The Aging Hippocampus: Cognitive, Biochemical and Structural Findings

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Aging is often accompanied by learning and memory problems, many of which resemble deficits associated with hippocampal damage. Studies of aging in nonhuman animals have demonstrated hippocampus-related memory decline, and point to a possible locus for impairments associated with normal and pathological aging in humans. Two well-characterized hippocampus-dependent tasks in nonhuman animal literature are the Morris water task (MWT) and the transverse patterning discrimination task (TPDT). We employed the virtual MWT and the TPDT to assess hippocampus-dependent cognition in humans. Magnetic resonance imaging and proton magnetic resonance spectroscopy were employed to measure hippocampal volume and neurochemistry respectively. Age-related deficits were observed in performance on both hippocampus-dependent tasks. This pattern of impairment was accompanied by decreased hippocampal NAA/Cre ratios and volume, both of which imply neuronal loss and/or decrease in neuronal density. Collectively, our results suggest that hippocampus undergoes structural and biochemical changes with normal aging and that these changes may represent an important component of age-related deterioration in hippocampus-dependent cognition.

Introduction

Cognitive ability is a crucial determinant of the quality and enjoyment of life of elderly people. Normal aging is often associated with cognitive decline in a number of domains, however, some cognitive processes are relatively unaffected. For example, verbal skills, implicit (procedural) learning, and semantic memory appear to be largely spared, while there are notable age-related deficits in episodic (declarative) memory, attention, working memory, and spatial learning (Kausler, 1994). Learning and memory impairments are characteristic of both normal and pathological aging, and although a great deal is known about the neurobiology of learning and memory, there is no consensus on the precise nature of underlying neurobiological changes. There is agreement, however, that many deficits accompanying normal aging resemble those seen following bilateral damage to the hippocampus (Geinisman *et al.*, 1995). Here we present novel evidence regarding age-related hippocampal morphological and biochemical variations that may represent an important component of an age-related cognitive deficit specific to the hippocampus.

The Morris water task (Morris, 1981) has been extensively employed in studying the relationship between hippocampal function and spatial learning and memory in rodents, and more recently in humans, providing a solid basis for cross-species comparisons (Hamilton *et al.*, 2002). Specifically, in the virtual version of the Morris water task (VMWT) participants are trained to navigate to a platform submerged in a circular pool of opaque water based on its fixed location to distal cues. Rodents with hippocampal damage have striking impairments in learning and remembering spatial information (Morris *et al.*, 1982; Sutherland *et al.*, 1982), and humans with temporal lobe damage, among other problems, show similar deficits in spatial information processing (Corkin, 1984). Hippocampal damage in rats (Alvarado and Rudy, 1992, 1995; Dusek and Eichenbaum, 1997), and monkeys (Alvarado *et al.*, 1995) also compromises certain forms of nonspatial learning, such as configural (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995) or relational (Eichenbaum, 1997) learning. For example, hippocampectomized rats are impaired in solving concurrent visual discriminations (Alvarado and Rudy, 1992), which are constructed so that each stimulus choice is ambiguous, and the problem could not be solved unless configural associations are formed. In contrast, elemental discriminations are typically spared, and configural or relational learning is not involved as stimuli are unambiguous, and, thus, do not require hippocampal circuitry. Astur and Sutherland (1998) found that normal, college-aged humans solve the TPDT using a configural strategy and rapidly learn elemental discriminations. Amnesic patients are impaired at configural learning in the TPDT, however, elemental learning is spared indicating a similar neurobehavioral pattern to that observed in rodents (Rickard and Grafman, 1998; Reed and Squire, 1999).

Age-related structural alterations in the hippocampus have been identified with diverse methods. Decreased neuronal count (Issa *et al.*, 1990), a decrease in the number of synaptic connections (Geinisman *et al.*, 1995), intracellular pathology (Raz, 1999), and affinity of neurofibrillary tangles for this region (Raz, 1999), all suggest that the hippocampal formation may be especially vulnerable to the effects of aging. The advent of neuroimaging technology, such as magnetic resonance imaging (MRI), has made *in vivo* assessment of neuroanatomy and brain function feasible. While age-related hippocampal volume reductions have been observed (Jernigan *et al.*, 1991; Golomb *et al.*, 1993), a host of studies reporting exceptions are not to be ignored (Sullivan *et al.*, 1995; Raz, 1996). Nevertheless, it is doubtful that hippocampus would be completely immune to the unfavorable effects of normal aging (Raz, 1999). Specifically, some MR volumetric studies of the hippocampus suggest a possible 'antero-posterior gradient of age-related vulnerability' in normal (Raz, 1999) and Alzheimer's populations (Petersen *et al.*, 1998). Also relevant are studies that have reported preferential involvement of posterior (equivalent to dorsal in rats,

rostral in birds) hippocampus in spatial navigation in rats (Moser *et al.*, 1993; Moser and Moser, 1998), birds (Couvillon and Bitterman, 1996), monkeys (Colombo *et al.*, 1998) and humans (Maguire *et al.*, 2000).

