Volumetric Correlates of Spatiotemporal Working and Recognition Memory Impairment in Aged Rhesus Monkeys

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Introduction

Memory processing dependent on the prefrontal cortex (PFC) and mediod temporal lobe is particularly susceptible to the effects of aging, although the onset and the degree of impairment vary widely across individuals (Rapp 1988; Bachevalier et al. 1991; Herndon et al. 1997). Neuron loss, as measured by changes in cell density, was once considered a primary cause of memory decline and an inevitable consequence of aging (Brody 1955). However, an age-related decrease in neuron density can occur without actual cell loss due to reduced perfusion-related shrinkage in aged brains compared with young ones (Haug et al. 1981). The use of stereological methods circumvents this confound by yielding estimates of the total number of neurons that are unbiased by differences in regional brain volumes (West 1993). Using stereology, it has been shown that neuron number is largely preserved during normal aging in both the PFC and mediod temporal lobe structures in macaque monkeys (Gazzaley et al. 1997; Merrill et al. 2000; Keuker et al. 2003, 2004; Smith et al. 2004). Nonetheless, substantial alterations of neuronal structural components do occur, including decreased dendritic branching, synapse number, and spine number (Jacobs et al. 1997; Duan et al. 2003; Kabaso et al. 2009; Dumitriu et al. 2010). Such changes likely disrupt neuronal plasticity and information processing required for learning and memory.

In conjunction with age-related changes in gray matter (GM) components, white matter (WM) degeneration has been widely reported in humans and monkeys. Age-related structural abnormalities are present in oligodendrocytes, such as bulbous swellings (Peters 1996), and in myelin, such as splitting and bulging of the sheaths (Feldman and Peters 1998). Also, myelinated fiber density and length decline from adulthood to old age (Tang et al. 1997; Marner et al. 2003; Bowley et al. 2010). These WM alterations may slow conduction velocity and “disconnect” GM regions critical for normal memory function (Warrington and Weiskrantz 1982; O’Sullivan et al. 2001). Based on these morphological changes, it has been hypothesized that normal cognitive aging arises from changes in both GM and WM components.

Previously, we demonstrated that decreases in overall cerebral volume predict age-related recognition memory impairment in the rhesus monkey (Shamy et al. 2006). The current study extended this analysis to a much larger number of subjects and tested whether regionally selective alterations of memory-related GM and WM components are coupled with multiple measures of cognitive aging. Using an established nonhuman primate model, young and aged rhesus monkeys were behaviorally characterized on tests of spatiotemporal working memory (delayed response [DR] and recognition memory (delayed nonmatching to sample [DNMS])). Using structural in vivo magnetic resonance imaging (MRI) scans, region of interest (ROI) analyses were conducted for the PFC, calcarine cortex, prefrontal WM, hippocampus, and striatum. Both traditional univariate and principal component (PC) analyses were performed to determine whether volumetric changes across this network of brain regions or alterations within individual brain regions better predict memory performance.

Materials and Methods

Subjects

Six young adult (9–12 years) and 14 aged (24–29 years) rhesus monkeys (Macaca mulatta) served as subjects. Groups were comprised of both females (3 young and 5 aged) and males (3 young and 9 aged). Six young and 6 aged monkeys were included in a previous volumetric study of the hippocampus (Shamy et al. 2006). All young females...
exhibited regular menstrual activity. One aged female was amenorrheic during behavioral testing and 3 had ceased cycling by the time of MRI acquisition.

Standard rations of laboratory primate chow were provided twice daily without the use of dietary restriction regimens. The colony was maintained on a 12/12-h light/dark cycle, and water was available ad libitum in the home cage. Environmental enrichment for all subjects was provided regularly consisting of behavioral testing, toys, fruits, and vegetables. Animals received regular veterinary clinical and physical exams and were judged to be generally healthy. Notably, aged rhesus monkeys do not develop the neuropathological hallmarks of Alzheimer's disease (AD) and instead provide a window on normal healthy aging, uncontaminated by neurodegenerative disease. Experimental procedures were conducted in accordance with National Institutes of Health guidelines following protocols approved by the UC Davis Institutional Animal Care and Use Committee and by the California National Primate Research Center affiliated with the University of California, Davis.

**Behavioral Characterization**

Monkeys were tested on a DR test of spatiotemporal memory as described in detail elsewhere (Rapp et al. 2003). This test is particularly sensitive to aging (Bachevalier et al. 1991) and to dorsolateral prefrontal cortex (dIPFC) lesions in young monkeys (Mishkin and Pribram 1956). Briefly, while the monkey watched through a clear Plexiglas screen of a manual Wisconsin General Testing Apparatus (Harlow and Bromer 1939), the experimenter baited one of 2 wells and then covered both with identical opaque plaques. The screen was then raised and the monkey was allowed to displace one of the 2 plaques for a reward. The rewarded location was randomized, and the left and right wells were baited equally often across trials within a test session. Subjects were tested for 30 trials a day, 5 days a week, until performance reached at least a 90% correct criterion, defined as 9 errors or less in 90 consecutive trials without a delay (0 s). Then, a 1-s delay was implemented by lowering an opaque screen after the wells were covered to hide the stimuli from view. Testing continued until monkeys again reached the 90% criterion. Once the performance criterion was achieved at both the 0- and 1-s delays, successively longer delays of 5, 10, 15, 30, and 60 s (90 trials each) were imposed. The primary memory demand of the DR task was to remember over a delay period which location was baited most recently, thus emphasizing spatiotemporal working memory.

Monkeys were also tested on a DNMS recognition memory task (Rapp and Amaral 1989, 1991; Rapp et al. 2003). This test is sensitive to aging (Prenty et al. 1987; Moss et al. 1988; Rapp and Amaral 1991; Moss et al. 1997), and performance is impaired in young subjects with lesions of various prefrontal and medial temporal regions (Mishkin 1978; Squire and Zola-Morgan 1991; Zola et al. 2000). Briefly, DNMS trials were initiated by presenting a sample object over the baited central well of the test tray in a manual test apparatus. After a predetermined interval, during which the stimuli were hidden from view, the sample object was presented together with a novel stimulus that covered to hide the stimuli from view. Testing continued until monkeys again reached the 90% criterion. Once the performance criterion was achieved at both the 0- and 1-s delays, successively longer delays of 5, 10, 15, 30, and 60 s (90 trials each) were imposed. The primary memory demand of the DR task was to remember over a delay period which location was baited most recently, thus emphasizing spatiotemporal working memory.

