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Radial Maze Performance in Young and Aged Mice: Neurochemical Correlates

DAVID BERNSTEIN AND DAVID S. OLTON

Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218

DONALD K. INGRAM,[1] STEVEN B. WALLER AND MARK A. REYNOLDS

Gerontology Research Center,[2] National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224

AND

EDYTHE D. LONDON[3]

Addiction Research Center, National Institute on Drug Abuse, c/o Baltimore City Hospitals Baltimore, MD 21224

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Learning Memory Aging Brain Hippocampus Cerebral cortex Acetylcholine Choline acetyltranferase Glutamic acid decarboxylase

BEHAVIORAL senescence in laboratory rodents is accompanied by numerous performance impairments in many learning and memory tasks [1,15]. For example, age-related performance deficits in rats have been demonstrated in radial mazes [3, 4, 19, 45]. This task involves successive selection of a number of arms radiating from the center of the maze in order to obtain a food reward. The optimal strategy is to choose each arm once and avoid returning to an arm from which the reward had been removed during a previous choice. Therefore, the animal must remember each response during a trial to perform accurately.

Choice accuracy in the radial maze has been proposed as a good measure of short-term working memory [36]. The poor performance of aged rats in this task is consistent with decrements in short-term memory of human and sub-human primates, and possibly reflects cholinergic dysfunction [5]. Central cholinergic systems have been implicated in radial maze performance of rats. Pharmacological treatments [47] and brain lesions [32] which disrupt cholinergic systems severely impair choice accuracy in this task. Also, the hippocampal activity of choline acetyltransferase (E.C.2.3.1.6., ChAT), the enzyme which catalyzes the biosynthesis of acetylcholine from choline and acetyl-coenzyme A, has been significantly correlated with choice accuracy for aged but not young Wistar rats [19].

The GABAergic system also seems to influence radial maze performance. The activity of the synthetic enzyme, L-glutamic acid decarboxylase (E.C.4.1.1.15., GAD) in the cerebral cortex, is positively correlated with choice accuracy in aged but not young Wistar rats [19].

The present study was designed to assess performance of mice (*Mus musculus*) in a radial maze. In a previous study it

[1]Requests for reprints should be addressed to D. K. Ingram.

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[3]Dr. London is a collaborative investigator of the Gerontology Research Center.

was observed that, unlike rats, young CD-1 mice did not perform above chance levels in an eight-arm radial maze [33]. In contrast, others reported successful radial maze learning in mice [40]. They also noted marked strain differences in performance. While C57BR/cDJ and BALB/cJ mice demonstrated learning, C57BL/6J mice did not perform above chance levels. Thus, one objective of the present study was to optimize test procedures in order to determine if learning on a radial maze could be demonstrated in at least one of two strains previously reported to be unable to perform above chance levels in this task. To this end, C57BL/6J mice were trained in a maze designed to enhance the salience of extramaze cues as is commonly done with rats [34]. Motivation for correct responding on the maze was enhanced by limiting the liquid available to the mice. The only liquid the mice received during testing was that which was available on the arms of the maze following a correct choice.

A second objective was to determine if aged mice were deficient in learning this task, as is the case for aged rats. Consequently, a group of aged (27–28 month) mice was included for comparison with the group of young mice (8 months).

A third objective was to determine the extent to which the young and aged mice differed in the activities of ChAT and GAD, and the extent to which the activities of these enzymes were correlated with choice accuracy. Activities of ChAT and GAD were measured in four brain regions: (a) the hippocampus, because damage to the hippocampus profoundly impairs performance on this task [35]; (b) the cingulate cortex, because oxotremorine, a direct acting cholinergic receptor agonist [25] which may enhance memory in mice [2], increases glucose utilization in this region [8]; (c) the sensorimoror cortex, because performance in the maze requires substantial sensorimotor integration, and levels of ChAT in the cortex were negatively related to motor performance in tests of strength and coordination in aged C57BL/6J mice [20]; (d) the frontal cortex, because some of the neocortical cholinergic deficits in Alzheimer's disease have been observed in this cortical region [17,41].

