

Sex differences in neurochemical markers that correlate with behavior in aging mice

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Abstract

Sex differences in neurochemical markers that correlate with behavior in aging mice NEUROBIOL AGING. We examined whether the enzymatic activities of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) were altered similarly with age in male and female mice, and whether these changes were correlated with age-related alterations in memory and anxiety. ChAT and GAD activities were measured in neocortex, hippocampus, and striatum of behaviorally characterized male and female C57BL/6 mice (5, 17, and 25 months). Generally, ChAT activity was increased, and GAD activity decreased, with age. However, disparate changes were revealed between the sexes; activities of both enzymes were decreased in 17-month males, whereas alterations in females were not observed until 25-months. Furthermore, enzyme-behavior correlations differed between the sexes; in males, ChAT activity was related to one behavioral task, whereas in females, activities of both enzymes were correlated with multiple tasks. Significant enzyme-behavior correlations were most evident at 17 months of age, likely the result of behavioral and enzymatic sex differences at this age. These data represent the first comprehensive report illustrating differential alterations of ChAT and GAD activities in aging male and female mice. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Over the past three decades, cholinergic neurons of the basal forebrain have been a primary focus of research investigating the neurobiology of age-related memory dysfunction. These neurons, which project to the neocortex and hippocampus, undergo considerable degeneration in normal aging [21,43] and Alzheimer's disease [14,15]. In particular, activity of the cholinergic synthetic enzyme choline acetyltransferase (ChAT) is diminished significantly in the neocortex and hippocampus of Alzheimer's brains [17], and these reductions have been associated with memory impairments in Alzheimer's disease [50, 53]. ChAT activity is also

a sensitive index of the functional integrity of cholinergic neurons in the aged rat brain. In aged rats, levels of ChAT activity in the hippocampus are correlated with spatial memory decline [4, 19] and compounds that alter hippocampal ChAT activity improve spatial memory [26]. Although cholinergic dysfunction certainly contributes to mnemonic decline in aging, it has become increasingly evident that age-related memory impairments are likely the result of alterations in multiple neurotransmitter systems [40]. GABAergic neurons in the basal forebrain also project to neocortex and hippocampus, and play an important role in modulating plasticity in both structures [20,59]. Although some evidence suggests reductions of the GABAergic synthetic enzyme glutamic acid decarboxylase (GAD) in normal aging [42] and Alzheimer's disease [53,54], age-related alterations in GAD and other GABAergic markers have not been documented as extensively as those of cholinergic markers. However, GAD is the rate-limiting step in GABA synthesis, and therefore, age-related alterations in GAD activity are likely to influence GABAergic transmission.

Few studies have examined age-related changes in ChAT and GAD activity in mice. Given the increased use of

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genetically altered mice in models of age-associated diseases such as Alzheimer's, it has become imperative to determine the magnitude of age-related neurochemical changes in mouse strains that commonly provide genetic background for transgenic lines. One such progenitor strain is the C57BL/6 [2]. The handful of studies conducted in C57BL/6 mice thus far do not provide a clear picture regarding the precise nature of age-related changes in either enzyme; previous reports indicate that ChAT and GAD activities are increased, decreased, or unaltered with age in the neocortex and hippocampus [9,58,60,63,64,65]. Despite findings of impaired spatial learning and memory in aged C57BL/6 mice [9,23,25,34], few previous neurochemical studies have measured ChAT and GAD activities in behaviorally characterized aged mice. Therefore, it is unclear whether altered enzyme activities are associated with age-related learning and memory decline in mice.

Furthermore, all previous investigations of age-related changes in ChAT and GAD activities in C57BL/6 mice have tested only males. Therefore, it is unknown whether these enzymes are affected by age in females. Cholinergic and GABAergic functions in the neocortex and hippocampus of females are modulated by the ovarian hormones estrogen and progesterone [30,57,67]. In young female C57BL/6 mice, neocortical ChAT activity fluctuates during the estrous cycle and neocortical GAD activity is decreased in response to ovariectomy [24]. Moreover, during early development and young adulthood, sexual dimorphisms in ChAT and GAD activities have been observed [16,24,39,55,56], as well as sex differences in response to cholinergic [6,55,56], GABAergic [52], and estrogen treatments [51]. Aging in the female C57BL/6 mouse is accompanied by significant reductions of estrogen and progesterone [46], whereas healthy C57BL/6 males do not experience age-related alterations of testosterone levels [47]. Thus, given the sensitivity of ChAT and GAD activities to sex hormones, the disparate loss of these hormones in aging mice raise the possibility that ChAT and GAD activities may be affected differentially by age in males and females. This sex-specific modulation of ChAT and GAD may have significant implications for cognition.

Our previous work in C57BL/6 mice suggests that age-related memory decline begins at an earlier age in females than in males [25]. Male and female mice, ages 5-, 17-, and 25-months, were tested in a battery of behavioral tasks to assess spatial and non-spatial (visual and olfactory) reference memory, anxiety levels, and general motor ability. Twenty-five month-old mice of both sexes exhibited impaired spatial and olfactory memory relative to 5 month-old mice, in the absence of deficits in motor activity or altered anxiety levels. Seventeen month-old females, but not males, displayed impaired spatial memory and heightened anxiety relative to young mice of both sexes. The premature deterioration of spatial memory in females coincided with age-related reductions in ovarian hormone cycling [25], suggest-

ing that plunging ovarian hormone levels during middle-age may trigger cognitive dysfunction in females.

The present study examined whether ChAT and GAD activities vary with age similarly in male and female mice, and whether these changes are associated with age-related behavioral dysfunction. Levels of ChAT and GAD activity were measured in the frontoparietal cortex, hippocampus, and striatum of male and female mice (5, 17, and 25 months old) previously tested in a battery of behavioral tasks including the Morris water maze, odor discrimination, and plus maze [25]. Although not typically considered critical for spatial memory, the striatum plays a role in the performance of some memory tasks, including the cued water maze task used in this study [10,18], and thus may be important for non-spatial memory. Therefore, we measured enzyme activity in the striatum to provide a potential neurobiological correlate for the cued task, as well as other motor tasks. Six indices created from measures of five behavioral tasks (the results of which are detailed elsewhere [25]) were correlated with ChAT and GAD activities to examine relationships between age, sex, memory, and enzyme activity.

2. Methods

2.1. Subjects

Male ($n = 36$) and female ($n = 36$) C57BL/6NIA mice were obtained from the National Institutes on Aging colony at Charles River Laboratories (Stoneridge, NY). Mice were tested at three ages, representing young, middle-aged, and aged time points: 5-months (10 male, 10 female), 17-months (10 male, 10 female), 25–26 months (16 males at 25-months, 10 females at 25-months, 6 females at 26 months; this group will hereafter be referred to as the 25-month group). Mice were housed up to 6/cage in a room with a 12:12 light/dark cycle, and were tested behaviorally and killed during the light phase of the cycle. Food and water were provided *ad libitum*, except during testing in the olfactory memory task, when food was available for approximately 6 h after completion of the daily test session. At the completion of testing in the olfactory task, mice were returned to an *ad lib* diet. After completion of all behavioral testing, the regularity of estrous cycling in females was assessed by vaginal lavage for ten days [25]. On the basis of the vaginal smears, females were categorized as exhibiting: regular cycling (estrus phase observed at least twice), irregular cycling (estrus phase observed once) and no cycling (absence of estrus or metestrus phases). All procedures conformed to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Care and Use Committee of Wellesley College.