In addition to structural visualization, MR allows an evaluation of the brain metabolite levels *in vivo* measured by non-invasive proton magnetic resonance spectroscopy (^{1}H-MRS). Studies in different patient cohorts (Brooks *et al.*, 1997, 1999) and normal populations (Jung *et al.*, 1999) have reported a strong relationship between neurochemical markers of brain integrity and neuropsychological function. Commonly detected metabolites include creatine (Cre), choline (Cho) and *N*-acetylaspartate (NAA). The Cre resonance provides a measure of cellular energy currency. The Cho-containing compounds are all integral components of membrane phospholipids and increased levels are taken as a sign of membrane breakdown, inflammation or demyelination, as seen in stroke (Olson *et al.*, 1992) and multiple sclerosis (Davie *et al.*, 1994). NAA is a putative neuronal marker as it is almost exclusively found in neurons (Moffett *et al.*, 1991). Decreased levels of NAA in the hippocampal region have been reported in post-traumatic stress disorder (Schuff *et al.*, 1997a), Alzheimer's disease (AD; Schuff *et al.*, 1997b) and normal aging (Schuff *et al.*, 1999), suggesting neuronal loss and/or decreased neuronal density in these conditions.

Despite reports of age-related neurochemical and structural hippocampal variations, little is known regarding the relationship between these variations and age-related decline in hippocampus-dependent learning and memory. The broad aim of the current study is to investigate hippocampus-dependent learning and memory in normal young and elderly humans, and to examine the relationship between hippocampus-dependent cognitive performance, and hippocampal anatomy and biochemistry. In order to reduce the potential effects of abnormal aging on the outcomes of this study we excluded elderly individuals who were positive for the ε4 variant of the Apolipoprotein E (APOE) allele, which is a risk factor for AD. While not a direct cause of AD, the APOE ε4 allele is perhaps indirectly implicated within the biological chain of events leading to AD and, thus, is considered a susceptibility gene for cognitive deficits and altered brain function in non-demented elderly. For example, Bookheimer *et al.* (2000) demonstrated that elderly with a genetic risk for AD show alterations in brain function even in the absence of gross morphological changes or cognitive deficits. Cognitive performance of young and elderly participants was evaluated on the TPDT and the VMWT in order to measure hippocampus-dependent learning. MRI was used to measure hippocampal structure and ^{1}H-MRS was used to quantify Cre, Cho and NAA in hippocampus.

Materials and Methods

Participants

A total of 16 young (age 20–39 years, mean = 26.1 years) and 16 elderly (age 60–85 years, mean = 77.6 years) participated in the study. Each group consisted of eight males and eight females. Young participants were University of New Mexico undergraduates who received research credits for a psychology class in return for their voluntary participation. Healthy elderly participants were recruited from the New Mexico Aging Process Study (NMAPS; Garry *et al.*, 1992). Each participant had normal or corrected to normal vision, and reported no history of neurological problems. In addition, elderly participants were screened by NMAPS using a battery of cognitive and physical measures, health habits and attitudes, morbidity, number of falls, diet, physical activity, nutritional status, and body composition. We obtained Mini Mental State Exam (MMSE) scores for our elderly subjects from the NMAPS. MMSE scores were quantified on the 100 point scale, with the lowest score of 95 (mean ± SD = 98.2 ± 1.56). When MMSE scores were converted from a 100 point scale to a more customary 30 point scale, the lowest score was 28.5 (mean ± SD = 29.5 ± 0.47). In addition, excluded were those with overt clinical conditions, such as coronary heart disease, significant peripheral vascular disease, insulin-dependent diabetes, hepatic disease, history of internal cancer requiring surgery, X-ray, or chemotherapy in the past 10 years, hepatitis, untreated hypertension, those taking medication (except for thyroid and estrogen replacement, or minor antihypertensives) and those with any history of psychiatric diagnosis (including depression and AD). Of further relevance to screening of the elderly participants is the polymorphism of the APOE gene. Presence of the APOE ε4 allele is a susceptibility gene known to influence age of onset and the underlying pathology of AD (Corder *et al.*, 1993). Due to recent discoveries linking memory decline in nondemented elderly to the ε4 allele of the APOE, all elderly participants selected for this study were typed as homozygous ε3 allele carriers. The Hixson and Vernier (1990) procedure was used for restricting enzyme isoform genotyping for the APOE alleles. Each participant gave informed consent in accordance with the guidelines for human research at the University of New Mexico.