MR Image Acquisition

Monkeys were anesthetized with ketamine (20 mg/kg, intra-muscular [i.m.]) and atropine (0.04 mg/kg, i.m.). The subject's head was then placed in an MRI-compatible stereotaxic frame and positioned within the standard quadrature radiofrequency coil used for human imaging. Eighty contiguous 1-mm coronal T1-weighted images of the whole brain were acquired for each monkey on a 1.5 T GE Signa Horizon LX NV/i MRI system (GE Medical Systems) with version 84M4 software, at the UC Davis Imaging Research Center, Sacramento, CA. A radio frequency-optimized gradient-recalled echo sequence (3D SPGR) was used with the following parameters: time repetition 21 ms, time echo 7.9 ms (full echo); flip angle 30°, field of view 16 × 16 cm, acquisition matrix 256 × 256, NEX 4 (no phase wrap option), and bandwidth 15.63 kHz. The gradient subsystem of the MRI system was measured prior to and throughout the study period. Local GE service personnel calibrated the subsystem monthly. Geometric accuracy was documented as stable to within ±0.15% (i.e., ±0.15 mm per 10 cm). Adjustments were not necessary to maintain that level of accuracy and precision throughout the study period.

**Image Processing**

Postacquisition processing was performed on an SGI Onyx 3400 system using the Analyze 5.0 software package (BIR) by experimenters blind with respect to the age and cognitive status of the subjects. Images were reconstructed into multiple 3D files for each subject. One file, used for hippocampal tracings, was reformatted to produce 0.3125-mm isotropic voxels and was aligned perpendicularly to the long axis of the hippocampus. Another file, used for segmentation of the cerebrum, WM, and ventricles, was reformatted to yield 0.3125 × 0.3125 × 1.0 mm voxels and was aligned along the horizontal line connecting the anterior and posterior commissures (AC–PC line). This file was manually edited in the coronal view to exclude brainstem, cerebellum, and nonbrain structures such as muscle, connective tissue, and skull. Finally, this file was reformatted to produce 0.3125-mm isotropic voxels and was used for the segmentation of prefrontal WM and semi-automated tracings of the PFC, calcarine cortex, and striatum.

**ROI Analysis**

Anatomical designations for the ROIs were guided by observations of postmortem histological material and prominent landmarks identifiable in MRI scans. Both semi-automated and manual tracing methods were used to create the ROIs. Unless otherwise noted, ROI tracings were completed in an anterior to posterior direction in coronal images and lateral to medial in sagittal sections by raters blind to condition. In order to maximize the accuracy of boundary placement (Geuze et al. 2005), the images were compared in multiple orientations throughout manual tracing. The Analyze “ROI module” was used to calculate the volume of the delineated brain regions. In addition to the individual regional measures described in the following sections, the ratio of prefrontal GM to WM was calculated to control for individual variability of PFC volumes.

**Intra- and Interrater reliability measures were previously published for the hippocampal (0.91–0.97; Shamy et al. (2006) and striatal (0.89–0.96; Matochik et al. 2000, 2004) tracing methods used in this study. In the development of the following methods, reliability was determined from repeated tracing of the ROI’s on 8 hemispheres and was calculated using Pearson r correlations. As expected based on variability in the clarity of identifiable landmarks in the macaque brain, interrater reliability was greatest for the dIPFC and anterior cingulate cortex (ACC) regions (0.93 and 0.95, respectively) and somewhat lower for the inferior frontal gyrus (IGF) and ventromedial prefrontal cortex (vmPFC) (0.89 and 0.85, respectively). Interrater reliability was similar for the dIPFC and ACC (0.94 for both) and for the IGF and vmPFC (0.91 and 0.88, respectively).

**Intracranial Volume**

The intracranial volume (ICV) was measured using semi-automated tracing methods, as shown in Figure 1. Tracing was initiated at a mid-sagittal section, and ICV borders were drawn dorsally along the inner table of the skull to the posterior limit of the cerebellum and ventrally along the base of the brain. Tracing continued laterally for each hemisphere on 2-mm-thick sections. The region was completed using the "propagate regions" tool in Analyze software program. Additional manual editing was completed as needed to ensure the accuracy of ICV boundary placement.

**Cerebrum, Ventricles, and WM**

Cerebral and ventricular volumes were acquired, as described by Shamy et al. (2006) (Fig. 2A,B). A Gaussian cluster multispectral analysis was
applied to the edited images to obtain automated boundaries of the cerebral and non-cerebral regions. This method segmented the ventricular volume as a nonbrain region, which was then manually reassigned as ventricular space. Segmentation of GM and WM was completed by first smoothing the images using a kernel size of 3x3x3 to decrease noise. A thresholding tool was then applied to segment the WM from GM and nonbrain regions.

Prefrontal Regions

Although the functional organization of the PFC has been widely debated (Fuster 1985, 2000, 2001; Barbas 2000; Miller and Cohen 2001), current perspectives agree that there are functional and anatomical differences between lateral, medial, and orbital PFC (Fuster 1985, 2000, 2001; Barbas 2000; Miller and Cohen 2001). Taking this into consideration, we quantified total prefrontal GM and WM volume and also divided the PFC into subregions. Regional boundary definitions were guided by an approximation of architectonic boundaries (Carmichael and Price 1994, 1995), known anatomical connectivity of prefrontal regions of the rhesus monkey (Goldman-Rakic et al. 1984; Petrides and Pandya 1999; Barbas 2000; Cavada et al. 2000), and what could be reliably segmented at the resolution of the available MRI images.