2.2. Behavioral procedures

Behavioral test procedures and data are detailed elsewhere [25] and summarized briefly here. All memory tasks tested reference memory, a type of memory for information that remains constant from trial to trial [49].

Morris water maze. Spatial and non-spatial reference memory were tested using spatial and cued versions of the water maze task. The spatial task tested the mouse's ability to use extramaze cues to locate a hidden escape platform and the cued task tested the mouse's ability to locate a visible platform utilizing local cues rather than extramaze cues. The hidden (spatial) or visible (cued) platform was placed in a circular tank (108 cm in diameter) filled with water (26°C). Mice were placed in the tank at four different points and allowed 120 sec to escape onto the platform. Mice were first tested in the spatial task for 5 days, during which the hidden platform remained in a constant location throughout testing. Six trials were conducted on each day; during trials 1–5 (platform trials), the platform was raised and available for escape, whereas during trial 6 (probe trial), the platform was lowered for a variable amount of time (20–40 sec) during which the mouse's search pattern was recorded. Following the spatial task, mice were tested in the spatial reversal task for 3 days, the results of which are described elsewhere [25]. Finally, mice were tested in the cued task for 3 days, in which a platform, made visible by lowering the water level and attaching a visual cue, was moved to a different quadrant for each trial of each session. Six platform trials were conducted per day. Swim time (sec), path length (cm), swim speed (cm/s), and time spent in a 14 cm wide corridor (measured from the start location to the platform) were recorded during platform trials in all tasks. Two measures were recorded during the probe trials in the spatial task: platform crossings (number of times/10 sec the platform location was crossed) and quadrant time (% of time spent in the quadrant formerly containing the platform).

Simple odor discrimination (SOD). In this olfactory reference memory task [8], the mice were trained to discriminate between two odors presented simultaneously. Powdered spices (cinnamon or curry) were mixed in sand and presented in small cups (2.5 cm in diameter). A 15 mg piece of chocolate was buried near the bottom of each cup. After pretraining, during which the food-restricted mice learned how to dig in the sand for the chocolate reward, mice were trained to associate one of the scents with chocolate. Testing was conducted in a clean mouse cage with bedding at one end and the two scent-filled cups at the other; only one cup was rewarded. Four trials of 5 min each were conducted for 3 days, and each mouse was assigned one scent that was rewarded consistently throughout testing. Choice accuracy (%), number of errors (digs) to the unrewarded cup, and latency (sec) to retrieve the chocolate were recorded.

Elevated plus maze. This task, commonly used to measure anxiety [38], consisted of a wooden maze with a central

platform and four arms radiating out in a plus shape. Two opposite arms had walls and were painted black, whereas the other opposite arms did not have walls and were painted white. The maze was elevated 90 cm above the floor. One session was conducted in which each mouse was placed in the central platform and allowed to freely explore the maze for 5 min. Five measures were recorded: number of closed arm entries, number of open arm entries, time spent in closed arms, time spent in open arms, and number of defecations in closed arms.

2.3. Enzyme activity assays

Each mouse was sedated with CO₂ [7], killed by cervical dislocation, and decapitated. The brain was removed immediately, and the frontoparietal cortex, hippocampus, and striatum were dissected bilaterally on ice. Tissue samples were weighed and stored at -70°C until the day of assay. Samples were resuspended in 50 mM TrisHCl and 0.02% Triton X-100, sonicated with a probe sonicator, and centrifuged for 10 min at 10,000× g. The supernatant was diluted 1:5 and designated as the crude extract. This crude extract was used for both assays. The protein content of the samples was measured using a Bio-Rad (Bio-Rad Laboratories, Hercules, CA) protein assay [11]. Enzyme activities were expressed as nmol of product/hr/mg protein. Female mice with intact estrous cycles were killed irrespective of estrous cycle phase. All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise noted.

Choline acetyltransferase. Activity of the enzyme ChAT, which synthesizes acetylcholine, was measured by the formation of [¹⁴C]Acetylcholine from [acetyl-1-¹⁴C]-acetyl-coenzymeA (55.7 mCi/mmol, New England Nuclear, Boston, MA) and choline based on the method of Fonnum [7,22]. Reactions contained 40 μl of crude extract and 300 mM NaCl, 50 mM NaH₂PO₄ (pH 7.4), 10 mM EDTA, 0.1 mM eserine, 0.05 mg/ml bovine serum albumin, 8 mM choline, 0.2 mM [¹⁴C]Acetyl CoenzymeA in a total volume of 80 μl. Samples were incubated for 20 min at 37°C, and the reaction was terminated by the addition of a 17:3 mixture of toluene and acetonitrile containing 5 g/liter Na-tetraphenylboron. After centrifugation for 1 min at 10000× g, the [¹⁴C]ACh product in the organic (top) phase was measured with a scintillation counter.

Glutamic acid decarboxylase. Activity of the enzyme GAD, which synthesizes gamma-aminobutyric acid (GABA), was measured from L-[1-¹⁴C]-glutamic acid (40–60 mCi/mmol, New England Nuclear, Boston, MA) using a [¹⁴C]CO₂ trapping technique [48]. Reactions contained 50 μl of crude extract and 0.5 M KH₂PO₄, 5 mM ethylenediaminetetraacetic acid, 1 mM 2-aminoethylisothiuronium bromide, 10 mM glutamate, 1 mM pyridoxal phosphate and L-[1-¹⁴C]-glutamic acid in a total volume of 100 μl. Samples were incubated for 1 h at 37°C in test tubes containing #32 glass fiber filters (Schleicher and Schuell, Keene, NH) coated with 0.5 M Solvable (Packard Instru-

Table 1
Summary of factor loadings in principal components analysis

Behavioral Index	Task	Measure	Sessions	Factor*
Spatial acquisition	Spatial	Swim time	1–3	4
		Pathlength	1–3	4
		Corridor	1–3	4
Spatial asymptotic	Spatial	Swim time	4–5	2
		Pathlength	4–5	2
		Corridor	4–5	2
Cued/SOD latency	Cued	Swim time	1–3	3
		Pathlength	1–3	3
		Corridor	1–3	3
	SOD	Latency	1–3	3
Swim speeds	Spatial	Swim speed	1–3	1
		Swim speed	4–5	1
SOD Accuracy	SOD	Swim speed	1–3	1
		Choice accuracy	1–3	5
Plus Maze	Plus Maze	Errors	1–3	5
		# closed-arm entries	—	—
		# open-arm entries	—	—
		Time in closed arms	—	—
		Time in open arms	—	—

All factor loadings between 0.66 and 0.93. SOD = simple odor discrimination; — indicates that the plus maze task was not included in the components analysis.

ments, Meriden, CT). Each filter was suspended at the top of the tube, just underneath a rubber stopper, which sealed the tube. The reaction was terminated by the injection of 15% trichloroacetic acid through the stopper. The tubes were incubated at room temperature for another 90 min to ensure complete release and absorption of [^{14}C]CO₂ into the filter paper. The filter papers were then removed from the tubes and placed in scintillation vials for measurement of the [^{14}C]CO₂ product with a scintillation counter.