METHOD

Subjects

Male C57BL/6J mice were used in two similar experiments conducted about 6 months apart. In the first experiment (A), the subjects were four young (8 month) and two aged (28 month) mice. In the second experiment (B), the subjects were six young (8 month) and four aged (27 month) mice. The mean lifespan of this strain has been estimated to be 26–27 months [14]. The mice were obtained at weaning from the Jackson Laboratory (Bar Harbor, ME) and raised in a vivarium at the Gerontology Research Center. At least 2 weeks before training began, they were moved to a vivarium in the Department of Psychology of the Johns Hopkins University. They were maintained on a 14-hr light:10-hr dark cycle.

The mice were housed in separate, metal cages. They were given food (Purina Lab Chow) and water without restriction before testing began. During testing (which was conducted Monday through Friday), food still was provided ad lib, but a water deprivation schedule was introduced. From Sunday afternoon until after the last trial on Friday, no water was present in the home cage, so that the only liquid the mice received was on the maze. From Friday afternoon through Sunday afternoon, unlimited water again was provided in the home cage. At the end of testing, and before the animals were killed, water again was provided ad lib.

Apparatus

In the first experiment, an eight-arm radial maze was constructed of wood, painted black. The central platform was 51 cm in diameter. Each arm was 667 cm long, 7 cm wide, and contained a cup 3 cm from its end. A wooden wall 25 cm high surrounded the central platform. At the entrance to each arm, a hole was cut into the wall. Access to each arm was controlled by a transparent Plexiglas guillotine door, which was attached by a string to a central control panel. The top of the maze was uncovered. The testing room was brightly illuminated, and it contained many visual stimuli, such as chairs, tables, and drawings on the walls.

A similar, elevated eight-arm radial maze was used in the second experiment. It had the same dimensions as the previous maze, but was not painted. It was located in a different room, which also was brightly lit and contained many objects.

Behavioral Procedure

Shaping. For 5 days in the first and 6 days in the second experiment, the mice were shaped to run to the ends of the arms to obtain isotonic saline. The cup at the end of each arm was filled with 0.4–0.6 ml of 0.9% (w/v) NaCl. The mouse was placed in the center of the maze, the doors were lifted, and the mouse was allowed to run throughout the maze for 10 min. After the mouse drank the saline at the end of an arm and returned to the middle of the maze, the cup was refilled. During shaping, the only liquid that the mice received was the 0.9% NaCl that they drank in the maze.

Training. At the start of each trial during both experiments, 0.05–0.1 ml of NaCl was placed in the cup at the end of each arm. The mouse was then placed into the center of the maze, the guillotine doors were opened, and the mouse was allowed access to all maze arms. A choice was recorded when the mouse had moved with all four paws into an arm. A drink was recorded when the mouse touched the cup of saline at the end of the arm. While the mouse was at the end of a maze arm, the doors to all arms were lowered except the one which the mouse had entered. When the mouse returned to the center of the maze, this last door was lowered, thus confining the animal to the center of the maze for 5 sec before the doors were lifted again. This procedure continued for 10 min or until the mouse made a total of twelve choices or drank the rewards at all eight arms. Three trials were given every testing day with at least 90 min between trials. After the last of the 36 test days, the mice were allowed unlimited access to water.

Neurochemical Assays

Between 3–5 days after testing, the mice were killed by cervical dislocation. The brains were removed quickly and dissected on an ice-cooled aluminum plate. The following brain regions were removed: hippocampus, frontal cortex, sensorimotor cortex, and cingulate cortex. The samples were frozen on solid CO_2 and stored in tightly sealed containers at −70°C until the time of assay.

Prior to assay, the samples were sonicated (Sonicator Cell Disruptor, Model W-220F, Heat Systems-Ultrasonic, Plainview, NY) in 20 volumes (w/v) of 0.05 M Tris-HCl, pH

7.4, containing 0.2% (v/v) Triton X-100. The resultant suspensions were centrifuged at 4°C for 15 min at 14,000 × g. ChAT (E.C.2.3.1.6.) activity in the supernatant was measured according to a previous method [7]. Standard incubations contained 60 mM choline chloride (Sigma Chemical Company, Inc., St. Louis, MO) and 0.1 mM [1-^{14}C]acetylcoenzyme A (2–5 mCi/mmole); Amersham Corp., Arlington Heights, IL). GAD (E.C.4.1.1.15.) activity was measured according to a previous method [48]. Standard incubations contained 2.5 mM L-glutamic acid (Sigma) with 5–10 nCi L-[1-^{14}C]glutamic acid (250 mCi/mmole; New England Nuclear Corp., Boston, MA). Enzyme activities were reported in terms of supernatant protein, which was assayed by a conventional method [26].