Virtual Morris Water Task (VMWT)

A circular pool located in the center of a square room comprised the virtual environment (Fig. 1*A*; for a detailed description, pictures of the environment, and testing procedure see Hamilton and Sutherland, 1999; Hamilton *et al.*, 2002). The distal cues were the only visual features of the environment disambiguating the spatial location of the platform. The presentation of the environment and data collection were controlled by an IBM-compatible laptop computer and a 14″ color monitor. Participants navigated using keyboard arrow keys. The participant's *x*, *y* coordinates were recorded every 100 ms for each trial allowing for latency and path length to locate the platform to be determined.

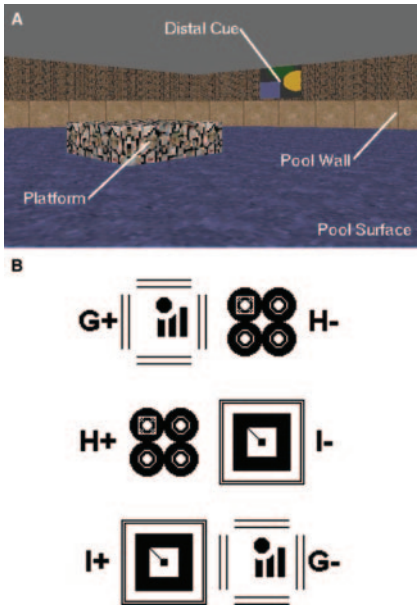

Figure 1. (*A*) A representative view of the virtual environment from the center of the pool. The pool surface, pool wall, room walls, and a single distal cue are visible. (*B*) The description of stimuli pairings for transverse patterning discrimination task. The problem was constructed so that each choice stimulus (G, H, I) was ambiguous; sometimes it was correct (+), and sometimes incorrect as follows: G+ versus H–, H+ versus I–, I+ versus G–.

The mode of presentation was largely allocentric in nature. Each participant received seven blocks (four trials each) of hidden platform training, followed by the 30 s no-platform probe trial and two blocks (four trials each) of visible platform training. In each trial, a limit of 60 s was allotted to locate the platform, after which the platform became visible, a discordant tone was sounded, and a visual message stating that the platform was now visible appeared. Regardless of whether the platform was located when it was visible or hidden, the participant remained on the platform for 10 s, after which the screen faded and a new trial began. A tone and a visual message stating that the platform was visible alerted participants at the beginning of each visible platform trial.

The participant's age, experience playing video games, any strategies the participant may have employed, a subjective rating of task difficulty, and whether the participant believed the platform and starting location to be fixed or variable were assessed by a questionnaire.

Transverse Patterning Discrimination Task (TPDT)

The TPDT was conducted in six phases using a stepwise approach (for a detailed description see Astur and Sutherland, 1998). Non-nameable stimuli were presented on a computer display in white with a blue background. Each trial began with a fixation cross, presented in the center of the display for 1 s followed by a stimulus pair presentation. Phases 1–3 consisted of elemental discriminations only. Stimulus pair A+B– was presented during phase 1. During phase 2 stimulus pair C+D– was presented in addition to the A+B– pair. In phase 3 the final elemental discrimination pair, E+F–, was presented in addition to the previous two pairs. Phases 4–6 were transverse patterning discriminations (Fig. 1*B*). First transverse patterning pair, G+H–, was presented during phase 4. During phase 5, stimulus pairs G+H– and H+I– were presented. During phase 6 the final stimulus pair, I+G–, was included in addition to the previous two pairs. Training continued for each phase until the participant made 11 correct responses out of 12 consecutive trials. A maximum of 400 trials was allotted to complete the task, after which the testing was discontinued.

Participants responded by pressing one of two designated keyboard keys that corresponded to the left or the right stimulus element of a pair. The key press cleared the stimulus pair from the display. Response evaluations were recorded to a file following a key press. Correct responses were followed by a presentation of word 'Correct' in the center of the display accompanied by a high-pitched tone for 1 s. Incorrect responses were followed by a word 'Incorrect' presented in the center of the display accompanied by a low-pitched tone for 1 s. One point was earned each time a correct response was given and a point was deducted for an incorrect response. Along with information whether a point was earned or deducted on the present trial, a running total as well as the correct/incorrect feedback message was displayed. After a 2 s delay the display was cleared and a 2 s intertrial interval followed.

Magnetic Resonance Imaging (MRI)

All studies were completed on a 1.5 T clinical scanner (Signa 5.4, GE Medical Systems, Waukesha, WI). The imaging protocol included a T_1-weighted axial series (fast-SPGR, T_E/T_R = 6.9/17.7 ms, flip angle = 25°, 1.5 mm thickness, no gap, 256 × 192 matrix) of the whole brain and a T_1-weighted coronal series (fast-SPGR, T_E/T_R = 6.9/17.7 ms, flip angle = 25°, 3.0 mm thickness, no gap, 256 × 192 matrix) oblique to the longitudinal axis of the hippocampus.