2.4. Creation of behavioral indices for correlation with neurochemistry

Indices for the water maze and simple odor discrimination tasks were based on a principal components analysis conducted with individual measures from these tasks [25]. A summary of this analysis is presented in Table 1. Because performance in most spatial task measures improved rapidly during early test sessions and reached asymptotic levels by session 4, mean values representing either acquisition (sessions 1–3) or asymptotic performance (sessions 4–5) were computed for spatial measures. Because asymptotic performance levels were not reached in the cued water maze and simple odor discrimination tasks, values for all three sessions were averaged. The platform crossings and quadrant time measures were not included in this analysis because they were not recorded in the cued task. In the principal components analysis, the initial factor pattern was rotated using a Varimax rotation algorithm and factors with eigenvalues >1 were retained in the analysis [25]. Measures from the plus maze were not included in the principal compo-

nents analysis because this analysis was intended to differentiate among different types of memory. However, four of the plus maze measures will be included here in a “plus maze” index (see below) to examine relationships between enzyme activities and anxiety.

In order to reduce spurious significant correlations resulting from comparisons of neurochemical values with many behavioral variables, the multiple measures of performance recorded for each task were reduced to six behavioral indices. Z-scores were calculated for each mouse for the measures listed in Table 1. These z-scores were then averaged based on the component analysis factor loadings to create six behavioral indices: “Spatial acquisition” (mean of swim time, path length, and corridor from sessions 1–3), “Spatial asymptotic” (mean of swim time, path length, and corridor from sessions 4–5), “Cued/SOD latency” (mean of swim time, path length, and corridor from the cued task sessions 1–3, and SOD latency), “Swim speeds” (mean of swim speeds from the spatial (sessions 1–3 and 4–5) and cued tasks), “SOD accuracy” (mean of SOD choice accuracy and # of errors), and “Plus maze” (mean of the # of closed and open arm entries, and the time in the closed and open arms). All indices combined measures of a single task except the Cued/SOD latency index, which combined cued task measures with SOD latency based on significant common loadings onto factor 3. Because lower values for swim time and path length indicate better performance, whereas lower corridor values indicate worse performance, the z-scores for the corridor measures were multiplied by –1 so that lower values would indicate better performance for the Spatial acquisition, Spatial asymptotic, and Cued/SOD latency indices. Similarly, in the SOD task, higher choice accuracies indicated better performance, whereas higher errors indicated worse performance. Therefore, the errors measure was multiplied by –1 so that higher values would indicate better performance for the SOD accuracy index. For the Plus maze index, the time-in-the-open-arms and #-of-open-arm-entry measures were multiplied by –1 so that lower values would indicate better performance. No alterations were made to swim speeds for the Swim speed index.

2.5. Data analysis

A series of four analyses was conducted to determine, 1) the effects of age and sex on ChAT and GAD activities in each of the three brain regions, 2) whether ChAT and GAD activities in each brain region were correlated with each behavioral index, 3) whether correlations between enzyme activities and behavioral indices within each sex remained correlated after partialling out common effects due to age, and 4) how much of the total variation in enzyme activities in each brain region could be accounted for by each behavioral index. First, two-way analyses of variance (ANOVAs, with Age and Sex as independent variables) were performed separately for each enzyme on values from the neocortex, hippocampus, and striatum. Fisher’s Protected Least Signif-

ificant Difference posthocs were performed on significant main effects of Age. Unpaired *t* tests were performed between males and females within each age group to examine sex differences within each age group. To more closely examine the pattern of age-related changes in ChAT and GAD activities within each sex, ANOVAs were also conducted separately for each sex for each brain region, with Age as the independent variable (these analyses will be referred to as “single-sex” ANOVAs).

To examine patterns of correlations between behavior and neurochemistry among the age groups and between the sexes (analyses 2–4 listed above), correlation and regression analyses were conducted separately for each age group and for each sex. By analyzing groups separately, rather than simultaneously including all animals, differences in patterns of correlation among groups are often revealed [4,5]. First, univariate correlations were performed between ChAT and GAD activities in each brain region and each behavioral index. The significance of these correlations was determined using Fisher’s *r*-to-*z*. Five sets of correlations were conducted: one for each age group and one for each sex. Next, to examine the unique relationships between behavior and cholinergic or GABAergic function within each sex irrespective of age, partial correlations were calculated between ChAT and GAD activities in each brain region and each behavioral index, with the common variance attributable to age removed. Two sets of partial correlations were conducted, one for females and one for males. Finally, to determine whether behavioral performance was a useful predictor of ChAT or GAD activity, stepwise multiple regression analyses were performed, including enzyme activities in each brain region and each behavioral index. Five sets of regression analyses were performed: one for each age group and one for each sex. Because statistically accounting for the multitude of pairwise correlations generated using a Bonferroni correction would result in an exceedingly stringent alpha level, this correction was not applied to the data. However, a more rigorous alpha level of 0.01 was utilized for all correlation

and regression analyses to minimize the incidence of Type I errors. ANOVA and univariate correlation analyses were conducted using the statistical packages SuperANOVA and Statview 4.51 (Abacus Concepts Inc., Berkeley, CA). Partial correlation and regression analyses were conducted using SPSS 6.1.1 (SPSS Inc., Chicago, IL).

3. Results

3.1. Subjects

All animals were in general good health; they were able to walk, swim, and complete all tasks. No evidence of tumors or other gross pathological abnormalities were present in any of the animals. Alopecia was evident in all 25 month-old females and to a lesser extent in 17 month-old females. However, skin lesions were not observed in any mouse tested. All mice showed normal righting and placing reflexes. Four mice (three 25 month-olds and one 17-month old) died prior to behavioral testing. Ninety percent (9/10) of 5-month females exhibited regular estrous cycling, compared to 20% (2/10) of 17-month females, and 0% (0/14) of 25-month females. Twenty percent (2/10) of 17-month females displayed irregular cycling. One hundred percent (14/14) of 25-month females exhibited no discernible estrous cycling, as did 60% (6/10) of 17-month females and 10% (1/10) of 5-month females. Radioimmunoassay analysis of serum from mice tested as part of a different experiment revealed the following mean levels of 17β -estradiol in the various cycling categories: regular cycling = 29.8 ± 7.8 pg/ml, irregular cycling = 21.9 ± 3.5 pg/ml, no cycling = 17.4 ± 1.8 pg/ml.

