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Region Selective Increase in Activities of CNS Cholinergic Marker Enzymes During Learning of Memory Tasks in Aged Rats

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NAKAMURA, S. AND T. ISHIHARA. *Region selective increase in activities of CNS cholinergic marker enzymes during learning of memory tasks in aged rats*. PHARMACOL BIOCHEM BEHAV **34**(4) 805–810, 1989.—The effects of learning memory tasks on activities of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) in the frontal cortex (FC), hippocampus (HC) and cerebellum of aged rat brains were studied in comparison with those of young adult rats. Aged rats were significantly inferior than young adult rats in both active avoidance (two-way shuttle box) and water-filled multiple T-maze learning. ChAT activity in the FC of aged rats was significantly increased after 5 days of training in an active-avoidance learning task. ChAT activity in the HC of aged rats was also significantly increased after 6 days of training in a water-filled multiple T-maze. These changes did not occur in young adult rats after either 2 or 5 days of active avoidance training, or in aged rats after 10 days of training, both of which were after the maximum level of learning of active avoidance task had been attained. AChE activity was significantly lower in the FC and HC of nontrained aged rats when compared with that of nontrained young adult rats. The reduced activity of AChE in both brain regions of nontrained aged rats rose to almost the same level as that in young adult rats in nontrained and trained states in an active avoidance task. From these findings, it is hypothesized that the task-dependent elevation in the activities of the central nervous system (CNS) cholinergic marker enzymes in trained aged rats may be compensatory changes to keep a relevant level of neurotransmission in the face of specific motor and/or cognitive insults.

Active avoidance learning Multiple T-maze learning Choline acetyltransferase Acetylcholinesterase
Frontal cortex Hippocampus Aged rats

NEURODEGENERATIVE disorders, such as senile dementia of the Alzheimer type, are associated with a decline in cognitive function (23). Hollander *et al.* (14) and Sherman *et al.* (22) have proposed that aged or senescent animals are a suitable model for the study of brain aging and senile dementia. Numerous studies have shown that cholinergic and cognitive functions are diminished in normal aged animals. For example, memory deficits in aged animals have been found in some behavioral tasks such as maze learning (2, 3, 27), a delayed alteration task (29) and passive avoidance learning (1,15). Several workers have shown that a reduction in cholinergic markers, such as choline acetyltransferase (ChAT) activity (26), high-affinity choline uptake (HACU) (24), and acetylcholine (ACh) release (6, 17, 19), occur in the aged rat brain. In contrast, some recent reports indicate that cholinergic function (e.g., HACU) is not necessarily reduced in behaviorally impaired aged animals performing the water maze task (8,12). Thus, there is not necessarily a correlation between cholinergic hypofunction and cognitive dysfunction occurring in aged animals. In order to investigate this discrepancy, it seems necessary to correlate learning tasks with the changes in biochemical markers in discrete brain regions.

In this study, we investigated the learning ability of young adult and aged rats in active avoidance and water-filled multiple T-maze learning tasks, and tried to correlate it with changes in cholinergic marker enzymes such as ChAT and acetylcholinesterase (AChE) in three brain regions (the frontal cortex, hippocampus, and cerebellum).

METHOD

Subjects

Rats used in this study were 3–4 months and 23–24 months old.

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TABLE 1

COMPARISON OF FLINCH THRESHOLD AND LOCOMOTOR ACTIVITY BETWEEN YOUNG ADULT AND AGED RATS

Animal	Flinch (mA)	Locomotor Activity (counts/10 min)
Young	0.54 ± 0.045 (12)	437 ± 25.2 (12)
Aged	0.61 ± 0.042 (10)	264 ± 22.3 (12)*

Values represent mean ± S.E.M.
( ) = sample size.
*$p<0.01$ compared to the young adult rats.

Male Fischer 344 (F344) rats were purchased from Charles River Japan at the age of 2 months (weight, 190–210 g). The rats were raised for 21 to 22 months in our animal facility at constant temperature (23 ± 1°C) and humidity (55 ± 5%), and with 12-hr light-dark cycles. Food and water were provided ad lib in their home cage.