The PFC was traced using semi-automated thresholding methods in the coronal plane from the anterior limit of the frontal pole (FP) to the arcuate sulcus. Premotor regions were removed in the sagittal plane, with additional editing performed in the coronal plane. Regions posterior to the termination of the principal sulcus and dorsal to the anterior limb of the arcuate sulcus were also removed. As shown in Figure 2C–L, the prefrontal ROI was segmented into 8 regions: the FP (area 10), the superior frontal gyrus (SFG; areas 9 and 8b), the ACC (areas 24 and 32), the dIPFC (area 46), the IFG (areas 12, 45, and 47), the vmPFC (areas 11, 13, and 14), the periaqueductal gyrus (8A; area 8A), and the prefrontal WM. The sum of the 7 GM regions was defined as the PFC GM volume. The ACC was manually outlined for both hemispheres on a parasagittal section to guide tracing of the genu of the ACC in the coronal plane. The boundaries were drawn dorsally along the cingulate sulcus, ventrally along the rostral sulcus, and the anterior limit was defined by connecting the 2 sulci. The ACC was then semi-automatically traced from the appearance of the sagittal tracing in the coronal plane along the dorsal portion of the ACC to the disappearance of the principal sulcus and along the ventral portion of the ACC to the end of the sagittal tracing. The dIPFC was traced in the coronal plane, inclusive of the dorsal and ventral bank GM along the entire extent of the principal sulcus. The ACC, the dIPFC, and the prefrontal WM were then imported into the PFC ROI file. This file was rendered in 3D and reoriented to the orbital view. The boundary of the IFG was traced approximately 2 mm medial to the lateral edge of the orbital surface from the anterior limit of the ACC to the posterior limit of the prefrontal ROI, thereby reassigning all GM voxels ventral to the dIPFC and lateral to the orbital surface boundary as the IFG. The ROI file was viewed again in the coronal plane and edited to further refine the dIPFC and IFG boundary. Area 8A was defined as the GM within the arcuate sulcus, exclusive of the dIPFC. The FP was assigned from the anterior tip of the brain until the appearance of the ACC, exclusive of the dIPFC. The SFG was defined as the cortex dorsal to the dIPFC from the FP to the appearance of the circular sulcus. Finally, WM regions were reassigned as prefrontal WM from the anterior limit of the brain to the posterior limit of the principal sulcus and the WM within the periaqueductal sulcus.

Calcarine Cortex

The volume of the primary visual cortex was measured using regional definitions similar to those in postmortem analyses described by Hof et al. (2000) (Fig. 3A–D). Human in vivo imaging studies and postmortem nonhuman primate studies have found preservation of occipital cortex with age (Hof et al. 2000; Raz et al. 2004). Therefore, we considered the region as a negative control for assessing the regional selectivity of any observed changes. The calcarine sulcus was...
Figure 2. Cerebral GM, WM, and ventricle (red) ROI segmentation (A, B). Coronal images of prefrontal ROIs (C–F). 3D surface renderings of the cortex (G–I) and white mater (J–L) ROIs. The PFC was segmented into the FP (purple), the SFG (red), the ACC (magenta), the dIPFC (green), the IFG (yellow), the vmPFC (blue), the perirhinal gyrus (BA; cyan), and the prefrontal WM (dark gray).
traced on every third coronal section and on the most anterior and posterior sections in which it appeared. The propagate regions tool in Analyze 5.0 was used to connect the traced sections into a 3D object. Additional manual editing was completed as needed.

**Striatum**

The volume of the caudate and putamen were measured using a technique similar to that described in Matochik et al. (2000, 2004) (see Fig. 3E,F). Age-related striatal atrophy has been reported (Matochik et al. 2000, 2004; Wisco et al. 2008), and therefore, we considered this region as a positive control for age-related volumetric atrophy. Briefly, the caudate and the putamen were traced on every fifth coronal section and on the most anterior and posterior sections in which these structures appeared. GM ventral to the anterior commissure was excluded, as was the tail of the caudate nucleus. The propagate regions tool in the Analyze 5.0 program was then used to connect the regions traced in the coronal plane. Additional manual editing was completed as needed.

**Hippocampus**

Detailed descriptions of the hippocampal tracing rules are reported in Shamy et al. (2006) (Fig. 3G,H). The boundary line of the hippocampus included the dentate gyrus, CA1–3 fields of the hippocampus, subiculum, presubiculum, and parasubiculum. The rostral and caudal limits of the hippocampus were traced in sagittal images, and the coronal plane was used to identify borders through the body of the structure. This method proved optimal for distinguishing between the rostral hippocampus and amygdala and between the caudal hippocampus and retrosplenial cortex.

**Data Analysis**

Univariate statistical analyses of regional volumes were performed using SigmaStat for Windows, version 3.1 (SPSS). To evaluate potential age effects on acquisition on each of the behavioral tests, Student’s t-tests were used. Mann-Whitney rank sum tests were employed when the distribution of the data failed tests of normality. To evaluate potential age effects on performance across increasing delays in the DR and DNMS tasks, repeated-measures analyses of variance (ANOVAS) were used. When statistically significant interactions were found, Holm-Sidak pairwise comparisons were computed. Student’s t-tests were used to assess the influence of age on cerebral and ventricular volumes. For regional analyses, 2-way ANOVAs were used to evaluate the potential influence of age on regional volume as a function of hemisphere and gender. Unless noted otherwise, the results did not differ as a function of these factors.

Pearson r correlation coefficients were used to explore potential relationships between the volumetric effects of aging and memory performance. Multiple sets of analyses were conducted to describe fully the nature of observed associations. First, 2 sets of correlations were computed for the raw values, one for the entire subject group and...
another for the aged subjects alone. This permitted assessment of relationships between brain structure and function that might become more tightly coupled later in the lifespan. Next, when the raw volumes were statistically correlated with behavior or age, normalized volume estimates were evaluated to account for individual differences in overall brain size. Specifically, the ROIs were adjusted using the analysis of covariance equation: volume adjusted = volume raw - b (the subject’s ICV - mean ICV of all subjects), where b is the slope of the regression line formed when the volume of the ROI is plotted against the ICV (Matochik et al. 2004; Raz et al. 2004). Pearson r correlations were computed between the resulting residual values and chronological age or the behavioral measures. Reported correlations are explicitly identified as “raw” and “normalized.” Standardization results in slight adjustments to the raw values that take into account overall differences in brain size while preserving individual variability in regional volume.