The numbers of mice included in the neocortical ChAT analyses were as follows: 5 month-old (10 male, 10 female), 17 month-old (9 male, 10 female), 25 month-old (15 male, 14 female). Samples sizes were similar for the hippocampal and striatal ChAT and GAD analyses except for the exclusion of one 5-month female due to protein values more than

Table 2
Age- and Sex-Related Impairments in Behavioral Indices

Index	5-Months	Index Z-score ^a 17-Months	25-Months
Spatial acquisition ^b	-0.67 ± 0.12	$-0.17 \pm 0.12^{*\ddagger}$	$0.60 \pm 0.18^{*\ddagger}$
Spatial asymptotic ^b	-0.19 ± 0.25	-0.04 ± 0.22	0.16 ± 0.14
Cued/SOD latency ^b	0.22 ± 0.33	0.11 ± 0.11	-0.20 ± 0.7
Swim speeds ^c	0.47 ± 0.23	$-0.10 \pm 0.20^*$	$-0.25 \pm 0.14^*$
SOD accuracy ^c	0.45 ± 0.21	0.06 ± 0.20	$-0.32 \pm 0.16^{*\ddagger}$
Plus maze ^b	-0.09 ± 0.10	$0.15 \pm 0.13^\ddagger$	-0.03 ± 0.12

^a All values are Z-scores that vary from 1 to -1.

^b Lower values indicate better performance, less anxiety

^c Higher values indicate faster speeds, higher accuracy

**p* < 0.05 vs. 5-month group.

† *p* < 0.05 vs. 17-month group.

‡ Significant difference between males and females (*p* < 0.05).

two standard deviations from other 5-month samples. One 25-month female and one 17-month male were excluded from the neocortical and hippocampal GAD analyses, respectively, because of lost tissue samples. Several mice were eliminated from the correlation analyses for a variety of reasons as follows. Five 25 month-old females and one 17 month-old female were excluded from the water maze analyses because of cataracts on one eye. Seven 5 month-old mice (4 females and 3 males) were excluded from either the water maze or odor discrimination analyses because they did not respond within the allotted time or because of missing data.

3.2. Behavior

Spatial and olfactory reference memory were impaired in 25-month mice. Age-group means for the behavioral indices are presented in Table 2 (see [25] for a complete description of age- and sex-related behavioral changes). Twenty-five month-old mice exhibited impaired spatial acquisition and olfactory reference memory relative to 5 month-old mice in the absence of deficits in spatial asymptotic performance, non-spatial (visual) reference memory, or anxiety. Sex differences in the 25 month-old group were absent in all tasks except the simple odor discrimination task, in which males exhibited lower choice accuracies but females exhibited more errors.

Spatial acquisition was deteriorated in females by 17 months, but was intact in males until 25 months. Seventeen month-old mice displayed intact spatial asymptotic performance, and non-spatial visual and olfactory reference memory (Table 2). As a group, they exhibited a spatial acquisition deficit less severe than that of 25 month-old mice, but displayed increased anxiety relative to both 5 and 25 month-old mice. However, considerable sex differences in spatial acquisition and anxiety were observed between 17-month males and females (see [25] for a detailed description). Briefly, 17-month females performed similarly to 25-month females in the spatial task (impaired relative to 5-month females), whereas 17-month males performed similarly to 5-month males (were significantly better than 25-month males). This pattern of results for individual spatial acquisition measures is illustrated in Fig. 1 for the path length measure, but was also evident in the swim time, quadrant time, and platform crossings measures (data not shown). The performance of 17-month females was significantly worse than that of 17-month males in the path length and corridor measures ($ps < 0.05$; Fig. 1).

Seventeen-month females were also more anxious than all other groups, including 17-month males. Seventeen-month males and females differed significantly in both the time and number of entries into the closed arms (data not shown).

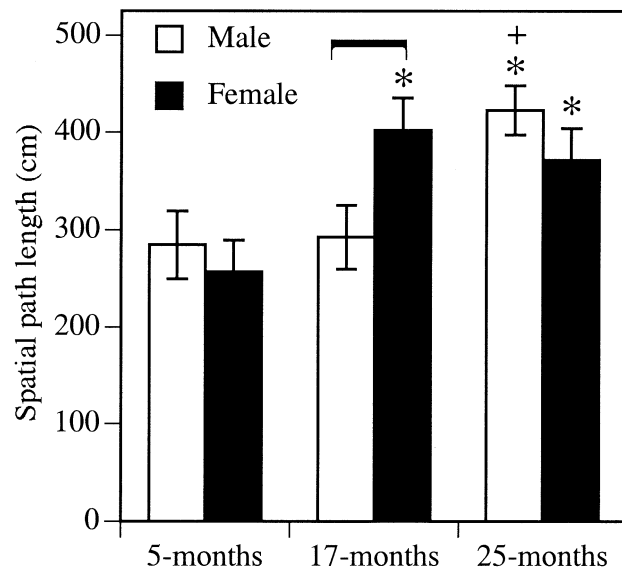


Fig. 1. Differential performance of 17-month males and female in spatial task acquisition, as illustrated by the spatial path length measure (lower numbers represent better performance). Seventeen-month females performed more like 25-month females in the spatial task, whereas 17-month males performed more like 5-month males. This pattern of results suggests an earlier onset of spatial memory decline in females than in males. Each bar represents the mean of all five test sessions. * represents a significant difference from same-sex 5-month group ($P < 0.05$). + represents a significant difference from same-sex 17-month group ($P < 0.05$). [indicates a significant sex difference between the 17-month groups.

3.3. Neurochemistry

Overall, ChAT activity increased but GAD activity decreased with age. The ChAT activities of each age group in each brain region are presented in Table 3. ChAT activity in all three regions was significantly affected by Age (neocortex: $F(2,62) = 40.39$, $P < 0.001$; hippocampus: $F(2,61) = 21.62$, $P < 0.001$; striatum: $F(2,61) = 5.6$, $P < 0.01$). Neocortical and hippocampal ChAT activities of the 25-month group were significantly higher than those of the 5-month group ($ps < 0.05$, posthocs). In contrast, the 17-month group exhibited lower hippocampal and striatal ChAT activities than the 5-month group. ChAT activity was significantly lower in all brain regions in 17 month-olds relative to 25 month-olds.

As illustrated in Table 3, GAD activities in the hippocampus and striatum were significantly decreased with age (hippocampus: $F(2,60) = 50.39$, $P < 0.001$; striatum: $F(2,61) = 12.69$, $P < 0.01$). Neocortical GAD activity was only marginally decreased with age ($F(2,61) = 3.1$, $P = 0.052$). In all three brain regions, 25-month mice had significantly lower GAD activity than did 5-month mice ($ps < 0.05$, posthocs). The hippocampal and striatal GAD activities of 25-month mice were also reduced relative to those of 17-month mice ($P < 0.05$). The 5- and 17-month groups differed only in terms of striatal GAD activity ($P < 0.05$), which was decreased in the 17-month group.

17-month males, but not 17-month females, exhibited

Table 3
ChAT and GAD activities in each age group

Enzyme	Brain Region	5-Months	17-Months	25-Months
ChAT	Neocortex	80.4 ± 3.4	73.0 ± 2.8	104.9 ± 2.6*†
	Hippocampus	92.5 ± 3.0	82.4 ± 2.8*	108.5 ± 3.3*†
	Striatum	315.9 ± 12.8	272.4 ± 10.4*	301.8 ± 5.7†
GAD	Neocortex	141.6 ± 6.4	131.8 ± 6.4	125.3 ± 3.1*
	Hippocampus	122.4 ± 3.33	114.3 ± 5.5	82.6 ± 2.4*†
	Striatum	160.2 ± 6.6	138.4 ± 6.7*	121.0 ± 4.8*†

Values represent the mean nmol product/hr/mg protein ± S.E.M.