Data Analysis

The number of correct choices (different arms) and of drinks taken in the first eight choices of each trial were determined for each block of six trials. The data from both experiments were combined and submitted to a 2 (experiment) by 2 (age) by 18 (blocks of 6 trials) analysis of variance (ANOVA) for unequal 'n,' with repeated measures on the last factor [9]. Age and regional comparisons of ChAT and GAD activities were conducted in separate 2 (age) by 4 (regions) ANOVAs for unequal 'n,' with repeated measures on the last factor. Where there were significant interactions, further analysis was made of the simple, main effects [49].

Correlations were determined for the relation of enzyme activities in each region to performance, as measured by correct responses during training. Because of the small sample sizes, the correlational analysis combined data across age groups and experiments, but controlled for differences attributed to age or experiment with a partial correlational technique [49].

RESULTS

Choice Accuracy

The mice demonstrated learning in both experiments, and there was no significant age difference in learning rate. In both experiments, the mice were quickly shaped to enter the arms and to drink isotonic saline from the cup at the end of each arm. When testing began, both groups performed at about chance level, which is 5.3 correct responses in the first eight choices [37]. As shown in Fig. 1, the mean number of correct responses steadily increased for young and aged mice in both experiments (A and B) and reached the same asymptote well above chance level.

The ANOVA of correct responses showed that performance was equivalent across experiments, as there was no significant main effect of experiment, $F<1.0$, and no significant interactions involving experiment, $ps>0.05$. The main effect of blocks was significant, $F(17,204)=15.48$, $p<0.0001$, which indicated enhanced learning with increased number of trials. However, the main effect due to age and the age by blocks interactions were both insignificant, $ps>0.05$. All other interactions also were insignificant, $ps>0.05$.

Drinks

Young and old mice demonstrated equivalent numbers of drinks throughout both experiments. The mean number of drinks steadily increased at nearly the same rate for both groups of mice (Fig. 2). The ANOVA for drink responses showed that the main effect of trial blocks was significant,

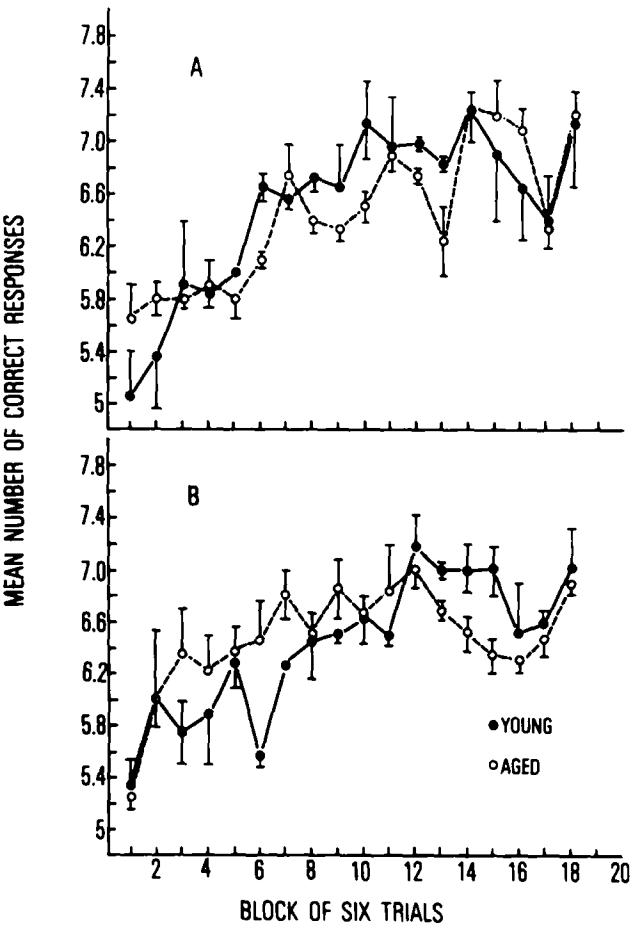

FIG. 1. Mean (±SEM) correct choices during first eight responses of young (8 month) and aged (27–28 month) male C57BL/6J mice in a radial maze during Experiment 1 (A) and Experiment 2 (B).