Lastly, when raw ROI volumes were significantly correlated with a behavioral measure, the raw volumes were controlled for age by using the analysis of covariance equation: volume adjusted = volume raw - b (age), where b is the slope of the regression line formed when the volume of the ROI is plotted against age. Pearson r correlations were computed between the resulting residual values and the behavioral data. Statistical significance was set at p < 0.05.

The current study, comprising detailed neuropsychological assessment and brain imaging analysis in 20 young and aged monkeys, is to our knowledge among the largest reported (see also Makris et al. 2010). Nonetheless, studies of aged nonhuman primates face the joint challenge of limited animal availability, high cost, and the need to minimize subject numbers. As a consequence, sample size is typically small in comparison with parallel research in people, and recognizing this limitation, marginal but nonsignificant correlations (i.e., 0.05 < r < 0.10) are denoted in Table 2 for informational purposes only.

Results of the present study were not corrected for multiple comparisons due to practical limitations on sample size in nonhuman primate research. It should be noted in this context, however, that the number of significant correlations observed between behavioral performance and regional volumetric results among the aged animals greatly exceeded that predicted by chance (see Results). Univariate analyses are best applied to obvious focal changes. However, there is a risk of “correcting away” such effects, resulting in type II errors. In contrast to univariate analyses, a multivariate analysis may better identify subtle and distributed age-related changes in the volumetric data. Multivariate and univariate analyses should be seen as complimentary since each is geared toward different aspects of the volumetric data.

A multivariate analysis was performed to evaluate whether changes across a network of brain regions better predicts age-related memory impairment. First, an age-related covariance pattern was obtained from the volumetric data that distinguished young from aged monkeys. To do this, a version of the Subprofile Scaling Model (SSM) (Moeller et al. 1987; Moeller and Strother 1991) was performed on the volumetric data. This technique has been used numerous times in clinical research to analyze both ROI and voxel-based morphometric data (Moeller et al. 1999; Scarmeas et al. 2004; Alexander et al. 2008). Briefly, log-transformed ROI values of all monkeys were pooled together and a principal component analysis (PCA) was performed. A PCA is commonly used to evaluate changes across a network of brain regions because it reduces a large data set and captures the major sources of variance by expressing it in terms of a few group-invariant PCs. In this study, we confined ourselves to the first 5 PCs since they accounted for 80% of the variance in the data. Thus, rather than using the bilateral ROI volumes for each monkey (24 values) only one number for each PC was needed per subject (5 values).

Next we performed 5 linear discriminant regressions in which group membership (young = 1 and aged = 0) was entered as the dependent variable, that is, a 20-component column vector with age status as a row entry for each monkey. The independent variables for these 5 regressions were the subject expression values of all possible contiguous subsets of the first 5 PCs for all monkeys and had a similar 20-component format per each independent variable. For the first regression, only PC1 was used, for the second regression PC1 and PC2 were used, and so on (regression 3: PCs 1–3; regression 4: PC1–4; regression 5: PC1–5). SSM removes whole-brain differences from the data prior to the multivariate decomposition. Since the volumetric data possesses absolute quantification, the whole-brain average for each subject was added as a separate independent variable in the regressions. From these 5 regressions the one that yielded the largest area under the receiver operating characteristic (ROC) curve was chosen. The ROC curve plots the fraction of false positives against the fraction of true positives when the threshold for criterion is defined across the entire data range. In this study, the false positives were defined as elderly monkeys whose network expression values fell above threshold, and, according to chronological age, were thus incorrectly classified as young. Likewise, the true positives were the correctly classified portion of young monkeys whose pattern expression fell above the threshold. The larger the area under the ROC curve, the better the discrimination between groups. When the ROC curve is unity, it indicates that the model has perfect discriminability. From the selected discriminant regression, a covariance pattern was then constructed using the obtained regression coefficients and applying them to the appropriate PCs. Neither was there an inferential judgment employed in the selection process of the PC set nor an iterative stepping procedure. By design, this one-shot “scanning” procedure selects a set of PCs without “data dredging” and minimizing P-level inflation.

Subsequently, subject expression of this covariance pattern was tested to determine whether it could account for certain aspects of performance on the DR and DNMS tasks. To provide summary measures for this correlation, the percent of correctly identified items at each retention interval was regressed against the 5 delay periods (5, 10, 15, 30, 60 s for DR; 15, 30, 60, 120, 600 s for DNMS). The regression weight, or “accuracy slope,” of this relationship quantifies how much additional information was gained or lost by imposing a delay. In the current experiment, this number is “negative,” indicating information loss, and has the unit of 1 percent/s. No memory impairment across increasing delays was indicated by a slope of 0, and greater memory impairment across increasing delays was indicated by more negative, or smaller, values. A brain-behavioral correlation was anticipated such that a more youthful profile should imply better memory across increasing delays. In order for this anticipated correlation to be positive, we defined both volumetric as well as behavioral measures such that increasing values signaled benign outcomes. Specifically, a “larger” pattern expression signaled a younger chronological age, and a larger accuracy slope signaled better performance across increasing delay lengths in the behavioral tasks.

Next, a bootstrap procedure was completed to estimate the reliability of each ROI’s loading in the covariance pattern (Efron and Tibshirani 1994). This resampling test was used primarily to visualize and approximate the true population variability underlying the data. The pattern derivation was performed 10 000 times from resampled data, including PCA and discriminant regression using the set of PCs selected earlier. This resampling scheme left the group assignment intact but sampled from the data with replacement. If 95% of the bootstrap samples yielded values either larger or smaller than zero, then the ROI loadings were deemed reliably positive or negative, respectively. Positive loadings indicated that ROI volumes were smaller in the aged monkeys, whereas negative loadings indicated that ROI volumes were larger in the aged monkeys. The critical variable in the bootstrap procedure was the overall expression of the pattern and not the individual loadings in the pattern. Therefore, even when individual loadings are not deemed reliable, the age-related pattern can still be successfully extracted from the covariance relationships—it just cannot be localized to a low number of key ROIs. The expression of the age-related pattern, computed as a dot product between the covariance pattern and the subjects’ volumetric data, is more stable than the individual ROI loadings (Habeck et al. 2005).