Intracranial volume was measured using the BET program (FMRIB Image Analysis Group, Oxford University, www.fmrib.ox.ac.uk/fsl). Automated *k*-means segmentation of the cerebrum was used to determine gray matter volume (GM), white matter volume (WM), cerebrospinal fluid volume (CSF) and those partial volume (PV) pixels that were composed of a mixture of GM and CSF (Petropoulos *et al.*, 1999). Because partial volume refers to the pixels that could not be exclusively assigned to GM or CSF, half of the PV pixel volume was assigned to GM and the other half to CSF. The total intracranial volume (ICV) was calculated by summing WM, GM, PV and CSF values.

Following segmentation of the coronal images, hippocampal volumetric analysis was performed. A graphical user interface (GUI) developed in our laboratory was used to visualize the images. While the measurements were automated, the GUI allowed the operator to inspect the quality of the segmentation procedure on each slice as measurements were performed, and to zoom in on any part of the picture for closer viewing. Measurements were taken from both the left and the right side of the brain, and total bilateral hippocampal volumes were recorded. The measurement was repeated for just the right hippocampus. In order to obtain left hippocampal volume, right hippocampal volume was subtracted from the bilateral hippocampal volume. The hippocampal measurements included the hippocampus proper, the subiculum and the dentate gyrus, while hippocampal white matter was excluded from the measurement due to the segmentation-based nature of this program.

The measurement started with the most posterior slice, where the crux of the fornix separated from the hippocampus. The most anterior slice was outlined using a set of criteria defined by Watson *et al.* (1992, and included hippocampus but not amygdala. The total hippocampal volume was calculated by summing the sectional areas available for measurement, an average of 12 (3 mm) slices. The obtained measurements for each slice were further grouped into three regions following the method of Maguire *et al.* (2000): posterior hippocampus (tail; three slices), body (six slices), and anterior hippocampus (head; three slices). There were no participants with <12 slices available for measurement. For those participants with >12 slices available for measurement, additional slices were added to either the anterior or posterior region according to anatomical boundaries (Duvernoy, 1988). For analysis purposes, the measurements were grouped into bilateral, right, left, anterior and posterior hippocampal volumes.

Magnetic Resonance Spectroscopy/Spectroscopic Imaging (MRS/MRSI)

Magnetic resonance spectroscopy data were collected in MRSI mode from a 15 mm thick axial slab that included both hippocampi using a PRESS sequence (T_E/T_R = 62/1500 ms, FOV = 20 cm, 24 × 24 phase encoding). Two additional single voxels were acquired in the left and right frontal white matter superior to the lateral ventricles (PRESS, T_E/T_R = 40/2000, 13 cm^3). All MRS/MRSI data were analyzed using LCModel (Provencher, 1993). The MRSI data were zero-filled to a 32 × 32 spatial matrix. An average concentration of NAA, Cho and Cre was taken from each voxel and values were expressed as ratios (NAA/Cre and Cho/Cre). Data from four participants (two young and two old) were excluded following the MRSI analysis because we were unable to obtain a reasonable fit due to poor signal to noise ratio.

Results

Cognitive Findings

All reported effects are significant at $P < 0.05$ unless otherwise stated and all tests were two-tailed. We assessed cognitive performance on the VMWT and the TDPT, which are spatial and non-spatial hippocampus-dependent tasks respectively. We observed a large deficit in performance of the elderly relative to young participants on both cognitive tasks. For the VMWT, behavioral measures on the hidden platform trials were grouped into seven blocks of four trials each for analysis purposes. Mean path length to locate the platform for each block per participant was calculated and subjected to an Age by Trial Block analysis of variance (ANOVA) using a multivariate approach to repeated measures (MANOVA). We found a significant Age by Trial Block interaction in which the elderly exhibited a place learning deficit compared with young adults [$F(6,180) = 2.52$, $P = 0.023$; Fig. 2]. We found no significant differences in the speed of swimming or in performance on visible platform trials (*P*-values > 0.1). In order to assess performance on a no-platform probe trial in the VMWT, we calculated mean latency and path length spent in the quadrant of the pool where the platform was originally located for each participant and subjected these measures

to separate one-way ANOVAs. We observed significant deficits in the performance of elderly compared with young adults on all VMWT probe trial dependent measures (P-values < 0.05; Fig. 3).

In addition, a multivariate analysis of covariance (MANCOVA) was performed with Age and Sex as independent variables, and the mean latency to locate the platform on each of the seven training blocks as a dependent variable controlling for the game playing experience and frequency. In addition to a significant Age main effect [$F(10,17) = 3.669$, $P = 0.009$], there was a significant effect of Sex [$F(10,17) = 5.203$, $P = 0.001$]. Overall, males had shorter latencies in locating the platform regardless of age. ANOVA with mean latency on the last training block as a dependent variable and sex as an independent variable controlling for age, hippocampal volume, and hippocampal NAA/Cre revealed a significant effect of Sex [$F(1,23) = 12.289$, $P = 0.002$]. There was no significant Sex effect in performance on the probe trial in either latency ($P > 0.687$) or distance ($P = 0.695$). The Age by Sex interaction was not statistically significant suggesting that the male superiority in navigation was similar for both age groups.