$F(17,204)=38.65$, $p<0.0001$, but that the main effect of age and the age by block interaction were insignificant, $ps>0.05$. The main effect of the experiment was significant, $F(1,12)=7.27$, $p<0.0001$, which indicated a generally higher level of drinking in Experiment 2; however, the experiment by block interaction was significant, $F(17,204)=3.31$, $p<0.0001$. Tests of the simple main effects (Winer, 1971) revealed that mice had significantly higher drink responses in Experiment 2 during blocks 1, 3, 12, and 13. The age by experiment by blocks interaction also was significant, $F(17,204)=1.78$, $p<0.03$. An analysis of simple, main effects suggested that the interaction was generated in Experiment 2 from significant age differences in the number of drink responses during trials 1, 3, and 4, in which the aged mice drank more, and during trial 15 when the young mice drank more, $ps<0.05$.

General Behavior

The mice tended to run along the perimeter of the central platform and avoid the open middle. They inhibited responses to the arms when the guillotine doors were lifted. When entering an arm, the mice paused and then quickly ran down the arm and drank the saline. When leaving an arm, the mice proceeded either directly to the entrance of another

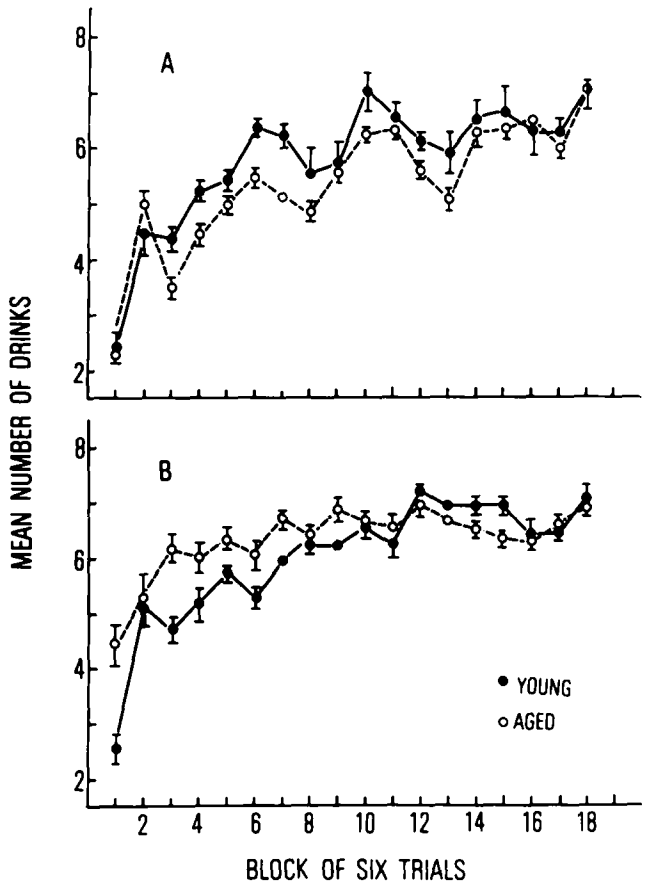

FIG. 2. Mean (±SEM) number of drinks during first eight responses of young (8 month) and aged (27–28 month) male C57BL/6J mice in a radial maze during Experiment 1 (A) and Experiment 2 (B).