Results

Behavioral Characterization

Means and standard errors for measures of the DR and DNMS tasks are reported in Table 1. Compared with DR performance observed in a much larger group of young monkeys, the subset available for imaging in this study performed somewhat less
accurately on average but within the normal range for both task acquisition and performance across increasing retention intervals (Rapp et al. 2003, 2004). Aged and young monkeys learned the DR task with 0- and 1-s delays at statistically equivalent rates, despite a substantial numerical difference at the 1-s interval (0 s, Mann–Whitney $U = 70.5, P = 0.380$; 1 s, $U = 47.0, P = 0.272$; Fig. 4A). Accuracy on the DR task declined in both groups with increasing delay length (main effect of delay: $F_{1,68} = 43.95, P < 0.001$). In this sample of subjects, performance was only numerically different as a function of age (main effect of age: $F_{1,68} = 3.12, P = 0.095$), nor was the interaction between age and delay length statistically reliable ($F_{1,68} = 0.57, P = 0.683$; Fig. 4B). Nonetheless, the overall pattern of results is qualitatively similar to many earlier studies demonstrating that DR performance is reliably impaired in aged monkeys (Rapp and Amaral 1991; Rapp et al. 2003). The lack of statistically significant group effects in the present case is primarily attributable to high variability among the young adults rather than preserved performance in the aged group (Fig. 4B).

Behavioral characterization in this sample of subjects yielded DNMS results similar to previous reports (Rapp and Amaral 1991; Rapp et al. 2004). Aged monkeys required many more trials than young monkeys to learn the DNMS procedure initially with a short 10-s delay (Mann–Whitney; $U = 24.0, P = 0.002$; Fig. 4C). When successively longer retention intervals were introduced, aged monkeys scored significantly worse than did young subjects (main group effect: $F_{1,68} = 18.95, P < 0.001$; Fig. 4D). The significant interaction between age and accuracy across delays ($F_{1,68} = 3.38, P = 0.014$) confirmed the result of studies in larger numbers of subjects demonstrating that aged monkeys scored comparably to young subjects at the 15-s retention interval ($t = 1.48, P = 0.143$) but displayed impaired recognition at longer delays (30 s, $t = 2.35, P = 0.022$; 60 s, $t = 2.02, P = 0.047$; 120 s, $t = 2.32, P = 0.024$; and 600 s, $t = 5.53, P < 0.001$). These behavioral data provided a basis for testing whether recognition memory impairment that occurs during normal aging is coupled with volumetric changes in the primate brain.

**Age-Related Volumetric Differences**

Means and standard errors for all ROIs are reported in Table 1, and correlations between the ROIs and behavioral measures are reported in Table 2. Volumes for individual subjects are plotted and correlations between the ROIs and behavioral measures are reported in Table 2. Volumes for individual subjects are plotted against age in Figure 5.

Volumetric assessment revealed that age-related atrophy in the rhesus monkey is not diffusely distributed but instead localized to selective brain regions. Neither cerebral nor ventricular volume was significantly different in the group of aged monkeys compared with young adults ($t = 0.24, P = 0.81$; Mann–Whitney: $U = 74.0, P = 0.386$). The lack of significant findings in the current study compared with our earlier report (Shamy et al. 2006) may be partially due to the addition of several subjects in their early 20s. At this age, degenerative changes are likely to be less widespread compared with monkeys in their late 20s and early 30s. Notably, in subjects over 20 years old, reduction of cerebral volume ($r = -0.54, P = 0.045$; standardized: $r = -0.67, P = 0.008$) and ventricular enlargement ($r = 0.61, P = 0.02$; standardized: $r = 0.60, P = 0.022$)

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**Table 1**

<table>
<thead>
<tr>
<th>ROI Volumes</th>
<th>Young</th>
<th>Aged</th>
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<tbody>
<tr>
<td>ICV</td>
<td>95953.3</td>
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<td>Cerebrum</td>
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<td>Ventricles</td>
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<td>Hippocampus</td>
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<td>10.0</td>
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Note: B, periarcuate gyrus.

*Age at MRI acquisition in years.

Number of trials to 90% criterion.

Mean percent correct across increasing delays.

Volumetric data in cubic millimeter.

Ratio in arbitrary units.

---

**Figure 4**

Mean trials to criterion (A, C) and recognition accuracy across longer delays (B, D) on the DR (top panels) and DNMS tasks (lower panels). Behavioral results for the subset of monkeys that participated in the present analysis were similar to findings for the much larger groups of young and monkeys tested over the course of this project. Aged monkeys learned the DR task as rapidly as young subjects without a delay (A). Despite a substantial numerical difference, the age-related impairment in DR accuracy with delays of 1 s and longer was not statistically reliable (B), primarily due to variability across the small number of young adults. The aged group exhibited robust deficits learning DNMS initially with a 10-s delay (C) and subsequently scored as well as adult controls at a 15-s retention interval (D). Reliable age-related deficits were when recognition memory was challenged with longer retention intervals (D).
Volume approached significance (correlation between chronological age and prefrontal WM年轻 groups. Among the aged individuals, the inverse correlation between total PFC volume and age was not statistically significant. Although there was no laterality effect for total PFC volume, the dLPFC and vmPFC were reliably correlated with age. As discussed in the study by Shamy et al. (2006) that included a subset of the monkeys examined here, the ventricular hypertrophy effect was attributable to outlier data from 2 aged monkeys with grossly enlarged ventricles.

Total PFC volume ($F_{1,18} = 0.03, P = 0.866$), PFC GM volume ($F_{1,18} = 0.62, P = 0.441$), and prefrontal WM volume ($F_{1,18} = 0.81, P = 0.38$) were not significantly different across the aged and young groups. Among the aged individuals, the inverse correlation between chronological age and prefrontal WM volume approached significance ($r = -0.53, P = 0.053$; standardized: $r = -0.52, P = 0.056$). Whereas the volume of prefrontal GM was not related to chronological age, the ratio of PFC GM to WM significantly correlated with age ($r = 0.55, P = 0.042$). Regional analysis of the PFC revealed selective reduction of the dLPFC ($F_{1,18} = 4.48, P = 0.048$) and preservation of the other PFC subdivisions in the aged group (FP: $F_{1,18} = 0.15, P = 0.706$; SFG: $F_{1,18} = 1.03, P = 0.324$; IFG: $F_{1,18} = 0.02, P = 0.902$; vmPFC: $F_{1,18} = 0.05, P = 0.832$; ACC: $F_{1,18} = 2.76, P = 0.114$; 8A: $F_{1,18} = 0.29, P = 0.611$). Volumes of the ACC and dLPFC significantly correlated with age across all subjects (raw: $r = -0.45, P = 0.045$; standardized: $r = -0.47, P = 0.037$; and raw: $r = -0.57, P = 0.008$; standardized: $r = -0.64, P = 0.003$, respectively), and for dLPFC volume, among the aged animals alone (raw: $r = -0.55, P = 0.043$; standardized: $r = -0.56, P = 0.036$). Correlations between the other PFC subregion volumes and age were not statistically significant. Although there was no laterality effect for total PFC volume, the dLPFC and vmPFC were larger in the right than in the left hemisphere across all subjects ($F_{1,18} = 16.65, P < 0.001$; $F_{1,18} = 10.51, P = 0.005$, respectively).