A chi-square analysis of the questionnaire data revealed that more of our younger participants adopted a place as opposed to a random strategy [$\chi^2(1) = 9.0$, $n = 32$, $P = 0.003$], and noticed that the platform location was in a fixed location relative to the cues [$\chi^2(1) = 5.4$, $n = 32$, $P = 0.02$]. Moreover, our younger participants had more experience playing video games [$\chi^2(1) = 9.0$, $n = 32$, $P = 0.003$]. Game playing experience significantly correlated with the VMWT performance ($r = -0.61$, $P < 0.001$), however, this relationship was not significant after we controlled for age ($P > 0.1$).

On the TPDT we trained participants on both configural (hippocampus-dependent) and elemental (hippocampus-independent) discriminations. For the TPDT, we measured the proportion of elderly compared with young adults that successfully completed the task, the number of trials required, and the number of errors made. Although 94% (15/16) of elderly and 100% (16/16) of young participants successfully completed elemental discriminations [$\chi^2(1) = 0.032$, $n = 32$, $P = 0.86$], only 19% (3/16) of the elderly compared with 100% (16/16) of the young solved the transverse patterning discriminations [$\chi^2(1) = 8.89$, $n = 32$, $P = 0.003$]. The elderly required significantly more trials to reach the criterion [$F(1,30) = 78.54$, $P < 0.001$] and committed significantly more errors [$F(1,30) = 49.45$, $P < 0.001$; Fig. 4].

The performances on the spatial and non-spatial hippocampus-dependent tasks correlated significantly with each other ($r = 0.79$, $P < 0.01$). The correlation between age and performance on each task was also significant (VMWT: $r = 0.77$, $P < 0.01$; TPDT: $r = 0.84$, $P < 0.01$). However, the partial correlation between VMWT and TPDT performance was still significant after we controlled for age ($r = 0.43$, $P = 0.017$).

Volumetric Findings

We hypothesized that elderly individuals would have smaller hippocampal volumes compared with young adults as assessed by MRI, and that smaller volumes would be correlated with poor performance on hippocampus-dependent tasks. We found significant age-related decrease in bilateral, right, left, anterior, and posterior hippocampal volumes (Fig. 5; ANOVA, P-values < 0.05), and no significant difference in intracranial volume (ICV; ANOVA, $P > 0.1$). Significant differences between the groups

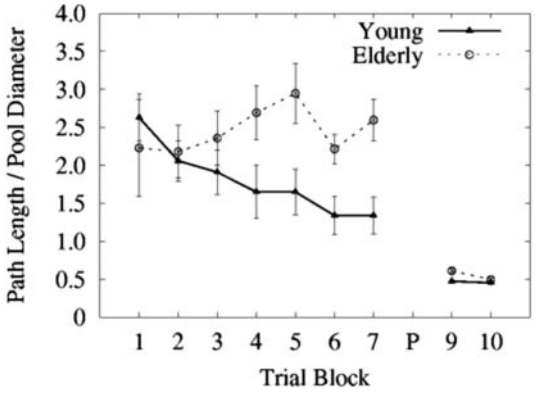

Figure 2. Performance of young and elderly on the hidden and visible trials of the virtual Morris water task. There were no significant differences in performance on trial blocks 1–3 between the young and the elderly ($P > 0.05$). On blocks 4–7 the elderly had significantly longer path lengths (P-values < 0.045) in locating the hidden platform. There were no significant group differences in path length to reach the visible platform (blocks 9–10).

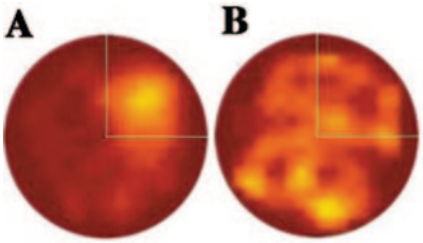

Figure 3. Performance on the probe trial of the virtual Morris water task. The search of the young group during the probe trial was focused on the correct quadrant as indicated by the concentration of yellow color inside the quadrant enclosed with white lines. Elderly searched all quadrants of the pool equally. The white lines delineate the correct quadrant of the pool in which the platform was located during training.