* $p < 0.05$ vs. 5-month group.

† $p < 0.05$ vs. 17-month group.

decreased ChAT and GAD activities in all brain regions. Table 4 presents the mean ChAT and GAD activities of all three ages, separated by sex. Five-month males and females differed only in hippocampal GAD activity, whereas 25-month males and females differed only in hippocampal ChAT activity. In all brain regions, 17-month males exhibited lower ChAT and GAD activities than 17-month females and 5-month males. The ChAT activity of 17-month males was also significantly reduced relative to 25-month males in all brain regions.

Sex differences in neocortical and striatal ChAT activity were evident at only one age as indicated by significant Age x Sex interactions (neocortex: $F(2,62) = 4.82, P < 0.05$; striatum: $F(2,61) = 3.82, P < 0.05$) in the absence of significant main effects of Sex. In both brain regions, 17-month males exhibited lower ChAT activity than 17-month females (neocortex: $t(17) = 4.1, P < 0.001$; striatum: $t(17) = 2.4, P < 0.05$). Sex differences were not observed in the 5- and 25-month groups in either brain region. These relationships are illustrated more clearly in Fig. 2A using neocortical ChAT activity as an example. This figure clearly shows that ChAT activity in females is not altered until 25-months, but in males is reduced at 17-months and elevated at 25-months. Unlike the other brain regions, sex differences in hippocampal ChAT activity were apparent at more than one age, as suggested by a significant main effect of Sex ($F(1,61) = 6.38, P < 0.05$) in the absence of a

significant Age x Sex interaction. Hippocampal ChAT activity differed between males and females at 17-months ($t(17) = 2.7, P < 0.05$) and 25-months ($t(27) = 2.3, P < 0.05$), but not 5-months. At both 17- and 25-months, males exhibited lower hippocampal ChAT activity than females.

GAD activity in all three brain regions was affected by sex as suggested by significant Age x Sex interactions (neocortex: $F(2,61) = 5.93, P < 0.01$; hippocampus: $F(2,60) = 13.08, P < 0.001$; striatum: $F(2,61) = 5.3, P < 0.01$). GAD activity in 17-month males was significantly lower than that of 17-month females in all three brain regions (neocortex: $t(17) = 3.5, P < 0.01$; hippocampus: $t(16) = 3.5, P < 0.01$; striatum: $t(17) = 2.9, P < 0.01$). This relationship is illustrated for neocortical GAD activity in Fig. 2B. GAD activity in all brain regions was similar between 25-month males and females, but was significantly higher in the hippocampus of 5-month males relative to 5-month females ($t(17) = -2.9, P < 0.05$). The main effect of Sex was not significant in any brain region, confirming sex differences primarily in the 17-month group.

Enzyme activities remained stable in females until 25 months, but were altered in males by 17 months. To more closely examine patterns of age-related decline in males and females, separate one-way ANOVAs were performed for each sex for each brain region. These single-sex ANOVAs also revealed sex differences in the pattern of age-related alterations for both ChAT and GAD activities. A summary

Table 4
Sex Differences in Enzyme Activities in the Neocortex, Hippocampus, and Striatum

Enzyme	Brain region	Males only			Females only		
		5-months	17-months	25-months	5-months	17-months	25-months
ChAT	Neocortex	80.7 ± 3.3	64.3 ± 2.9*	108.5 ± 4.0*†	80.1 ± 6.1	80.8 ± 2.8	101.1 ± 3.0*†
	Hippocampus	92.7 ± 3.6	75.5 ± 3.5*	101.9 ± 5.0†	92.4 ± 5.1	88.7 ± 3.3	115.6 ± 3.4
	Striatum	330.0 ± 12.9	249.0 ± 8.4*	300.5 ± 8.4*†	300.2 ± 22.7	293.5 ± 15.8	303.1 ± 7.8
GAD	Neocortex	147.8 ± 8.8	113.2 ± 8.0*	124.2 ± 3.6*	135.4 ± 9.3	148.6 ± 6.2	126.7 ± 5.4
	Hippocampus	130.0 ± 4.1	97.5 ± 6.6*	84.5 ± 3.8*	113.9 ± 3.8	127.5 ± 5.5*	80.5 ± 2.8*†
	Striatum	170.4 ± 8.4	121.2 ± 7.0*	115.3 ± 7.3*	148.9 ± 9.5	153.8 ± 8.6	127.1 ± 6.0†

Values are mean ± S.E.M.

Sex differences within an age group are highlighted in bold.

* $p < 0.05$ vs. same-sex 5-month group.

† $p < 0.05$ vs. same-sex 17-month group.

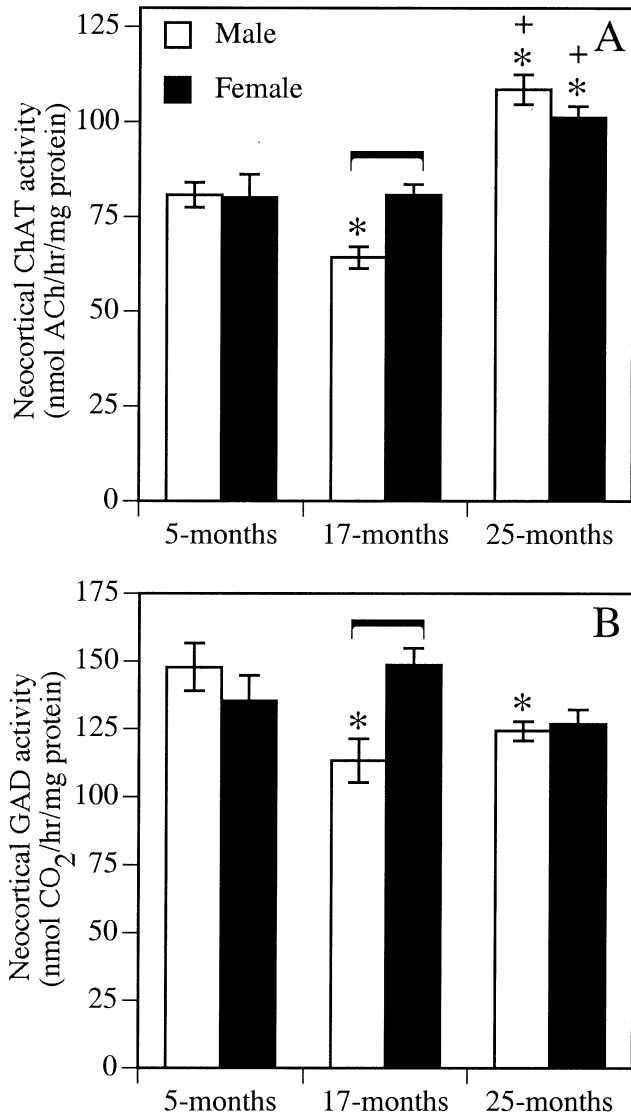


Fig. 2. Neocortical ChAT (A) and GAD (B) activity in males and females at each age. 17-month males exhibited significantly lower neocortical ChAT and GAD activities than 17-month females and 5-month males ($ps < 0.01$). * represents a significant difference from same-sex 5-month group ($P < 0.05$). + represents a significant difference from same-sex 17-month group ($P < 0.05$). [indicates a significant sex difference between the 17-month groups.