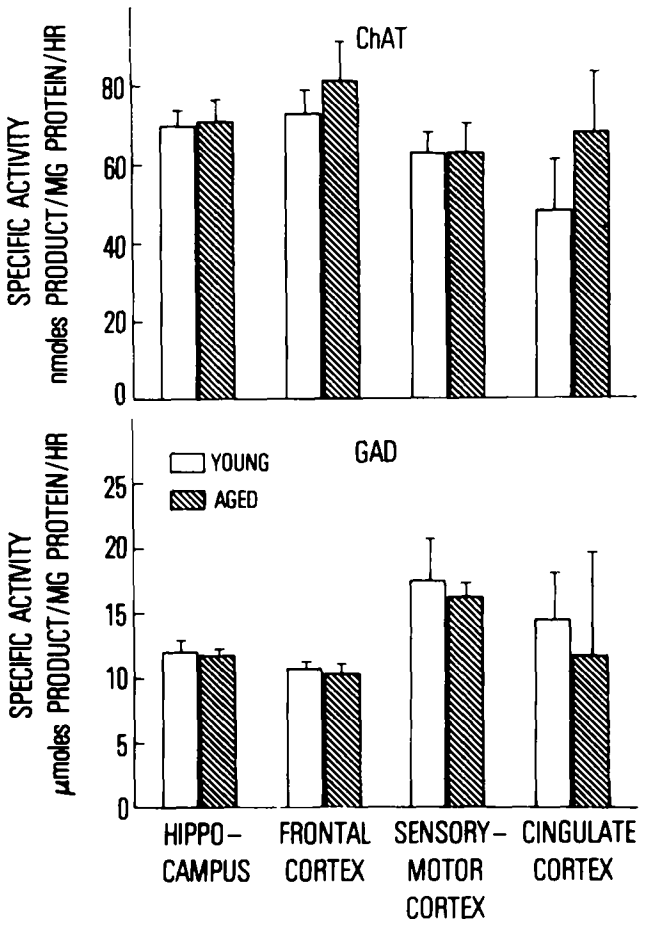

FIG. 3. Mean (±SEM) estimates of the activities of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) in selected brain regions of young (8 month, n=10) and aged (27–28 month, n=6) male C57BL/6J mice following performance in a radial maze.

arm or walked around the periphery of the center and waited for the doors to be lifted. Toward the end of a trial, the mice appeared to be more cautious about entering arms and took longer to make a choice after the doors were opened.

Enzyme Activities

Age and regional comparisons of the activities of ChAT and GAD are presented in Fig. 3. The results of the ANOVAs for the activities of each enzyme revealed no significant main effects of age or region and no significant age by region interactions with respect to any parameter, $ps > 0.05$.

Individual correct responses during blocks 1–6 (trials 1–36) and blocks 7–12 (trials 37–72) were correlated with individual estimates of regional enzyme activities. The trials were split into these portions to compare the patterns of correlation during early training, when the mean level of performance was not reliably above chance (5.3 correct responses) and during later trials, when it exceeded the chance level. Performance during blocks 13–18 was at an asymptotic level with fewer errors and, therefore, was of less interest.

The pattern of correlations is presented in Fig. 4. While only three of the correlation coefficients reached statistical significance ($ps < 0.05$), a definite pattern was evident. The most consistent result was a positive correlation between ChAT activity in the cingulate cortex and choice accuracy, a correlation that was greater than 0.6 and significant, regarding both phases of training. The other significant result was a negative correlation between ChAT activity in the sensorimotor cortex and choice accuracy in the first set of trials. All the other correlations of ChAT activity and performance were small and insignificant.

GAD activity was not significantly correlated with choice accuracy in any comparison; however, choice accuracy tended to be positively correlated with GAD activity in the cingulate cortex and negatively correlated with GAD activity in the sensorimotor cortex. This pattern paralleled that observed for ChAT activity.

DISCUSSION

The results of these experiments demonstrate that: (a) C57BL/6J mice can solve a spatial discrimination on an eight-arm radial maze; (b) young and old mice learn at

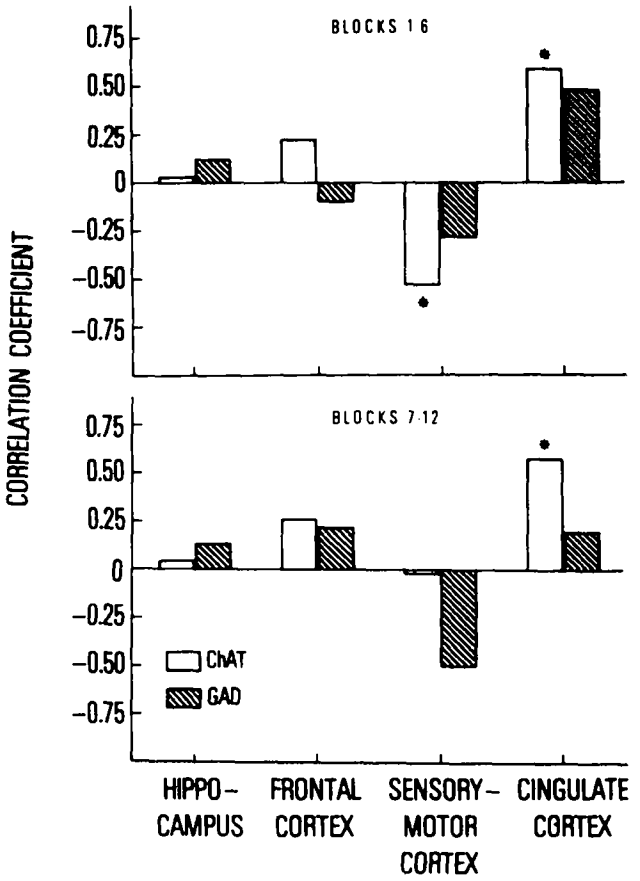

FIG. 4. Partial correlation (controlling for age and experiment) of the number of correct responses in the radial maze and the activities of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) in selected brain regions of young (8 month, n=10) and aged (27–28 month, n=6) male C57BL/6J mice (*denotes $p<0.05$).