Flinch Threshold Test

Each rat was put into a test box (33 × 25 × 30 cm) and given an AC current shock through the grid floor for 3 sec every 30 sec. A "flinch" was defined as a crouching response. The minimum shock intensity at which rats flinched was recorded.

Locomotor Activity

The apparatus, an experimental chamber (60 × 60 × 30 cm, OUCEM-86 Biomedica Ltd., Osaka, Japan) consisting of 7 photobeams crossing at right angles, was placed in a soundproof box. The light was attached to the ceiling of the soundproof box 20 cm above the floor of the experimental chamber. Animals were put into the experimental chamber and locomotor activity was recorded as the number of crossings of the photobeam during a 10-min period.

Active Avoidance Response

The apparatus used was a shuttle box with two identical compartments, separated by a hurdle (Biomedica). A noise (2.8 kHz, 70 dB) generated by a buzzer at the ceiling of the shuttle box was given for 5 sec as the conditioned stimulus. If the rat crossed into the other compartment during the conditioned stimulus, an avoidance response was recorded. Otherwise, a foot shock (1.0 mA) was delivered for 5 sec through the grid floor as an unconditioned stimulus. Each rat was given 20 trials daily with fixed intertrial intervals of 20 sec.

Water-Filled Multiple T-Maze Task

The apparatus used was a water tank (150 × 150 × 30 cm) equipped with a Biel-type multiple T-maze (maze pathway, 15 cm wide) with 5 choice points and a straight channel (5). The tank was filled with water (22 ± 2°C, 22 cm deep). An inclined board was placed at the goal, where a rat could escape out of the water. Rats were trained in the multiple T-maze task. The experimental conditions were the same as described elsewhere (18). In brief, on the first day, rats were put into the straight channel three times a day to examine if they could swim from the starting point to the goal without drowning. On the next day, maze training was started. For the first 3 days, they were subjected to orthodromic training and then to reversal training for the next 3 days. Latency (in seconds) in reaching the goal-point and the number of errors in choice were recorded. Each rat was given 4 training trials daily.

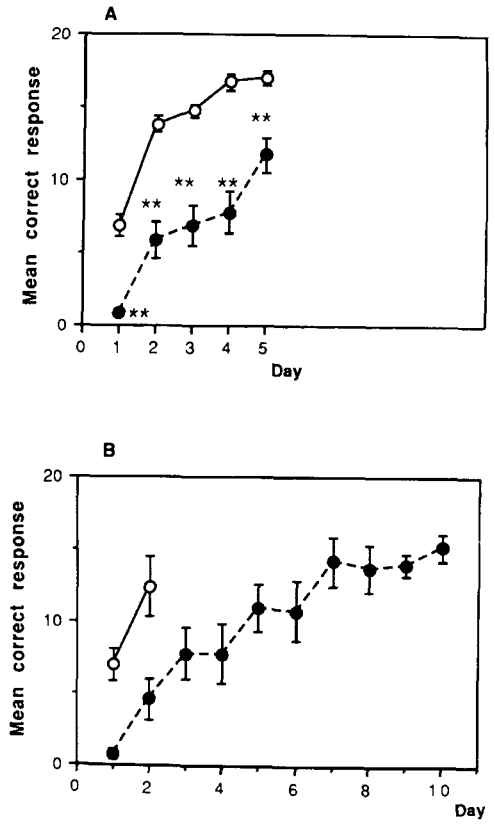

FIG. 1. Acquisition of active avoidance learning in young adult and aged Fischer 344 rats. (A) Five days of training in active avoidance learning. ○: Young adults (n = 12); ●: aged (n = 12). (B) Two or 10 days of training in active avoidance learning ○: Young adults (n = 5); ●: Aged (n = 5). **$p<0.01$ compared to the young adult rats.