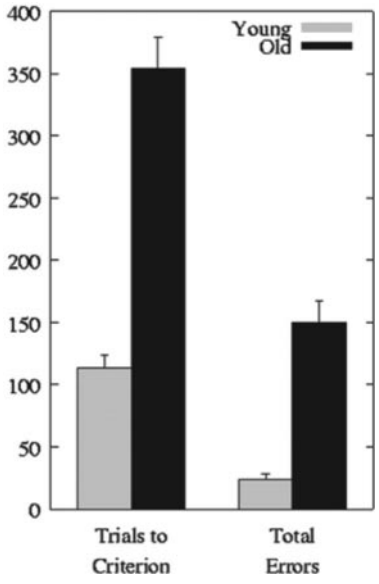

Figure 4. Performance on the transverse patterning discrimination task. Elderly required a significantly larger number of trials to reach the criterion and committed a significantly larger number of errors.

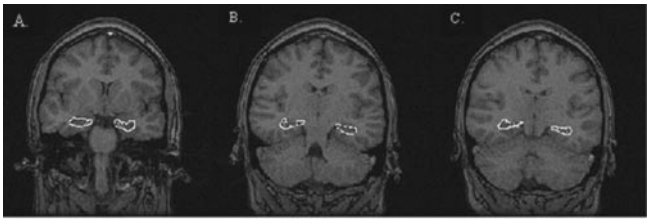

Figure 5. Hippocampal area measured throughout the coronal plane is outlined with white border for each representative slice. (*A*) The anterior hippocampus (head). (*B*) The body of the hippocampus. (*C*) The posterior hippocampus (tail).

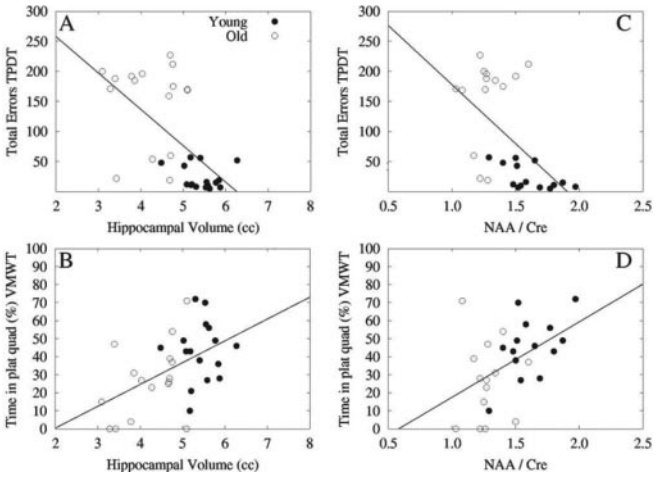

Figure 6. Correlations between performance of young (black circles) and elderly (open circles) on TPDT and VMWT with hippocampal volume (*A*, *B*) and biochemistry (*C*, *D*) respectively. Larger hippocampal volume was correlated with fewer errors on the TPDT ($P = 0.01$; *A*), and higher percentage of time spent searching the correct quadrant of the pool on the probe trial of the VMWT ($P = 0.01$; *B*). Higher hippocampal NAA/Cre values were correlated with fewer errors on the TPDT ($P = 0.01$; *C*) and with higher percentage of time spent searching in the correct quadrant of the pool during probe trial on the VMWT ($P = 0.05$; *D*).

persisted on all measures, except for anterior hippocampus ($P > 0.05$), after we normalized hippocampal volumes to ICV. We also found that anterior hippocampal volumes were significantly larger than posterior hippocampal volumes [paired *t*-test; $t(31) = 10.49$, $P < 0.001$].

Consistent with our hypothesis, larger total hippocampal volumes were associated with better performance on each task (Fig. 6*A*,*B*). Further, both anterior (VMWT: $r = -0.48$, $P < 0.01$; TPDT: $r = -0.48$, $P < 0.01$) and posterior (VMWT: $r = -0.33$, $P < 0.05$; TPDT: $r = -0.39$, $P < 0.05$) hippocampal normalized volumes significantly correlated with performance on both cognitive tasks.

Biochemical Findings

We hypothesized that elderly participants would have lower NAA/Cre than younger adults, and that lower hippocampal NAA/Cre would correlate with poor performance on hippocampus-dependent tasks. Participants underwent ^{1}H-MRS/MRSI in order to obtain metabolite levels from hippocampus and frontal white matter (which served as a control region). Consistent with our hypotheses, we found significantly lower NAA/Cre values in both hippocampal (Fig. 7*B*) and frontal white

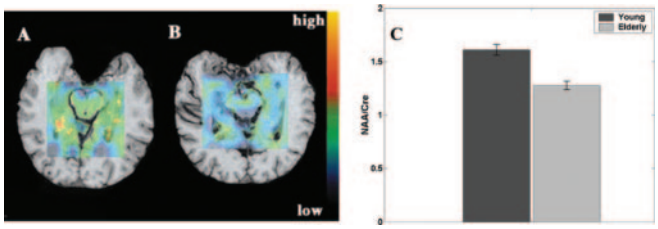

Figure 7. Anatomical image of a representative (*A*) young and (*B*) elderly participant with NAA map overlay. Higher levels of NAA are found in the hippocampal region of our (*A*) young participant (as indicated by bright green color), compared with the hippocampal region of (*B*) elderly participant (as indicated by dark green and mainly blue color). (*C*) Hippocampal NAA/Cre in young and elderly participants. Elderly have significantly lower levels of NAA/Cre compared with young participants.

