction brings about working memory deficit in this task. On the other hand, it is known anatomically or histologically that there is degeneration of cholinergic neurons in the basal forebrain and septum in aged rats (26–28), like that observed in aged humans or patients with Alzheimer’s disease (29, 30). Moreover in the present study, pretest PHY treatment dose-dependently improved the impaired performance of aged rats (Fig. 3). Taken these data together, the present results suggest that there is a functional deficit in the central cholinergic systems in aged rats.

In conclusion, a deficit in working memory retention was demonstrated in aged rats. The present results may be functional evidence for cholinergic dysfunction in aged rats. Stimulation of cholinergic systems could only slightly
but not significantly potentiate working memory performance of young rats with intact central cholinergic systems, but the same treatment could improve the impaired working memory of aged rats, whose central cholinergic function seemed to be deteriorated.

REFERENCES

1 Lippa, A.S., Pelham, R.W., Beer, B., Critchley, D.J., Dean, J. and Bartus, R.L.: Brain cholinergic dysfunction and memory in aged rats. Neurobiol. Aging 1, 13–19 (1980)
2 Rapp, P.R., Rosenberg, R.A. and Gallagher, M.: An evaluation of spatial information processing in aged rats. Behav. Neurosci. 101, 3–12 (1987)
3 Gage, F.H., Dunnett, S.B. and Bjorklund, A.: Spatial learning and motor deficits in aged rats. Neurobiol. Aging 5, 43–48 (1984)
4 Spangler, E.L., Chanchich, M.E., Curtis, N.J. and Ingram, D.K.: Age-related impairment in complex maze learning in rats: relationship to neophobia and cholinergic antagonism. Neurobiol. Aging 10, 133–141 (1989)
5 Ingram, D.K., London, E.D. and Goodrick, C.L.: Age and neurochemical correlates of radial maze performance in rats. Neurobiol. Aging 2, 41–47 (1981)
6 Furukawa, S. and Iwasaki, T.: Deficits in radial-arm maze learning in aged rats. Japan. J. Psychol. 60, 192–195 (1989)
7 Beatty, W.W., Bierley, R.A. and Boyd, J.G.: Preservation of accurate spatial memory in aged rats. Neurobiol. Aging 6, 219–225 (1985)
8 Ordy, J.M., Thomas, G.J., Volpe, B.T., Dunlap, W.P. and Colombo, P.M.: An animal model of human-type memory loss based on aging, lesion, forebrain ischemia and drug studies with the rat. Neurobiol. Aging 9, 667–683 (1988)
9 Dunnet, S.B., Evenden, J.L. and Iversen, S.D.: Delay-dependent short-term memory deficits in aged rats. Psychopharmacology (Berlin) 96, 174–180 (1988)
10 Willig, F., Palacios, A., Monnmaur, P., M’harzi, M., Laurent, J. and Delacour, J.: Short-term memory, exploration and locomotor activity in aged rats. Neurobiol. Aging 8, 393–402 (1987)
11 Wallace, J.E., Krauter, E.E. and Campbell, B.A.: Animal models of declining memory in the aged: short-term and spatial memory in the aged rat. J. Gerontol. 35, 355–363 (1980)
12 Aggleton, J.P., Blindt, H.S. and Candy, J.M.: Working memory in aged rats. Behav. Neurosci. 103, 975–983 (1989)
13 Beninger, R.J., Wirsching, B.A., Jhamandas, K. and Boegman, R.J.: Animal studies of brain acetylcholine and memory. Arch. Gerontol. Geriatr. Supp. 1, 71–89 (1989)
14 Zola-Morgan, S. and Squire, L.R.: Medial temporal lesion in monkeys impair memory on a variety of tasks sensitive to human amnesia. Behav. Neurosci. 99, 22–34 (1985)
15 Aggleton, J.P. and Mishkin, M.: Memory impairments following restricted medial thalamic lesions in monkeys. Exp. Brain Res. 52, 199–209 (1983)
16 Aggleton, J.P., Nicol, R.M., Huston, A.E. and Fairbrain, A.F.: The performance of amnesic subjects on tests of experimental amnesia in animals: delayed matching-to-sample and concurrent learning. Neuropsychology 26, 265–272 (1988)
17 Olton, D.S. and Schlosberg, P.: Food-searching strategies in young rats: Win-shift predominates over win-stay. J. Comp. Physiol. Psychol. 92, 609–618 (1978)
18 Dennis, W.: Spontaneous alternation in rats as an indicator of the persistence of stimulus effect. J. Comp. Psychol. 28, 305–312 (1939)
19 Dunnet, S.B.: Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria- fornix on delayed matching in rats. Psychopharmacology (Berlin) 87, 357–363 (1985)
20 Spencer, D.B., Pontecorvo, M.J. and Heise, G.A.: Central cholinergic involvement in working memory: Effect of scopolamine on continuous nonmatching and discrimination performance in the rat. Behav. Neurosci. 99, 1049–1065 (1985)
21 Hest, A., Stroet, J., Harren, F. and Feenstra, M.: Scopolamine differentially disrupts the behavior of male and female Wistar rats in delayed nonmatching to position procedure. Pharmacol. Biochem. Behav. 35, 903–909 (1990)
22 Brito, G.N.O., Davis, B.J., Stopp, L.C. and Stanton, M.E.: Memory and the septo-hippocampal cholinergic system in the rat. Psychopharmacology (Berlin) 81, 315–320 (1983)
23 Stanton, M.E., Thomas, G.J. and Brito, G.N.O.: Posterodorsal septal lesions impair performance on both shift and stay working memory tasks. Behav. Neurosci. 98, 405–415 (1984)
24 Hepler, D.J., Olton, D.S., Wenk, G.L. and Coley, J.T.: Lesions in nucleus basalis magnocellularis and medial septal area of rats produce qualitatively similar memory impairments. J. Neurosci. 5, 866–873 (1985)
25 Chrobak, J.J., Hanin, I. and Walsh, T.J.: AF64A (ethylcholine aziridinium ion), a cholinergic neurotoxin, selectively impairs working memory in a
multiple component T-maze task. Brain Res. 414, 15–21 (1987)

26 Altavista, M.C., Rossi, P., Bentivoglio, A.R., Crociani, R. and Albanese, A.: Aging is associated with a diffuse impairment of forebrain cholinergic neurons. Brain Res. 508, 51–59 (1990)

27 Kadar, T., Silbermann, M., Brandeis, R. and Levy, A.: Age-related structural changes in the rat hippocampus: correlation with working memory deficiency. Brain Res. 512, 113–120 (1990)

28 Fischer, W., Victorin, K., Bjorklund, A., Williams, L.R., Varon, S. and Gage, F.H.: Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. Nature 329, 65–68 (1987)

29 McGeer, P.L., McGeer, E.G., Suzuki, J., Dolman, C.E. and Nagai, T.: Aging, Alzheimer’s disease, and the cholinergic system of the basal forebrain. Neurology 34, 741–745 (1984)

30 Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T. and DeLong, M.R.: Alzheimer’s disease and senile dementia: loss of neurons in the basal forebrain. Science 215, 1237–1239 (1982)

31 Santucci, A.C., Kanof, P.D. and Haroutunian, V.: Effect of physostigmine on memory consolidation and retrieval processes in intact and nucleus basalis-lesioned rats. Psychopharmacology (Berlin) 99, 70–74 (1989)

32 Gower, A.J.: Enhancement by scovorine and physostigmine of retention of passive avoidance response in mice. Psychopharmacology (Berlin) 91, 326–329 (1987)