Neurochemical Parameters

ChAT and AChE activities. Five groups of both young adult and aged rats were subjected to the ChAT assay; a naive nontrained group, a group trained for 5 days in the active avoidance task (AAT), a group trained for 2 (young adult rats) or 10 days (aged rats) in the AAT and a group trained for a total of 6 days (3-day orthodromic and subsequent 3-day reversal) in the multiple T-maze task, a group that did and a group that did not receive a foot shock in the absence of conditioned stimuli (aged rats). The animals were killed, the brains rapidly excised, and the frontal cortex (FC), hippocampus (HC), and cerebellum were dissected out by the methods of Glowinski and Iversen (13).

ChAT activity was assayed by the method of Fonnum (11). In brief, tissue samples were homogenized in 20 vol. of ice-cold 10 mM EDTA (pH 7.4) containing 0.5% Triton X-100. The reaction mixture contained 10 μM [^{14}C]acetyl coenzyme A (CoA; 53 mCi/mmol, New England Nuclear Corp.), 50 mM sodium phosphate buffer (pH 7.4), 600 mM sodium chloride, 40 mM EDTA, 0.1 mM physostigmine, 8 mM choline chloride, 200 μM acetyl-CoA, and the tissue homogenate (about 80 μg of protein) in a total volume of 100 μl. The sample was incubated for 30 min at 37°C. Radioactivity was counted by liquid scintillation spectrometry.

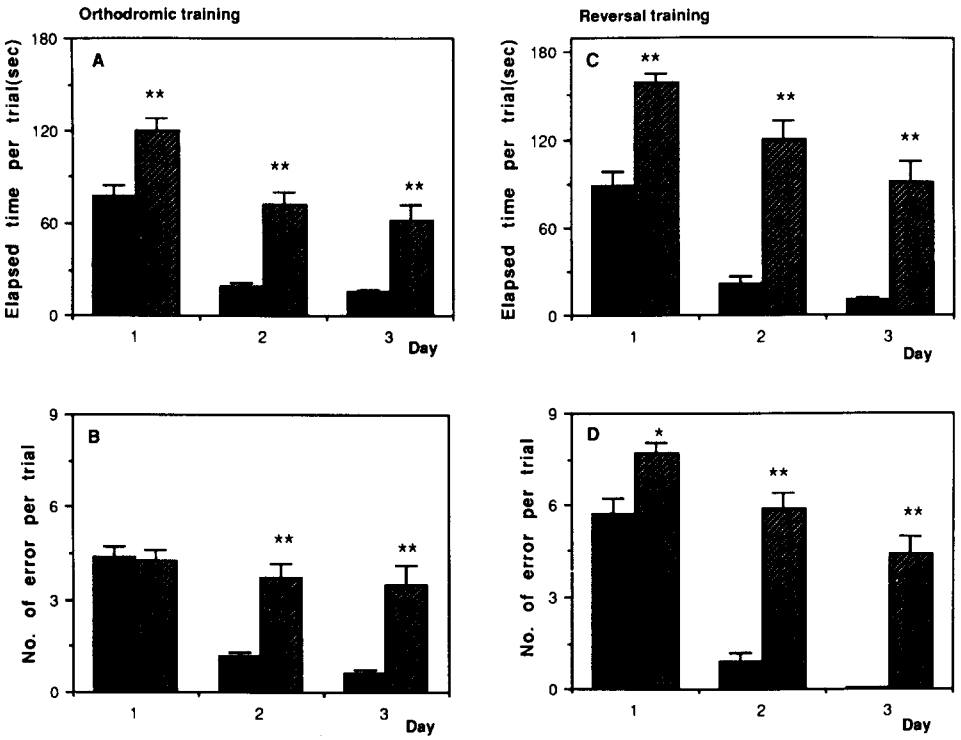

FIG. 2. Acquisition of orthodromic and reversal maze learning in young adult and aged rats. (A,B) Orthodromic training; (C,D) Reversal training; (A,C) Elapsed time per trial; (B,D) Number of errors per trial. Time for one training trial was limited to 180 sec. Solid bars: young adult rats (n = 16); stippled bars: aged rats (n = 14). *$p<0.05$ and **$p<0.01$ compared to the young adult rats.

Two other groups of both young adult and aged rats, one of which was not trained and the other which was trained for 5 days in the AAT, were subjected to AChE assay.