similar rates in this task; and (c) correct responding is positively correlated to ChAT activity in the cingulate cortex and negatively correlated to ChAT activity in the sensorimotor cortex.

Our observation that C57BL/6J mice performed above chance levels in a radial maze contrasts with findings from two previous studies [33,40]. In the first study, the maze used was smaller, more enclosed, and constructed with opaque doors [33]. The maze in which C57BL/6J mice had performed poorly [40] was made of clear plastic, but was much smaller. The larger, open maze used in the present experiment could have made the task easier by enhancing the salience of extramaze cues, which are the most effective discriminative stimuli of rodents in spatial tasks [34].

In both previous studies with mice, food deprivation and food reward were used. In a preliminary experiment with food deprivation to motivate the mice and food at the end of each arm as the reward, little learning was observed. Other factors in the present study that might have enhanced learning opportunity include: a long shaping period, three trials each test day, the confinement to the center of the maze between choices to interrupt response habits. Thus, our procedures obviously facilitated performance; however, it is evident that C57BL/6J mice learn more slowly than do rats in this paradigm [37] and more slowly than at least two other mouse strains [40].

Age-related performance impairments have been observed in previous studies with rats on this type of maze [19,45] and with mice in tasks such as passive avoidance [6, 23, 24], active avoidance [10], and complex T-mazes [11,12]. Thus, we expected an age-related impairment in this task. However, we found no age difference in two separate experiments.

The discrepant findings may reflect species differences in the decline of working memory. Furthermore, the age-related decline in the performance of rats might result not from impaired short-term retention, but from a decline in visual discrimination [45]. Procedural differences also may be important. The maze in the present study may not have been complex enough to manifest age differences [13]. It had only 8 choices, while the mazes used for the rats often had 12 or 16 choices [3, 4, 19]. Another critical procedural variable might be the time interval during which working memory is taxed. For example, in studies of passive avoidance retention in rats and mice, no age differences are observed when the test-retest interval is less than 2 hr, but differences appear when the interval is increased [23,50]. Working memory in the current maze task was required for only a few minutes. If the task duration had been lengthened by instituting longer response delays, an age difference might have been manifested. Such an outcome was found by increasing the intertrial interval up to 10 min in a spontaneous alternation task for rats [50]. Increasing the response interval up to 15 min also decreased performance in a radial maze task; however, aged rats were not affected differentially [45].

Confinement to the center of the maze was intended to assure that the age difference reflected a memory impairment rather than possible age differences in response strategies. However, two previous studies [19,45] reporting an age-related impairment in radial maze performance did not link it to differences in response patterning; whereas, another study [4] has demonstrated age differences in response strategies. The procedures for assessing this hypothesis were quite different among the studies.

The motivational manipulation also must be considered. We estimated that the mice were consuming about 1.5–2.5 ml of fluid daily, which is about 30–50% of their normal daily intake of 4–5 ml, but they were replenished over the weekends. This regimen apparently produced a high motivational level, as judged by the quick responses in the radial maze. As mentioned above, we had little success with food rewards in the radial maze during pilot studies. Under a less severe fluid deprivation schedule (22 hr), age differences in male C57BL/6J mice were observed in complex T-maze performance [18].

As a final methodological consideration, the relatively small sample sizes (ns=10 and 6 for the young and aged groups, respectively) should be acknowledged. The small sample of aged animals might not be representative of typical performance for this age group in this task. However, data that might counter this argument were not presented with the results. We included in the analysis only those aged mice that survived the 7 weeks of training and were sacrificed for neurochemical assay. Actually, nine aged animals began training, so there was substantial mortality. Regarding this observation, one might contend that only the healthiest mice survived, and this selective factor indeed biased the sample.

Based on an examination of data from the nonsurvivors, though, we observed no marked differences in performance prior to death compared to the performance of the survivors. The mean number of correct responses at time of death was 6.7, which is close to asymptotic level for young mice.

