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White matter integrity correlates of implicit sequence learning in healthy aging

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Abstract

Previous research has identified subcortical (caudate, putamen, hippocampus) and cortical (dorsolateral prefrontal cortex, DLPFC; frontal motor areas) regions involved in implicit sequence learning, with mixed findings for whether these neural substrates differ with aging. The present study used diffusion tensor imaging (DTI) tractography to reconstruct white matter connections between the known gray matter substrates, and integrity of these tracts was related to learning in the alternating serial reaction time task (ASRT) in younger and healthy older adults. Both age groups showed significant sequence learning (better performance to predictable, frequently occurring vs. less frequent events), with an age-related difference in the late learning stage. Caudate-DLPFC and hippocampus-DLPFC tract integrity were related to ASRT sequence learning, and these brain-behavior relationships did not differ significantly between age groups. Additionally, age-related decreases in caudate-DLPFC tract integrity mediated age-related differences in late stage sequence learning. Together, these findings complement studies of gray matter substrates underlying implicit sequence learning, and provide evidence for similar white matter integrity-sequence learning relationships in younger and healthy older adults.
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Implicit learning refers to acquiring sensitivity to regularities in the environment that occurs without awareness (Seger, 1994). This ubiquitous process plays a role in skills ranging from language acquisition (Kuhl, 2004) to social intuition (Lieberman, 2000), which involve extracting predictable words from speech streams and non-verbal cues from social interactions, respectively. Implicit learning is essential for adapting to new people, places, and technologies; particularly in aging, as explicit forms of learning decline (Craik and Jennings, 1992).

The present study focused on implicit probabilistic sequence learning, a form of implicit learning that involves extracting regularities from a series of events. This type of learning can be assessed with the alternating serial reaction time (ASRT) task (Howard and Howard, 1997), in which participants view four open circles on the screen and respond to the location of the circle that fills in black. Unbeknownst to participants, every second event follows a repeating pattern and intervening events are randomly determined. Sequence-specific learning can be seen as faster reaction time and/or higher accuracy to the predictable, pattern events compared with random events.

Behaviorally, implicit sequence learning is sometimes spared in aging. For example, older adults learn as well as younger adults in studies using sequences with simple de-

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terministic structure, where single items (0^{th} order structure) or pairs of items (1^{st} order structure) occur more frequently than others (Curran, 1997; Frensch and Miner, 1994; Howard and Howard, 1989). However, age-related deficits often appear when more complex probabilistic sequences are used, such as those in which the lowest level of regularity to be learned spans three (2^{nd} order structure) or four (3^{rd} order structure) consecutive trials (Bennett et al., 2007; Curran, 1997; Howard and Howard, 1997; Howard et al., 2004). When present, the magnitudes of these age-related differences are greater in later stages of learning, as younger adults continue to learn after older adults reach a plateau.

The neurobiological substrates of implicit sequence learning include frontostriatal networks (Curran, 1995; Doyon et al., 2009; Seger, 2006), which comprise projections between the striatum (caudate, putamen) and frontal regions such as the dorsolateral prefrontal cortex (DLPFC), and primary, supplementary (supplementary motor area: SMA), and premotor areas. There is also evidence that medial temporal lobe (MTL) regions such as the hippocampus and parahippocampal gyrus (entorhinal, parahippocampal, and perirhinal cortices) are involved in implicit learning of complex sequences (Rose et al., 2002; Schendan et al., 2003). Whereas the putamen and frontal motor regions appear to support the motor demands of the task (e.g., response preparation and execution), both the caudate and MTL have been implicated in the formation of associations necessary for learning (e.g., stimulus-stimulus and stimulus-response associations) (Curran, 1995). These two structures may be recruited at different stages of learning, with the MTL being active earlier than the caudate (Schendan et al., 2003). Together, these findings suggest that the MTL may be essential for rapid association formation in early learning stages, whereas the caudate is involved in forming associations into later stages of learning.

Previous functional Imaging Research has examined age group differences in the neural correlates of implicit sequence learning. In two separate studies, task-related activity was observed in expected regions for younger and older adults (e.g., striatum, frontal regions, hippocampus). However, one of these studies found that the magnitude of these effects did not differ with age group (Daselaar et al., 2003), whereas the second study reported an age-related decrease in task-related activity in the prefrontal cortex and putamen (Aizenstein et al., 2006). The latter study further revealed significant hippocampal activity early in learning and frontostriatal activity (DLPFC, putamen) throughout learning, but only for the younger group. These mixed findings indicate that the effect of aging on brain-sequence learning relationships remains unresolved.