The activity of AChE was measured by the method of Ellman *et al.* (10). In brief, the tissue sample was homogenized in 50 vol. of 100 mM phosphate buffer (PB, pH 8.0). A 300-μl portion of this homogenate and 100 μl of 10 mM dithiobisnitrobenzoic acid were added to a reaction tube containing 2.7 ml of 100 mM PB. The sample was incubated at 37°C. Finally, 20 μl of 75 mM acetylthiocholine iodide was added and the changes in absorbance were recorded. The changes in absorbance per minute were calculated and the AChE activity was represented as the rate, in moles of substrate hydrolyzed per minute per gram of tissue. Protein was assayed by the method of Lowry *et al.* (16).

Statistics

To analyse data from the behavioral and biochemical studies, Student's *t*-test and analysis of variance (ANOVA) and Duncan's multiple range test were used.

RESULTS

Locomotor Activity and Flinch Threshold

Locomotor activity in aged rats was significantly less than in young adult rats ($t = 5.14$, $p<0.01$), although the flinch threshold was virtually identical in young adult and aged rats (Table 1).

Behavior Test

Active avoidance learning. The mean correct responses (MCR) increased as training session increased in both young adult and aged rats (Fig. 1A). However, the rate of increase in aged rats was significantly lower than that of young adult rats throughout the five test sessions, $F(1,22) = 35.5$, $p<0.01$. At session 5, MCR was 80% in young adult rats, and about 60% in aged rats (Fig. 1A). The difference was statistically significant, $F(1,22) = 18.2$, $p<0.01$. The session number required to achieve 60% or more MCR for young adult rats was 2, while that for aged rats was more than 7 (Fig. 1B).

Multiple T-maze learning. The swimming speed of aged rats was slightly less than that of young adult rats (young rats, 15.2 ± 1.5 sec/150 cm; aged rats, 20.9 ± 2.4 sec/150 cm), but the difference was not statistically significant.

In orthodromic training, the elapsed time per trial was significantly longer in aged rats than in young adult rats during the first to third test sessions, $F(1,28) = 45.6$, $p<0.01$ (Fig. 2A). The mean number of errors per trial in aged rats was also significantly greater than in young adult rats, $F(1,28) = 23.2$, $p<0.01$ (Fig. 2B).

In reversal training, the elapsed time was also longer for aged than for young adult rats, $F(1,28) = 72.9$, $p<0.01$ (Fig. 2C). Young adult rats generally reached the starting point without entering the blind alley except in the first test session, while aged rats continued to enter the blind alley during the first to third test sessions, $F(1,28) = 67.3$, $p<0.01$ (Fig. 2D).

Neurochemical Analysis

Because aged rats were found to be significantly inferior to young adult rats in both active avoidance tasks and water-filled multiple T-maze tasks, we measured the activities of ChAT and AChE as described previously.

TABLE 2

COMPARISON OF CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN THE 3 SELECTED BRAIN REGIONS OF YOUNG ADULT AND AGED RATS

Animal	N	State	ChAT Activity (nmol/mg protein/hr) Frontal Cortex	Hippocampus	Cerebellum
			A) Active Avoidance Task		
Young adult	6	Nontrained	43.1 ± 1.1	54.2 ± 2.7	6.3 ± 0.3
	6	Trained	42.5 ± 0.6	55.2 ± 2.7	6.1 ± 0.4
Aged	7	Nontrained	43.3 ± 1.0 *	54.3 ± 1.1	6.3 ± 0.4
	7	Trained	52.7 ± 2.1 *	55.0 ± 2.9	6.0 ± 0.4
			B) Water-Filled Multiple T-Maze Task		
Young adult	6	Nontrained	43.1 ± 1.1†	54.2 ± 2.7	6.3 ± 0.3
	6	Trained	42.2 ± 1.8	50.8 ± 2.9 *	6.7 ± 0.4
Aged	7	Nontrained	43.3 ± 1.0	54.3 ± 1.1 *	6.3 ± 0.4
	7	Trainied	45.1 ± 1.0	64.9 ± 3.0	6.5 ± 0.2

Each of 3 brain regions was obtained from rats in a nontrained state and from rats after either 5 days of active avoidance training in a shuttle box or 6 days of training in a water-filled multiple T-maze. Each value represents the mean ± S.E.M. N, Number of animals used.