matter regions in the elderly (ANOVA; *P*-values < 0.05). There were no age-related differences in Cho/Cre values (ANOVA; *P*-values > 0.1). Both right [$t(27) = 5.51$, $P < 0.001$] and left [$t(26) = 5.86$, $P < 0.001$] NAA/Cre were higher in the frontal white matter than in the hippocampus. Frontal and hippocampal Cho/Cre were not significantly different (*P*-values > 0.1). A paired *t*-test revealed no significant hemispheric differences between frontal or hippocampal NAA/Cre ratios (*P*-values > 0.1). We also found a significant correlation between higher right ($r = 0.52$, $P < 0.01$) and left ($r = 0.61$, $P < 0.01$) hippocampal NAA/Cre and larger respective volumes.

We computed zero-order correlations in order to assess the relationship between task performance and neurochemistry. Better performance on both hippocampus-dependent tasks significantly correlated with higher hippocampal NAA/Cre (Fig. 6*C*,*D*). We also found a significant correlation between frontal white matter NAA/Cre and some performance measures on each of the tasks. Specifically, right frontal NAA/Cre significantly correlated with latency to reach the platform on the VMWT ($r = -0.36$, $P = 0.05$), total trials needed to reach the criterion ($r = -0.41$, $P = 0.02$), and total errors committed ($r = -0.37$, $P = 0.04$) on the TPDT. Similarly, left frontal NAA/Cre correlated with total trials to reach criterion ($r = -0.407$, $P = 0.02$), and total number of errors ($r = -0.36$, $P = 0.05$) on the TPDT. However, partial correlations between performance on the two tasks and hippocampal NAA/Cre controlling for frontal neurochemistry were significant (*P*-values < 0.05), whereas partial correlations between frontal neurochemistry and cognitive performance were not significant after we controlled for hippocampal neurochemistry (*P*-values > 0.09).

Two separate multiple regression/correlation (MRC) analyses were performed using a stepwise approach with total hippocampal volume, hippocampal NAA/Cre, and age as independent variables in order to further assess hippocampal contribution to performance on hippocampus-dependent tasks. Performance on the VMWT served as a dependent variable in the first MRC analysis, and performance on the TPDT served as a dependent variable in the second MRC analysis. For VMWT, performance significantly correlated only with hippocampal NAA/Cre and age (*P*-values < 0.007), but not hippocampal volume ($P = 0.09$). Subsequently, only hippocampal NAA/Cre and age were included in predicting performance. This can be explained by the fact that both volume and NAA measurements represent an amount of available viable tissue, and the two measurements may have been somewhat redundant, even though the correla-

tion for total hippocampal volume and performance on the VMWT was approaching significance ($P = 0.091$). Hippocampal NAA/Cre accounted for a significant amount of variance in performance on the VMWT [$R^2 = 0.225$, $F_{Change}(1,25) = 7.538$, $P = 0.011$]. Age did not account for a significant amount of variance over and above hippocampal NAA/Cre ($P = 0.363$). MRC analysis performed with TPDT performance as a dependent measure revealed that total hippocampal volume accounted for a significant amount of variance in predicting performance on the TPDT [$R^2 = 0.259$, $F_{Change}(1,26) = 9.076$, $P = 0.006$]. NAA/Cre accounted for a significant amount of variance in predicting performance on the TPDT over and above total hippocampal volume [$R^2 = 0.505$, $F_{Change}(1,25) = 12.42$, $P < 0.002$]. Age accounted for a significant amount of variance over and above total hippocampal volume and NAA/Cre in predicting performance on the TPDT [$R^2 = 0.738$, $F_{Change}(1,24) = 21.39$, $P < 0.001$).

Discussion

The overall aim of this study was to examine the relationship between hippocampal biochemistry and morphology and cognitive performance in normal aging. In order to assess age-related cognitive variations, participants were tested on spatial (VMWT) and non-spatial (TPDT) hippocampus-dependent tasks. We observed large cognitive deficits in performance of elderly relative to young participants on both hippocampus-dependent tasks (learning the hidden platform location on the VMWT, and transverse patterning discriminations), consistent with well established age-related decline in cognitive performance reported in previous studies of aging. However, there were no differences in performance on hippocampus non-dependent conditions (visible platform trials of the VMWT and elemental discriminations). The performances on the VMWT and TPDT were significantly correlated, hence the cognitive deficits observed in the elderly were not specific to a particular test format. We suggest that the observed deficit in performance on the VMWT was not solely caused by the inherent constraints of virtual environments, such as the lack of locomotor-based proprioceptive and vestibular cues, and a narrower filed of view, since all of our participants were deprived of such information. The observed deficits in spatial navigation also were not sensory-motor or motivational in nature given that there were no differences in the speed of swimming or performance on visible platform trials.