In addition to the distributed gray matter regions identified above, white matter connecting these neural networks may also be a critical neural substrate of implicit sequence learning in healthy aging. One structural imaging study of older adults demonstrated that the degree of periventricular and deep white matter hyperintensity (i.e., white matter lesion) burden was related to implicit sequence learning performance (Aizenstein et al., 2002). Unfortunately, these findings lack regional specificity and they overlook the potential role of normal-appearing white matter in implicit sequence learning. Therefore, a preferred method is diffusion tensor imaging (DTI) tractography, which can be used to investigate the integrity of white matter in specific tracts with known functional properties. DTI measures the diffusion, or movement, of molecular water, which is summarized for each voxel as an ellipsoid where the eigenvalues $\lambda 1$, $\lambda 2$, and $\lambda 3$ indicate the rate of diffusion along its three principal axes (Basser et al., 1994; Pierpaoli and Basser, 1996). Tractography analyses can then reconstruct white matter tracts, which are assumed to follow the trajectory of the primary diffusion directions ($\lambda 1$) of adjacent voxels (see Jones, 2008 for an overview of tractography methods), and measures of white matter integrity can be calculated from these tracts using the DTI-based eigenvalues.

Fractional anisotropy (FA) is a commonly used measure of integrity that refers to the coherence of the orientation of water diffusion, independent of rate. It is calculated as the fraction of total diffusion that is anisotropic, which is derived from the normalized variances of the three eigenvalues (Basser and Pierpaoli, 1996). Higher FA values indicate more consistent diffusion, and thus better integrity of the underlying tract. In healthy aging, significant decreases in FA are often reported, with the age group differences being largest in frontal tracts (Madden et al., 2009a; Sullivan and Pfefferbaum, 2006). When accompanied by an age-related decrease in cognitive performance, a few studies have shown that individual differences in FA from underlying white matter tracts mediate age group differences in cognitive tasks involving attention and executive functions (Madden et al., 2009b; Perry et al., 2009; Zahr et al., 2009). However, these effects have not been examined for implicit cognitive processes such as implicit sequence learning.

To address gaps in earlier research, the present study pursued two aims regarding relationships between ASRT performance and DTI tractography-based measures of integrity in younger and healthy older adults. The first aim was to examine white matter integrity correlates of implicit sequence learning using correlations between FA from three bilateral subcortical-cortical tracts and performance on the ASRT, and assess whether these FA-sequence learning relationships varied with the stage of learning and with age group. We expected that sequence-specific learning (see ASRT data analysis section of the Methods) would relate to FA in the hippocampus-DLPFC tracts at early learning stages and FA in the caudate-DLPFC tracts at all learning stages, but not to FA in the putamen-SMA tracts because the latter regions are not involved in sequence-specific association formation. Based on the mixed findings from earlier functional imaging studies, we did not make specific predictions regarding the difference in these FA-sequence

learning relationships across age groups. The second aim was to assess age group differences in white matter integrity and implicit sequence learning, and determine whether individual differences in integrity mediated age group differences in learning. We hypothesized that older adults would show reduced FA, especially in frontal tracts that are more susceptible to aging (e.g., Davis et al., 2009), and smaller learning effects, especially in later learning stages, compared with younger adults, with evidence in support of mediation.

1. Method

1.1. Participants

Fourteen Georgetown University undergraduate students (18.9 ± 0.7 yr old, 9 female) and 14 older adults (67.6 ± 3.1 yr old, 63–72 yr, 10 female) who responded to advertisements in the *Washington Post* Health Section were recruited. All participants gave informed consent, and received either payment or course credit for their participation. The Georgetown University Institutional Review Board approved the experimental procedures.

Prior to participation, individuals were screened for conditions that would affect their ability to complete the computer-based task (e.g., uncorrected vision, arthritis, and back problems that would make it difficult to see items presented on the computer screen or comfortably push the response buttons) or prevent them from being able to enter the MRI scanner (e.g., being pregnant, having ferrous metal implants, having difficulty lying in the supine position for 30 min, and being claustrophobic). Participants were also screened for conditions that would influence their cognitive functioning and/or contribute to white matter pathology (e.g., stroke, dementia, diabetes, and uncontrolled depression or hypertension). Based on responses to a health screen questionnaire and performance on an extensive neuropsychological test battery (Table 1), no participant was excluded from the study.