of these differences is provided in Table 5. Among females, neocortical and hippocampal ChAT activity remained stable until 25 months of age, at which point they were increased ($ps < 0.05$, posthocs). The main effect of Age was significant for both structures (neocortex: $F(2,31) = 9.68$, $P < 0.001$; hippocampus: $F(2,30) = 15.97$, $P < 0.001$). Striatal ChAT activity and neocortical GAD activity were unaffected by age. Hippocampal GAD activity was altered in females by 17 months of age, at which point it was increased, but was then decreased by 25-months below both 5- and 17-month levels ($ps < 0.05$, posthocs). Striatal GAD activity in females was unaltered until 25 months of age, at which point it was decreased relative to 17-month females

($P < 0.05$, posthocs). The main effect of Age was significant for both hippocampal and striatal GAD activity (hippocampus: $F(2,30) = 40.86$, $P < 0.001$; striatum: $F(2,30) = 3.74$, $P < 0.05$).

Among males, activities of both enzymes were altered by 17-months in all brain regions (Table 5). Seventeen-month males exhibited lower ChAT and GAD activities than 5-month males in all brain regions and lower ChAT activity than 25-month males in all three brain regions ($ps < 0.05$, posthocs). GAD activity in all three regions remained reduced in 25-month males relative to 5-month males ($ps < 0.05$, posthocs); GAD was similarly decreased in 17- and 25-month males. Among 25-month males, ChAT activity relative to 5-month males was increased in the neocortex, unaltered in the hippocampus, and decreased in the striatum. The main effect of Age was significant for both ChAT and GAD in the neocortex (ChAT: $F(2,31) = 36.99$, $P < 0.001$; GAD: $F(2,31) = 6.63$, $P < 0.01$), hippocampus (ChAT: $F(2,31) = 8.38$, $P < 0.01$; GAD: $F(2,30) = 26.91$, $P < 0.001$), and striatum (ChAT: $F(2,31) = 14.09$, $P < 0.01$; GAD: $F(2,31) = 14.67$, $P < 0.001$).

3.4. Correlations between behavioral indices and neurochemistry

Because relationships between neurochemistry and behavior have been previously reported to vary among different age groups [4], a series of correlation and regression analyses were performed separately for each age group. As indicated in the methods section, six behavioral indices were used for correlation to minimize the total number of comparisons. Lower index values indicated better performance for the “spatial acquisition,” “spatial asymptotic,” and “cued/simple odor discrimination latency” indices, but less accurate performance for the “simple odor discrimination accuracy” index. Lower values also indicated slower swim speeds and less anxious plus maze behavior.

Relationships between behavior and neurochemistry varied with age. Univariate and multivariate relationships for each age group are presented in Table 6 (relationships in which the univariate correlation coefficient (r) was not significant for any age group are excluded from the table). Patterns of significant neurochemistry-behavior correlations differed among the age groups. No relationships were observed between behavior and neurochemistry at 5 months. Only one univariate correlation was significant at 25-months (that between “plus maze” and hippocampal GAD activity), and this index did not account for a significant amount of variability in hippocampal GAD in the stepwise regression analysis. In contrast to the few significant correlations in the 5- and 25-month groups, several univariate correlations were significant in the 17-month group. Among 17 month-olds, significant correlations were limited to indices of spatial water maze performance, particularly the “spatial asymptotic” index, which accounted for a significant amount of the variance in both ChAT and GAD activ-

Table 5
Summary of Sex Differences in Enzyme Activities and Behavioral Indices

Enzyme or behavioral index	Brain region	Males only		Females only	
		17-months	25-months	17-months	25-months
ChAT	Neocortex	↓	↑↑	—	↑↑
	Hippocampus	↓	—↑	—	↑↑
	Striatum	↓	↓↑	—	—
GAD	Neocortex	↓	↓	—	—
	Hippocampus	↓	↓	↑	↓↓
	Striatum	↓	↓	—	↓
Spatial acquisition*		—	↑↑	↑	↑
Plus maze†		—	—	↑	—

Filled arrows indicate an increase or decrease relative to the same-sex 5-month group, open arrows indicate an increase or decrease relative to the same-sex 17-month group, and dashed lines indicate no difference from the same-sex 5-month group.

* Elevated values represent poor performance.

† Elevated values represent more anxious behavior.

ities. Interestingly, all significant correlations in the 17-month group were positive, indicating that low activity for both enzymes was associated with good spatial memory.

Relationships between behavior and neurochemistry also varied by sex. Because males and females (particularly 17 month-olds) differed in the magnitude of neurochemical alteration with age, separate correlation and stepwise multiple regression analyses were conducted on each sex to determine whether different relationships between neurochemistry and behavior would emerge. Significant univariate and multivariate relationships are presented in Table 7. Males and females did not exhibit any common behavior-neurochemistry relationships. Among males, significant correlations were positive and were limited to relationships between the spatial water maze and ChAT activity; low neocortical and striatal ChAT activities (Fig. 3A) were associated with good spatial memory. Among females, both ChAT and GAD activities were correlated with behavior. Low hippocampal ChAT activity in females was associated with more anxiety (Fig. 3D) whereas low hippocampal GAD activity was associated with better spatial asymptotic performance.

4. Discussion

The results of the present study demonstrate that ChAT and GAD activities in the neocortex, hippocampus, and striatum are altered with age in both male and female C57BL/6 mice. However, disparate patterns of change were revealed between the sexes; both enzymes were decreased by 17-months in males, whereas alterations in females were not apparent until 25-months. Significant enzyme-behavior relationships were limited primarily to the 17-month group. In addition, the pattern of enzyme-behavior correlations differed between the sexes; in males, only ChAT activity and spatial task indices were related, whereas in females, both enzymes were related to behavior. These findings indicate differential age-related modulation of ChAT and GAD activity in males and females, a phenomenon that may contribute to sex differences in particular cognitive processes.

4.1. Alterations in males

Relative to 5-month males, 17-month males exhibited significant reductions of ChAT and GAD activity in all

Table 6
Significant Univariate Correlations Between Neurochemistry and Behavioral Indices in Each Age Group

Behavioral index	Enzyme	Brain region	5-Months	17-Months	25-Months
Spatial asymptotic	ChAT	Striatum	-0.21	0.62*‡ (38%)	0.21
	GAD	Neocortex	0.05	0.69*‡ (48%)	-0.38
	GAD	Hippocampus	0.51	0.60*	0.22
	GAD	Striatum	0.11	0.73*‡ (53%)	-0.24
Swim speeds	ChAT	Neocortex	-0.14	0.62*‡ (22%)	-0.39
Plus maze	GAD	Hippocampus	0.13	-0.15	0.47*

Numbers in parentheses indicate the % of variance in a given enzyme activity accounted for by the associated behavioral measure listed in column one (as measured in the regression analyses).