Our findings also suggest male superiority in spatial navigation regardless of age. Furthermore, the sex difference persisted even after we controlled for age, total hippocampal volume and hippocampal NAA/Cre, suggesting that brain regions other than hippocampus may be contributing to sex difference in performance. It is not possible, however, to delineate from the data gathered here why men outperform women regardless of age. Further research is needed in order to address the nature of sex differences in virtual navigation.

The fact that the NAA/Cre values decreased with age, while Cho/Cre remained stable in both the frontal white matter and the hippocampus, suggests that the observed reductions are primarily driven by the NAA decrease and not the variations in Cho and/or Cre containing compounds. Also, significantly lower hippocampal, compared with frontal, NAA/Cre indicates that the observed reduction in hippocampal NAA was not solely caused by a global age-related deterioration.

Furthermore, we found a significant correlation between frontal white matter NAA/Cre and specific performance measures on each of the tasks, suggesting limited involvement of frontal regions in successfully completing the two hippocampus-dependent tasks. Considering their role in planning and decision making, the contributions of frontal regions to the network supporting successful performance on the two cognitive tasks should not be surprising (Maguire *et al.*, 1998). A persistence of the correlation between performance on both hippocampus-dependent tasks and hippocampal NAA/Cre, even after controlling for frontal neurochemistry, however, is more compelling.

Our structural findings indicating reduction in hippocampal volume with age are consistent with many earlier reports (for a review see Raz, 1999). Sample heterogeneity has been identified as one possible cause of variable results in the volumetric literature and recently the polymorphism of the APOE gene has been given increasing attention. Our sample of elderly was carefully selected to reduce variability. For example, only homozygous APOE ε3 elderly carriers were studied, and many health parameters were ruled out as a source of group differences. One caveat always present with MRI research is that any image-based volumetric measurement is methodology dependent (Jack, 1994), as there are several different volumetric procedures available varying in technique, sensitivity, and operator subjectivity, all contributing to the complexity of interpretation of the results across different studies. Our technique, while limited to measuring only hippocampal gray matter, is an automated measure requiring very little operator input. Notwithstanding the limitations, smaller hippocampal volumes correlated significantly with lower NAA/Cre. While we cannot conclusively determine the origin of hippocampal age-related volume reduction without postmortem tissue analyses, our NAA findings suggest it may be largely neuronal in nature.

Our findings of larger anterior compared with posterior hippocampi are consistent with previously reported larger anterior regions in the normal subjects (Raz, 1999; Maguire *et al.*, 2000), and also suggest that anterior hippocampus is rather resilient in the face of normal aging (Raz, 1999). A large decrease in anterior hippocampal volume has however been reported in AD patients (Raz, 1999), suggesting that anterior hippocampus may be susceptible to the degenerative change associated with AD and may aid in discriminating between normal aging and individuals destined to develop dementia. A larger number of subjects, and more conservative technical and anatomic boundary criteria should be employed in future studies investigating a discriminatory power of anterior hippocampus as a diagnostic tool in AD. Furthermore, differential age-related anterior hippocampal atrophy may be associated with the nature of cortical inputs to anterior hippocampus and more posterior regions (Van Hoesen *et al.*, 1972; Rosene and Van Hoesen, 1977; Witter *et al.*, 1986). Moreover, a regional expansion in the posterior hippocampi has been reported in groups of people occupationally dependent on their navigational skills, such as the London taxi drivers (Maguire *et al.*, 2000), and may not be detectable in a sample of the general population.

Alterations in long-term potentiation (LTP) have been reported in aged rats (Barnes *et al.*, 2000; Almaguer *et al.*, 2002). Specifically, aged rodent hippocampus fails to exhibit an experience-dependent increase in the amount of spatial information it transmits (Shen *et al.*, 1997), suggesting that a functional plasticity deficit within hippocampus could be a major factor in

age-related memory impairment. It is possible that the observed structural and biochemical hippocampal changes are involved in altering hippocampal plasticity that in turn may be reflected in age-related cognitive deficits. However, we can only speculate on the relationship between structural and biochemical changes, and alterations in plasticity. The data presented here do not provide any direct evidence to bear on the issue of plasticity or dendritic branching.

Our combined behavioral and imaging study of a carefully selected aging sample sheds some light on hippocampal aging, as well as the neurobiological substrates of human hippocampus-dependent learning and memory. We have provided support for variations linked specifically to the hippocampus, even though regional brain differences caused by aging are thought to be rather subtle compared with global brain deterioration. Collectively, our results suggest that normal aging is associated with hippocampal structural and biochemical changes, and that these changes may constitute an important component of age-related deficits in hippocampus-dependent learning and memory.

Notes

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