The aged monkeys exhibited significantly reduced striatal volume compared with their younger counterparts ($F_{1,18} = 5.27, P = 0.034$), consistent with previous findings from Matocchik et al. (2000). When the caudate and putamen were considered separately, group differences only approached

### Table 2

<table>
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<th></th>
<th>Age</th>
<th>DR Acq 1-s</th>
<th>DR Ave</th>
<th>DNMS Acq 10-s</th>
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<td>0.42*</td>
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<td>0.48*</td>
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<tr>
<td>Hippocampus</td>
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<td>0.19</td>
<td>0.52*</td>
<td>-0.57*</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Note: 8A, periarcuate gyrus.
*Number of trials to 90% criterion with 1-s delay.
*Mean percent correct across 5- to 90-s delay.
*Mean percent correct across 10- to 600-s delay.
*P ≤ 0.05; *0.05 ≤ P ≤ 0.10.

Figure 5. When chronological age was plotted against ROI volumes (A–H), age was significantly predictive of dLPFC (G), striatal, putamen, and caudate (M) volumes across the entire subject group. In the aged group alone, age was correlated with cerebral (A), prefrontal WM (C), the ratio of prefrontal GM to WM (D), the dLPFC (G), and ACC (H) volumes. Regression lines indicate significant correlations between regional volume and age. St, striatum; Put, putamen; Cd, caudate; Hipp, hippocampus.
statistical significance ($F_{1,18} = 2.88, P = 0.107; F_{1,18} = 4.21, P = 0.055$, respectively). Chronological age was inversely correlated with the volume of the striatum (raw: $r = -0.55, P = 0.012$; standardized: $r = -0.71, P < 0.001$), caudate (raw: $r = -0.49, P = 0.028$; standardized: $r = -0.52, P = 0.019$), and putamen (raw: $r = -0.47, P = 0.058$; standardized: $r = -0.62, P = 0.004$). The right striatum and putamen were significantly larger than the left when all subjects were considered in the analysis ($F_{1,18} = 14.90, P = 0.001; F_{1,18} = 21.48, P < 0.001$, respectively), but these effects were not reliable for the aged subjects alone.

As reported in Shamy et al. (2006) and confirmed in this larger sample, hippocampal volume failed to correlate with age ($F_{1,18} = 1.17, P = 0.294$). There was also no significant correlation between hippocampal volume and age among monkeys older than 20 years. Correlations with calcarine cortex volume were not statistically reliable for either the aged group alone or all subjects considered together.

### Volumetric Correlates of DR Performance

In contrast to a priori expectations, PFC volume was largely unrelated to the status of spatiotemporal working memory, whereas volumes of the hippocampus and striatum correlated with individual differences in various aspects of DR performance in aged monkeys (Table 2). Specifically, cerebral, ventricular, and PFC volumes failed to correlate with any measure of DR performance. In contrast, hippocampal volume correlated with the number of trials to criterion on the DR task with a 1-s delay across all subjects (raw: $r = -0.52, P = 0.022$; standardized: $r = -0.53, P = 0.019$) and in the aged group alone (raw: $r = -0.57, P = 0.04$; standardized: $r = -0.71, P = 0.007$; Fig. 6A,C). When hippocampal volume was controlled for age, the association remained significant for the entire subject sample ($r = -0.52, P = 0.022$) but not for the aged animals alone. Hippocampal volume among the aged monkeys also positively correlated with DR accuracy averaged across increasing retention intervals (5–60 s, raw: $r = 0.59, P = 0.034$; standardized: $r = 0.36, P = 0.220$; Fig. 6D), and this effect remained significant when chronological age was included as a covariate ($r = 0.56, P = 0.045$). Among all subjects, performance across delays on the DR task was additionally coupled with putamen (raw: $r = 0.48, P = 0.038$; standardized: $r = 0.28, P = 0.250$) and overall striatum volumes (raw: $r = 0.46, P = 0.048$; standardized: $r = 0.29, P = 0.221$; Fig. 6B), although neither association remained reliable when the influence of age was controlled.

### Volumetric Correlates of DNMS Performance

A novel observation in this study is that DNMS performance was coupled with the volumes of multiple brain regions outside the hippocampus, including subdivisions of the PFC and striatum. Volumetric correlations with DNMS acquisition and recognition accuracy across delays are shown in Figures 7 and 8, respectively, and listed in Table 2. Acquisition of the DNMS task with a short delay was negatively correlated with ACC...
(raw: $r = -0.48$, $P = 0.038$; standardized: $r = -0.51$, $P = 0.026$) and dIPFC volumes (raw: $r = -0.57$, $P = 0.012$; standardized: $r = -0.64$, $P = 0.003$) in the PFC. The dIPFC values also correlated with the average recognition accuracy across longer retention intervals (raw: $r = 0.55$, $P = 0.015$; standardized: $r = 0.5$, $P = 0.030$). For the aged subjects considered alone, IFG volume was the only PFC measure that was related to task acquisition (raw: $r = -0.57$, $P = 0.044$; standardized: $r = -0.69$, $P = 0.009$), but total PFC volume ($r = 0.61$, $P = 0.027$; standardized: $r = 0.52$, $P = 0.07$), PFC GM ($r = 0.58$, $P = 0.036$; standardized: $r = 0.519$, $P = 0.069$), and prefrontal WM ($r = 0.60$, $P = 0.032$; standardized: $r = 0.43$, $P = 0.142$) all correlated with average DNMS performance. Subregion analysis revealed that the relationship between prefrontal GM and recognition accuracy was largely accounted for by correlations for the SFG (raw: $r = 0.61$, $P = 0.027$; standardized: $r = 0.54$, $P = 0.059$), the dIPFC (raw: $r = 0.6$, $P = 0.031$; standardized: $r = 0.47$, $P = 0.107$), and the vmPFC (raw: $r = 0.59$, $P = 0.032$; standardized: $r = 0.56$, $P = 0.046$). The contribution of aging was critical to these relationships as none of the identified associations remained significant when chronological age was included as a covariate in the analysis.