*Significantly different from nontrained aged rats or training young adult rats at $p<0.01$.

ChAT Activity

ChAT activity in nontrained young adult and aged rats. There was no significant difference in ChAT activity in the three brain areas examined in young adults (n = 6) and aged rats (n = 7) in the nontrained state (Table 2A).

Effects of 5-day training in active avoidance task and 6-day training in a water-filled multiple T-maze task on ChAT activity in young adult and aged rats. In an active avoidance learning task, ChAT activity significantly increased in only the frontal cortex of aged rats over that of nontrained aged rats and also of trained young adult rats in the last or fifth training ($p<0.01$, Table 2A). In a water-filled multiple T-maze learning task, the enzyme activity significantly increased in only the hippocampus of aged rats over that of nontrained aged rats and also of trained young adult rats at the last or sixth training ($p<0.01$, Table 2B).

Effect of 2- or 10-day training in an active avoidance task on ChAT activity in young adult and aged rats. Measurement after almost the same level of MCR in a successfully completed active avoidance learning task (that is, in the second session for young adult rats and in the tenth session for aged rats) showed that ChAT

TABLE 3

COMPARISON OF CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN YOUNG ADULT AND AGED RATS

Animal	N	State	Active Avoidance Task ChAT Activity (nmol/mg protein/hr) Frontal Cortex	Hippocampus	Cerebellum
Young adult	5	Nontrained	40.8 ± 0.8	50.8 ± 3.7	6.5 ± 0.5
	5	Trained (2 days)	42.5 ± 1.5	49.0 ± 2.4	6.5 ± 0.5
Aged	5	Nontrained	43.5 ± 0.8	53.2 ± 1.3	6.5 ± 0.5
	5	Trained (10 days)	43.7 ± 0.6	52.0 ± 4.5	6.3 ± 0.3

Each of 3 brain regions was obtained from rats in a nontrained state (control) and from rats subjected to 2- or 10-day active avoidance training. Each value represents the mean ± S.E.M. N, Number of animals used.

TABLE 4

COMPARISON OF ACETYLCHOLINESTERASE ACTIVITY IN YOUNG ADULT AND AGED RATS

Animal	N	State	Rates (moles/l per min × 10^{-6} per g of tissue) Frontal Cortex	Hippocampus	Cerebellum
Young adult	6	Nontrained	6.27 ± 0.12	8.46 ± 0.14	4.00 ± 0.07
	6	Trained	6.32 ± 0.27	8.55 ± 0.19	3.87 ± 0.10
Aged	5	Nontrained	5.71 ± 0.09*	7.82 ± 0.18†	3.79 ± 0.07
	5	Trained	6.24 ± 0.28	8.16 ± 0.09	3.85 ± 0.02

Each of 3 brain regions was obtained from rats in a nontrained state (control) and from rats subjected to 5-day active avoidance training. Each value represents the mean ± S.E.M. N, Number of animals used.

*, †Significantly different from control at $p<0.05$ and $p<0.01$, respectively.

activity in the frontal cortex of aged rats was comparable to that for young adult rats (Table 3).

Effect of foot shock on ChAT activity in aged rats. There was no significant difference in ChAT activity in the FC of aged rats [shock off, 41.3 ± 2.85 nmoles/mg/hr (n = 5); shock on, 43.3 ± 1.11 nmoles/mg/hr (n = 5)].

AChE Activity

In a nontrained state, AChE activity in both the frontal cortex and hippocampus of aged rats was significantly lower than that of young adult rats (Table 4). However, at the fifth training session in active avoidance learning, the enzyme activity in the affected brain regions of aged rats was almost identical to that for the young adult rats (Table 4).

DISCUSSION

In the present study, we found that aged rats had significantly impaired acquisition of the active avoidance response in a two-way shuttle box.