The effects of aging on ChAT and GAD activities in the mouse brain are unclear. Previous studies have demonstrated all possible relations between increasing age and hippocampal ChAT activity: (a) increased levels [46], (b) no differences [42], and decreases [44]. In the present study, no age-related differences in ChAT and GAD activities were found in any of the four brain regions that were examined, including the hippocampus. The discrepancies may arise from genuine strain differences, precluding a homogeneous perspective on the neurochemistry of aging [46]. Alternatively, they may reflect procedural differences. The mice used in the relevant studies had many different behavioral experiences. Some were experimentally naive virgins [44], others were experimentally naive but had been used for breeding [42], while others had undergone extensive experimental testing [46]. The maze training in the present study apparently could influence age-related differences in the neurochemical markers. Alterations in cholinergic parameters, such as increased concentrations of acetylcholine or enhanced choline uptake, have been induced by a learning experience in rats [31,39].

Evidence for a hippocampal component in radial maze performance of rats was obtained in studies of rats with fimbria-fornix lesions [35]. These lesions, which disrupt the cholinergic innervation to the hippocampus, are associated with decreased choice accuracy on the maze [35]. Choice accuracy in the radial maze correlated with hippocampal ChAT activity in aged but not young Wistar rats [19]. In the present study with mice, however, a relation between performance and hippocampal ChAT was not found. One of the previous studies also observed no relation between hippocampal ChAT activity and strain differences in radial maze performance by mice [40]. In other studies comparing mouse strains, hippocampal ChAT was correlated with behavior. The correlation was positive for performance in a continuous reinforcement bar press task emphasizing long-term retention [21], and negative for performance in a similar task emphasizing short-term acquisition [22]. Studying individual differences within the C57BL/6J strain, previous investigators had found a significant positive correlation between hippocampal muscarinic receptor densities and 24-hr retention in a step-down passive avoidance task, but not in a step-through passive avoidance [24]. Thus, the relation between cholinergic neurochemical markers in the hippocampus and performance by mice in learning and memory testing paradigms still requires clarification.

ChAT activity in the cingulate cortex was positively correlated with performance in the present study. Findings from past studies suggest that in rats and cats, the major effect on performance in learning tasks resulting from lesions to this cortical region [29], to its afferent pathways [43], or to adjacent regions [28] is impaired acquisition in two-way active avoidance. Performance in one-way active avoidance is affected only marginally [38] or not at all [27]. No significant effects of cingulate cortex lesions on the performance of rats were observed in a food-motivated, Lashley-III maze [43]. In contrast, performance in passive avoidance tests requiring inhibition of responses may be enhanced by such lesions [29]. Apparently, performance in learning situations involving approach-avoidance conflicts, as in two-way active avoidance, seems to be most affected by damage to the cingulate gyrus or its connections [16]. While such tasks involve aversive motivational manipulations, this type of approach-avoidance conflict in the appetitively-motivated radial maze task may be reflected in the ability to inhibit responses to arms that were entered previously. Moreover, our data suggest cholinergic involvement of the cingulate cortex in this behavior. This suggestion agrees with the observation that oxotremorine, a cholinergic agonist with purported memory-enhancing effects [2], increases glucose utilization, a measure of local functional activity, in this brain region [8]. Another finding that suggests a role of cingulate cortex cholinergic systems in memory is the significant decrement of ChAT in layers II and III of cingulate cortex autopsy samples from Alzheimer's patients [17].

In the present study, ChAT activity in the sensorimotor area of the frontoparietal cortex was negatively related to radial maze performance, but only during the early phase of training. A previous study of this mouse strain also showed an inverse relation between performance in tests of strength and coordination and ChAT in cortical samples containing frontoparietal and temporal areas [20]. Thus, the negative relation between ChAT and maze performance may reflect impaired psychomotor abilities in mice that have high ChAT activities in the sensorimotor cortex [20].

In summary, C57BL/6J mice can learn to perform a spatial discrimination in a radial arm maze when the task is designed to enhance performance. The lack of an impairment in the aged mice relative to the young mice in this task suggests that aging in the mouse does not affect performance in the same way as in rats; or, alternatively, the particular task parameters used here were not sufficiently sensitive to measure these effects. Finally, significant correlations between regional activities of ChAT and GAD and maze performance may help identify critical brain systems involved with performance in this task by mice.

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