Striatal correlates of DNMS performance included a significant inverse relationship between learning the task initially and total volume of the striatum (raw: $r = -0.52$, $P = 0.024$; standardized: $r = -0.68$, $P = 0.001$), which was largely attributable to a coupling between acquisition scores and the volume of the caudate (raw: $r = -0.54$, $P = 0.017$; standardized: $r = -0.61$, $P = 0.005$). Smaller striatum (raw: $r = 0.057$, $P = 0.01$; standardized: $r = 0.54$, $P = 0.016$), caudate (raw: $r = 0.54$, $P = 0.017$; standardized: $r = 0.52$, $P = 0.021$), and putamen volumes (raw: $r = 0.47$, $P = 0.043$; standardized: $r = 0.39$, $P = 0.096$) were also associated with lower average recognition accuracy across increasing memory delays. Similar to the findings for the PFC, these effects appeared related to the joint influence of age on both striatal volume and DNMS performance.

Finally, for the aged subjects alone, smaller cerebrum volume ($r = 0.66$, $P = 0.014$; standardized: $r = 0.57$, $P = 0.043$) and enlarged ventricles ($r = -0.68$, $P = 0.011$; standardized: $r = -0.68$, $P = 0.011$) were coupled with impaired recognition accuracy. The correlation between ventricular volume and DNMS performance remained significant when 2 outliers with gross ventricular enlargement were omitted from the analysis ($r = -0.79$, $P = 0.004$) and when the influence of age was evaluated ($r = -0.55$, $P = 0.05$).

**Multivariate Analysis**

Results of the multivariate analysis are displayed in Figure 9. A PCA was performed on the combined ROI values of all monkeys and selected the set of first 3 PCs to construct a covariance pattern. Overall, most of the loadings in the covariance pattern were predictive of better performance across delays. The unequal-samples significant result, $T = 2.54, P < 0.05$, such that a younger profile was predictive of better performance across delays. The bootstrap procedure was then used to identify ROIs with a robust loading in the covariance pattern. Four such ROIs were found. The first, the right prefrontal white matter (rWM) had a smaller rWM volume in the young monkeys compared with the aged monkeys (i.e., negative loading in covariance pattern). The remaining 3 areas, bilateral putamen (rPut, lPut) and left ACC, had significantly increased volumes in the young compared with aged monkeys (i.e., positive loadings in covariance pattern). Overall, most of the loadings in the covariance pattern were predictive of better performance across delays.
pattern were positive, indicating larger mean structural volumes in the young monkeys compared with elderly monkeys. This resulted in an average value of the loadings in the covariance pattern of +0.07. Normalization of the loadings in the covariance pattern was such that the Euclidean norm was unity.

To test whether there was a residual correlation between task performance and ROI volumetric measures that was unaccounted for by the covariance pattern and task performance, the data were residualized with respect to the covariance pattern. Further, the covariance pattern was checked to determine whether there were ROI-wise associations between the residuals and the accuracy slopes from both DR and DNMS tasks. No significant association was found at an uncorrected $P$ level of 0.05, which indicated that the covariance pattern did indeed capture the major source of age- and task-related variance in the data.

**Discussion**

The present study used univariate and multivariate analyses to investigate potential volumetric alterations across multiple brain regions and their relationship to age-related memory impairment in the rhesus monkey. Results of the univariate analyses revealed that aging is not associated with gross structural change uniformly distributed throughout the macaque brain. Dorsolateral PFC and striatal volumes were smaller in aged monkeys compared with young adults. Additionally, there was a negative correlation of prefrontal WM volume with age selectively among the older subjects. In contrast, relative volumetric preservation was noted in other PFC subregions, the calcarine cortex, and the hippocampus. The overall pattern of results was similar to findings from studies of human brain aging, in which much larger numbers of subjects are typically available for analysis. For example, age-related volumetric decline in prefrontal GM (Raz et al. 1997, 2004, 2005; Salat et al. 1999, 2001; Tisserand et al. 2002), prefrontal WM (Raz et al. 1997, 2004; Salat et al. 2001), and striatum (Raz et al. 1995, 2005; Matochik et al. 2000, 2004) also has been documented in elderly people, together with stability of the calcarine cortex (Raz et al. 1997). In contrast to findings in nonhuman primates (Shamy et al. 2006), however, hippocampal atrophy (Kaye et al. 1997; Raz et al. 1995, 1998; Tisserand et al. 2000) also has been documented in human aging, suggesting a possible contribution of incipient AD (Soininen et al. 1994; Laakso et al. 1998; Sullivan et al. 2005), vascular disease (Raz et al. 2007), or metabolic disease (Anan et al. 2010).

In humans, prefrontal GM and WM volume measurements reportedly predict impairments of recall, executive function, and spatial working memory (Raz et al. 1998; Head et al. 2002; Gunning-Dixon and Raz 2003), whereas compromised integrity of the medial temporal lobe, particularly the hippocampus, is coupled with declarative memory deficits (Raz et al. 1998). Corresponding comparisons for the current sample of monkeys indicated that PFC volume correlated with recognition accuracy (i.e., DNMS), hippocampal volume correlated with spatiotemporal memory in aged subjects (i.e., DR), and striatal
volume correlated with measures of both spatiotemporal and recognition memory. Chronological age largely accounted for the relationships between recognition memory and PFC volume, whereas the relationship between hippocampal volume and DR performance was independent of age. Studies evaluating structural MR images in humans and macaque monkeys have revealed that cerebral volume is moderately predictive of age-related recognition memory impairment (Coffey et al. 2001; Shamy et al. 2006). Additionally, an inverse correlation was found in the current study between ventricular volume and recognition memory performance. These findings raise the possibility that age-related recognition memory impairment might result from atrophy distributed across multiple memory-related structures.