Sensitivity to electric shock as an aversive stimulus, which is an important factor in avoidance learning task, in aged rats was identical to that in young adult rats, indicating that the impairment in the active avoidance task is not due to a decrease in sensory function. However, locomotor activity was significantly lower in aged rats than in young adult rats. This result indicates that impairment of active avoidance task may be partially due to a decline in motor function of aged rats.

Learning ability in young adult and aged rats was also examined in water-filled multiple T-maze task. Although swimming ability in a straight channel was identical in the two age groups, performance in the water maze was apparently impaired; that is, aged rats had a higher elapsed time and number of errors. The greater elapsed time may reflect decreased locomotor activity. Therefore, the impairment of aged rat's learning ability may be mainly due to a disruption of cognitive function. These behavioral findings indicate that some biochemical changes might occur in the brain during acquisition of a given kind of learning in aged rats.

Upon neurochemical examination, we measured ChAT and AChE activities as indices of brain function, and found no difference in basal ChAT activity in the FC and HC between young adult and aged rats in a nontrained state. However, significantly lower AChE activity was noted in the FC and HC of nontrained aged rats when compared to that of nontrained young adult rats. Similar observations of ChAT activity have been presented by Dravid *et al.* (9) and Sherman *et al.* (24); that is, there is no change or only a slight decrease during aging in the activity of ChAT in the HC and cortex of F344 rats. However, Springer *et al.* (25) found that ChAT activity in Ammon's horn and the subiculum was significantly lower in aged rats. The discrepancy might be due to age and strain differences; that is, Springer *et al.* used 40-month-old Sprague-Dawley rats, and we used 24-month-old F344 rats.

It is interesting to note that ChAT activity was significantly and selectively increased in a "task-dependent" manner over the basal level in the FC of aged rats after 5 days of active avoidance learning and in the HC of aged rats after 6 days of water-filled multiple T-maze learning. Furthermore, there was no difference in ChAT activity in the FC and HC between young adult and aged rats after the criteria for active avoidance learning was almost attained, and AChE activity in the FC and HC of aged rats reached the levels equivalent to that in young adult rats during acquisition of active avoidance learning. We also found that in aged rats that were tested under the same conditions in the active avoidance task without giving the conditioned stimuli, there was almost no increase in ChAT activity in FC. In contrast, Gallagher *et al.* (12) and Decker *et al.* (8) have reported that in aged rats in which memory impairment was observed in the water maze task, they could not observe any effect of training on HACU. Collectively, these findings suggest that during aging of Fischer 344 rats, the presynaptic cholinergic marker enzyme activity may remain normal and the postsynaptic marker enzyme activity is slightly, but significantly, decreased in the nontrained naive state, but the post- as well as presynaptic elements respond positively with regionally selective increases to behavioral training until a certain level of memory is reached.

We found no increase in cholinergic marker enzyme activities in either the FC or HC of young adult (3–4 months) Fischer 344 rats at any state of training. Conversely, Gallagher *et al.* found the training in Morris water maze to reduce high-affinity choline uptake (HACU) in the HC of young adult (4–5 months) Long-Evans rats (12). In contrast, there are several reports showing that the learning of T-, radial- or water-maze or active avoidance tasks increase the cholinergic activity in the HC and FC of young adult (3–5 months) Sprague-Dawley, Long-Evans, or Wistar rats (8, 20, 28). We have no relevant data or information to explain this discrepancy. Schmidt *et al.* (21) have obtained data showing that the HACU and turnover rate of ACh increases in the HC of Z-M, but not of Fischer 344 rats following foot-shock stress.

From the present behavioral and biochemical findings, it is hypothesized that the regionally selective and "task-dependent," but unregulated increase in the presynaptic and the age-related decrease in postsynaptic cholinergic markers may reflect compensatory responses of the system to keep ACh concentration in the synaptic cleft normal in the face of increased demand for motor and/or cognitive information processing during specific learning tasks in aged rats with deficient cognitive function. This compensation would have to occur in relation to such an underlying change as the age-related decrease in the number of cholinergic neurons in the basal forebrain (4), from which FC or HC are innervated (7), and decreased AChE activity in the FC and HC of aged rats as shown in the present study.

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