* $p < 0.01$, univariate correlation.

‡ $p < 0.01$, accounts for significant amount of variance in stepwise regression analysis.

Table 7
Significant Univariate Correlations Between Behavioral Indices and Neurochemistry in Each Sex

Behavioral index	Enzyme	Brain region	Male	Female
Spatial acquisition	ChAT	Neocortex	0.53*	0.32
Spatial asymptotic	ChAT	Striatum	0.43* [†]	-0.11
	GAD	Hippocampus	0.04	0.39 ^{†‡} (22%)
Plus maze	ChAT	Hippocampus	0.07	-0.32 ^{†‡} (22%)

Numbers in parentheses indicate the % of variance in a given enzyme activity accounted for by the associated behavioral measure listed in column one (as measured in the stepwise regression analyses).

* $p < 0.01$, univariate correlation.

[†] $p < 0.01$, partial correlation controlling for common effects of age.

[‡] $p < 0.01$, accounts for significant amount of variance in regression analysis.

three brain regions (see Table 5). In the case of GAD, this activity remained diminished at 25-months. In contrast, ChAT activity in 25-month males was elevated above 17-month levels in all brain regions. The cause of the uniform down-regulation of ChAT and GAD activities in all brain regions at 17-months is unclear. GAD activity is decreased in both 17- and 25-month males, suggesting a permanent age-related reduction in several brain regions that may reflect a degeneration of GABAergic function in mice. This reduction does not appear to directly influence memory, as spatial and olfactory memories were disrupted in 25-month, but not 17-month, males relative to 5-month males. In

contrast to GAD, ChAT activity in 25-month males was elevated in all brain regions relative to 17-month males, indicating an up-regulation of ChAT with advanced age. Interestingly, the reduced ChAT activity in 17-month males of the current study was associated with intact spatial and olfactory memory, whereas the elevated ChAT activity in 25-month males was associated with mnemonic decline. This relationship is consistent with previous studies of aging male rats which show correlations between elevated ChAT activity and impaired spatial water maze performance [4,26]. Up-regulated ChAT activity in aged rodents may reflect a compensatory mechanism to counteract age-related degeneration of other cholinergic parameters such as muscarinic receptor binding [36,60,65] or choline uptake [58]. In middle-aged male rats, cholinergic agonist treatment decreases hippocampal ChAT activity and improves spatial memory [26], suggesting that increased ChAT activity in aging rodents may actually contribute to memory decline, either by interfering with normal synaptic function or by inducing supra-optimal release of acetylcholine.

Our neurochemical data are consistent with some previous findings in male C57BL/6 mice, but not others, due to the inconsistent nature of this literature. For example, in previous investigations of male mice ranging from 24–32 months of age, neocortical, hippocampal, and striatal levels of ChAT activity have been reported to be increased [58, 64,65], decreased [60,63], and unaltered [9,58,60] relative to young mice. The few studies addressing age-related changes in GAD activity generally have found no alterations with age [64], although increased striatal GAD activity has been reported in 24-month males [60,65]. The varied findings of this and previous investigations in the C57BL/6 strain are curious, and perhaps highlight the importance of methodological considerations in the study of neurotransmitter alterations with aging. Sex differences cannot explain the discrepancies in the previous C57BL/6 literature, as all studies used males exclusively. However, several other explanations may be considered. First, differences in dissection procedures may contribute to the apparent inconsistencies, particularly in the neocortex, where regional boundaries in mice are not pronounced compared to other mammals. Second, differences in handling and other behavioral experience prior to tissue collection, which reportedly alter cholinergic [41] and GABAergic function in rats [12], may have influenced the observation of age-related enzyme alterations. Previous studies of ChAT and GAD activity in C57BL/6 mice have utilized animals with a variety of behavioral experiences, from those who were experimentally naive [63,64,65] to those with extensive experimental experience [9,60]. Furthermore, among the studies in which mice had behavioral experience prior to tissue collection (including the present study), the behavioral tasks were vastly different (e.g. [9], [60]). Thus, the distinct characteristics of each behavioral task may have differentially altered enzyme activity levels. Finally, differences in assay procedures may have led to the discrepancies

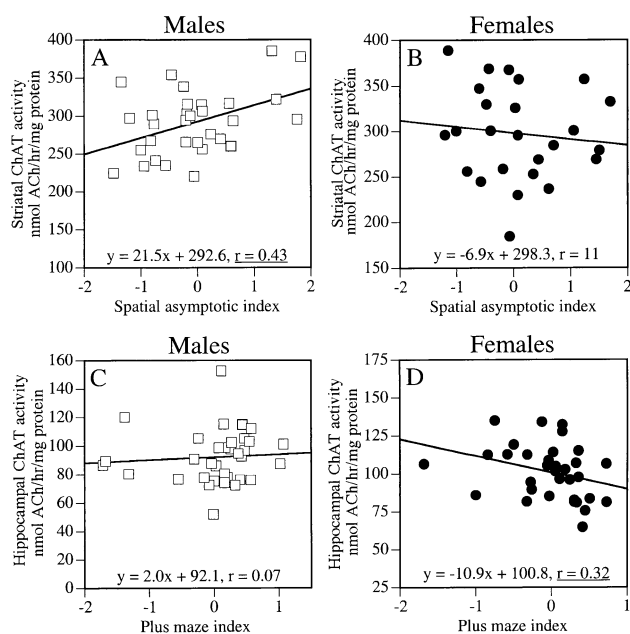


Fig. 3. Scatter plots of behavior-Chat activity correlations in males and females. Striatal ChAT activity vs. the “spatial asymptotic” index in males (A) and females (B). Hippocampal ChAT activity vs. the “plus maze” index in males (A) and females (B). Regression equations and correlation coefficients (r) are presented at the bottom of each figure; significant r values are underlined. Lower spatial asymptotic and plus maze index values indicated better water maze and less anxious performance.

among these studies. Although this possibility may explain absolute differences in values between studies, this would not likely obscure relative age-related differences. The potential importance of these seemingly minor methodological discrepancies within one mouse strain underscores the need for careful consideration when extrapolating to other mouse strains or species.

4.2. Alterations in females

This study is the first, to our knowledge, to examine age-related changes in ChAT and GAD activity in female C57BL/6 mice. Our findings suggest that ChAT and GAD activities in females are predominantly stable until 25 months of age, at which point neocortical and hippocampal ChAT activities increase, and hippocampal and striatal GAD activities decrease. The GAD activity reductions observed in 25-month females are consistent with some data from male rats [1] and aged humans [42], but are contrary to other studies in male rats and mice [35,60,61,64]. However, the fact that reductions in this study were observed in both males and females in the current study lends support to the notion that GABA synthesis is generally depressed in aged C57BL/6 mice. In contrast to GAD activity, neocortical and hippocampal ChAT activity was significantly elevated in 25-month females relative to 5- and 17-month females. This finding is consistent with the elevated ChAT observed in our 25-month males (relative to 17-month males) and with several other studies of male mice [58,64,65]. As previously mentioned, this increased ChAT activity may reflect a compensatory up-regulation of acetylcholine synthesis to maintain adequate cholinergic function at advanced ages.