To consolidate the regionally selective univariate findings under a single neural substrate that could account for both age as well as task performance, a multivariate analysis was conducted. An age-related covariance pattern was found whose expression distinguished young from aged monkeys and also correlated with impairments across delays on the DNMS task. Overall, the loadings of individual brain regions were positively weighted, indicating that smaller volumes were predictive of an older profile and impaired recognition memory performance. The most reliable regional volumes within the network included the putamen bilaterally and the left ACC, which had a positive loading, and the rWM, which had a negative loading. As monkeys age, expression of this pattern decreases and this change across the network of brain regions predicts greater DNMS impairment.

A related study from Alexander et al. (2008) combined voxel-based morphometry (VBM) with SSM to evaluate age-related structural changes in the context of memory performance and included results from a subset of subjects analyzed here. In contrast to the present analysis, in which volumes were quantified using manually acquired ROIs, VBM is a method in which brains are morphed into a single template space, and comparisons of image density are made between subjects on a voxel-by-voxel basis (Alexander et al. 2008). Reduced GM concentration reported in VBM analyses is often interpreted as atrophy (Keller et al. 2002; Wessels et al. 2006, but see Eriksson et al. 2009), and in this context, manually traced ROI volumetric measurements are sometimes treated as a standard for validating VBM results (Kubicki et al. 2002). However, observations from VBM compared with manual ROI analyses can differ significantly in magnitude or location within the same subjects (Good et al. 2002; Testa et al. 2004; Giuliani et al. 2005; Kennedy et al. 2009), perhaps partly attributable to decreased sensitivity of ROI analyses to focal changes (Voormolen et al. 2010) or alignment and smoothing artifacts in VBM analyses (Eriksson et al. 2009).

To our knowledge, this is the first pair of complementary studies to use VBM and ROI analyses to assess relationships between age-related structural and cognitive changes in rhesus monkeys. Across both investigations, dlPFC was reduced, together with preservation in the visual cortex. Concomitant reductions in GM were also reported in the vmPFC, IFG, and superior temporal cortex, alongside preservation in several regions outside the scope of our ROI analysis. Higher expressions of this pattern were correlated with DR performance and DNMS acquisition. Notably, striatal GM was reportedly preserved in the VBM assessment (Alexander et al. 2008), contrary to data obtained in the current study and by Matoschik et al. (2000). As discussed previously (Alexander et al. 2008), this may be due in part to the lower accuracy of anatomical parcellation in VBM assessment compared with manually traced ROIs. A corresponding advantage of VBM, however, is that by surveying the entire brain unconstrained by ROI boundaries, this approach can enhance sensitivity for detecting localized differences in GM. This decreased sensitivity to local changes in ROI tracing methods may partly explain the failure to find significant correlations between age and vmPFC and IFG volumes in the current study. On the other hand, the computation of ROIs using anatomical landmarks is less prone to potential errors induced by image preprocessing which can influence group-level results in structural as well as functional neuroimaging (Strother et al. 2004; Strother 2006). Furthermore, ROI computation increases the signal-to-noise ratio, which is beneficial regardless of whether a univariate or multivariate analysis is employed.

For completeness, we used SSM on our ROI data to see whether a single age-related dimension in the data could be established that also had predictive power with respect to behavioral performance on the DNMS task. After residualizing the volumetric data with respect to the pattern, no significant brain–behavioral or age correlations persisted, suggesting that the pattern indeed captured the main source of age- and task-related variance in the results. Inferring biological meaning from the correlative relationships in the covariance pattern is difficult. Instead, our analysis identifies topographic commonalities in age-related volumetric change across all monkeys, without postulating any “interaction” or “communication” among the implicated brain areas.

The pattern of correlations we report between regional brain volume and behavior has precedence in previous studies of aged nonhuman primates. The findings are also notably different, however, than might be predicted from a standard neuropsychological framework, on the basis of the behavioral effects of experimental lesions in young subjects. Similar to the current results, for example, hippocampal glucose metabolism, measured by $^{18}$F-fluorodeoxyglucose positron emission tomography (FDG PET) imaging, correlates with DR performance among young and aged monkeys (Eberling et al. 1997). Given that DR emphasizes spatiotemporal memory, such correlations may arise from task demands on hippocampus-dependent processing capacities, including the spatial (Baylis and Moore 1996; Beason-Held et al. 1999; Lavener et al. 2006) and temporal organization of memory (Buckmaster et al. 2004; Alvarado and Bachevalier 2005; Lavener et al. 2006). With respect to our findings for the PFC whereas an earlier stereological quantification of histological material failed to detect reliable associations between dlPFC volume and DR performance measures in aged monkeys (O'Donnell et al. 1999), several studies have reported that DNMS performance is coupled with various age-related alternations in the dlPFC (reviewed in Luebke et al. 2010), including changes in myelinated axons in the deep layers of area 46 (Moore et al. 2005), monoamine receptor binding (Moore et al. 2005), the electrotonic structure of both long and local projection neurons (Kabaso et al. 2009), and firing rates of layers II and III neurons (Chang et al. 2005). Recognition is understood to comprise a multicomponent process, and these correlations may reflect the reported differential involvement of PFC subregions to the relevant capacities, including recollection, familiarity, and novelty detection (Daselaar et al. 2006).
Overall, our findings demonstrate that normal cognitive aging in nonhuman primates is associated with regionally selective morphometric alteration distributed across a network of memory-related regions. The additional implication is that normal brain aging comprises a distinct neurobiological setting in which cognitive outcomes are not easily predicted from the known functional organization of the affected areas. Thus far, in vivo imaging studies in behaviorally characterized monkeys have been primarily cross-sectional and are therefore susceptible to cohort effects. An advantage of this approach is the ability to obtain MRI scans at multiple time points, and an important future direction is to determine whether longitudinal change occurs across this network of brain regions and if the rate of change predicts trajectories of cognitive aging in rhesus macaques.

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References