Table 5 illustrates sex differences in enzyme activity and behavior. It is clear that sex differences observed in ChAT and GAD activities are most robust at 17 months of age. Interestingly, this is also the age at which sex differences in spatial acquisition and anxiety are predominant; seventeen-month females exhibit impaired spatial acquisition (see Figure 1) and heightened anxiety relative to 17-month males, as well as 5-month mice [25]. The spatial acquisition performance of 17-month females more closely resembles that of 25-month females, whereas that of 17-month males resembles that of 5-month males, which we interpret as indicating an earlier onset of spatial memory decline in females relative to males. However, the reverse appears to be true for ChAT and GAD activities: the onset of neurochemical alterations occurs at an earlier age in males than females. It is possible that the reduction of ChAT and GAD activities in the 17-month male brain is an adaptive mechanism meant to balance other age-related changes in neural function. By down-regulating ChAT and GAD activities, the middle-aged male brain may maintain optimal levels of neurotransmitter functioning, which may allow it to sustain spatial memory abilities similar to young mice. As previously mentioned, pharmacological reduction of ChAT activity in 17-

month male rats improves spatial memory in the water maze [26], suggesting that down-regulation of ChAT in middle-age may enhance memory. Perhaps, the middle-aged female brain is not capable of this type of compensation, and the failure to down-regulate ChAT and GAD activities in middle-age is detrimental to spatial memory and anxiety.

What could account for differential compensatory abilities in males and females? In our previous study, we report that 80% of the 17-month females tested in the current study were exhibiting irregular or non-existent estrous cycling [25]. It is, therefore, possible that the age-related loss of estrogen and/or progesterone cycling contributes to sex differences in both the brain and behavior. In C57BL/6 females, neocortical ChAT activity fluctuates during the estrous cycle and neocortical GAD activity is reduced by ovariectomy [24]. In rats, basal forebrain ChAT mRNA [27] and hippocampal GAD immunoreactivity [45] are modulated by estrogen and progesterone, as are hippocampal dendritic spine density [68], long-term potentiation [66], and neurogenesis [44]. Perhaps the considerable reduction of these hormones during middle-age triggers changes in the female brain that diminish its compensatory abilities. If it cannot adapt to the changing nature of middle-age, the female brain may be less able to process spatial and anxiety-provoking stimuli, leading to impaired performance in both behavioral paradigms. This notion is supported by a recent finding that ChAT mRNA in aged female rats is affected by long-term ovarian hormone loss beyond the effects of normal aging [28]. Moreover, long-term estrogen and progesterone replacement in ovariectomized aged female rats enhances significantly spatial working memory [29]. Together, these findings suggest that the loss of ovarian hormones in females exacerbates ongoing age-related neurobiological deterioration. Our data point toward the possibility of enhancing spatial memory and reducing anxiety in middle-aged females by using hormone replacement and/or drug treatments that can down-regulate ChAT and GAD activity in the neocortex, hippocampus, or striatum.

4.3. Correlations within each age group

Different patterns of enzyme-behavior correlations were apparent in each age group. Among 5 month-olds, no relationships were evident between activity of either enzyme and behavior. Among 25 month-olds, only one correlation was observed, that between hippocampal GAD activity and plus maze performance. Finally, among 17 month-olds, ChAT and GAD activities in all regions were correlated solely with spatial water maze indices.

In the 17-month group, GAD activity in all brain regions was associated with spatial asymptotic performance such that lower GAD activity was related to better performance. This finding may suggest that reduced GABA synthesis leads to decreased inhibitory activity in these three structures, which may then contribute to enhanced spatial memory. This notion is consistent with pharmacological studies

in which GABAergic antagonists augment spatial working memory in rats [33] and reference memory tested in avoidance paradigms in mice [13,37]. ChAT activity in the neocortex of 17 month-olds was associated with the swim speeds index, whereas striatal ChAT activity was associated with the spatial asymptotic index. The directions of the enzyme-behavior relationships indicate that lower ChAT activity in 17 month-olds was related to better performance. As mentioned, this relationship is consistent with previous reports in middle-aged male rats [4,26].

Our finding in 25 month-olds of few enzyme-behavior relationships in accord with a previous report in mice [9], but is inconsistent other reports in rats of significant relationships between ChAT activity and spatial memory [3,19]. These discrepancies may reflect species differences in the role these enzymes play in modulating memory with age. It is also curious that enzyme-behavior correlations that were significant at 17-months were no longer significant at 25-months. However, this result is perhaps not surprising, given that brain-behavior correlations at 17-months were certainly influenced by the sex differences that resulted from ongoing ovarian hormone reductions in females. As previously mentioned, putative sex differences in compensatory abilities may underlie the sex differences observed in acquisition of the spatial task and undoubtedly influence the behavior-neurochemistry correlations. In contrast, sex differences at 25-months in enzyme activities and behavior were minimal, and thus did not likely contribute to relationships between these variables.

It is unclear whether age-related alterations in ChAT and GAD activities occur independently of each other, or whether a change in one enzyme precipitates compensation in the other. The anatomical connectivity of cholinergic and GABAergic septal basal forebrain neurons that project to the hippocampus provides clear potential for interaction between these neurotransmitters. The activities of both septal populations are affected by intraseptal administration of cholinergic and GABAergic compounds; for example, intraseptal infusion of the GABAergic agonist muscimol diminishes basal forebrain cholinergic activity [31] and hippocampal acetylcholine release [32]. Because GABAergic interneurons are present in the hippocampus in great abundance relative to cholinergic interneurons [62], the hippocampal GAD activity measured in the present study reflects both extrinsic and intrinsic activity, whereas hippocampal ChAT activity likely represents functioning of extrinsic basal forebrain cholinergic projections. Thus, these potential interactions cannot be pinpointed in the present report, but likely exist and are worthy of further investigation.

4.4. Correlations within each sex

Disparate patterns of enzyme-behavior correlations also emerged between the sexes. Among males, the only significant correlations observed were between neocortical and

striatal ChAT activity and both spatial water maze indices. Both of these relationships were positive, suggesting that lower ChAT activity in males was related to better spatial memory. Among females, hippocampal ChAT and GAD activities were associated with the spatial asymptotic and plus maze indices; hippocampal ChAT was related to the plus maze index, whereas hippocampal GAD was associated with spatial asymptotic performance. Overall, the differential relationships between enzyme activity and behavior in males and females are intriguing. The fact that significant correlations were limited to ChAT activity in males, but were evident with both enzymes in females, may suggest sex differences in the roles of the cholinergic and GABAergic systems in modulating behavior.

5. Conclusions

These data show, for the first time, that ChAT and GAD activities are differentially affected by age in male and female mice. Furthermore, disparate relationships between enzyme activity and behavior may provide a biological basis for understanding sex differences in spatial memory and anxiety. Our data suggest that strategies for cognition enhancement will likely differ between the sexes; e.g. cognition enhancement in middle-aged female, but not male, mice may be achieved with drug treatments that down-regulate ChAT and GAD activity. Finally, these findings underscore the need for examining both sexes in studies of aging.

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