1.2. General procedure

Participants completed 3 days of testing. On the first day, they completed screening procedures (informed consent, biographical and health screen questionnaires, and MRI safety form) and the MRI scanning protocol. On the second and third days of testing, participants completed the comprehensive neuropsychological test battery (see Table 1) and a computer-based implicit sequence learning task, the ASRT.

1.2.1. MRI scanning protocol

Participants were scanned using the 3.0 Tesla MRI system (Siemens Magnetom Trio, Erlangen, Germany) at Georgetown University's Center for Functional and Molecular Imaging. An imaging technician positioned participants in the scanner, laying them in the supine position with a circularly polarized head coil. A mirror mounted on the head coil allowed them to watch cable television programming during scanning. Fitted padding was used to minimize head movements.

A high resolution T1-weighted structural scan (MPRAGE) was acquired first with the following parameters: scan time = 7: 23 minutes, TR = 2,300 ms, TE = 2.94 ms, TI = 900 ms, 9° flip angle, one slab, 160 sagittal slices with a 1.0 mm slice thickness, and FOV = 256 × 256 mm with a 256 × 256 matrix resulting in an effective resolution of 1.0 mm^3 isotropic voxels. A neurologist reviewed these images, and no participant exhibited a clinically significant structural abnormality that was atypical for their age (e.g., lesions, excessive atrophy). Thus, future references to normal-appearing white matter refer to the absence of gross white

Table 1
Neuropsychological test results

	Cognitive processing	Younger adults	Older adults	*t*
MMSE	Screen for dementia	29.9 ± 0.3	29.4 ± 0.7	−2.7*
WAIS-III vocabulary	Vocabulary	62.6 ± 6.0	68.0 ± 5.3	2.6*
WAIS-III digit symbol coding	Processing speed	91.9 ± 13.9	61.6 ± 13.3	−5.9**
WAIS-III digit symbol pairing	Cued recall	16.0 ± 3.3	11.1 ± 4.9	−3.1*
WAIS-III digit symbol recall	Free recall	8.1 ± 1.2	7.0 ± 1.4	−2.4*
WAIS-III digit span forward	Working memory	11.8 ± 2.5	11.6 ± 2.2	NS
WAIS-III digit span backward	Working memory	7.0 ± 2.5	8.9 ± 2.9	NS
COWAT-FAS sum	Verbal fluency	46.1 ± 11.4	47.2 ± 12.9	NS
USC-REMT Free recall correct	Free recall	32.5 ± 5.1	25.4 ± 5.2	−3.4*
USC-REMT Free Recall Repetitions	Free recall	2.4 ± 2.5	2.9 ± 3.5	NS
USC-REMT Free recall intrusions	Free recall	0.5 ± 0.9	1.0 ± 0.9	NS
WJ-III Word Attack SS	Reading ability	92.6 ± 8.3	101.1 ± 2.7	3.6*
WJ-III word identification SS	Reading ability	108.6 ± 10.4	112.7 ± 5.0	NS

Notes. All scores are given as mean ± SD, with neuropsychological test scores based on raw data except where standard scores (SS = age-adjusted standard score with a mean of 100 and standard deviation of 15) are noted. Independent sample t tests show group effects (* $p < .05$, ** $p < .001$, NS = not significant). Three participants did not complete the COWAT-FAS and USC-REMT tests. MMSE = Mini Mental State Examination; WAIS-III = Wechsler Adult Intelligence Scale, 3rd Ed.; COWAT-FAS = Controlled Oral Word Association Test-FAS; USC-REMT = University of Southern California-Repeatable Episodic Memory Test; and WJ-III = Woodcock-Johnson, 3rd Ed.

matter artifacts as detected with the high resolution MPRAGE.

Two 35-direction diffusion weighted echo planar imaging sequences were then acquired using gradient values of b = 0 and b = 1,000 second/mm^2 applied in 35 orthogonal directions. Each acquisition had the following parameters: scan time = 4: 39 min, TR = 7,700 ms, TE = 100 ms, 55 axial interleaved slices with a 2.5-mm slices thickness with no gap, and FOV = 240 × 240 mm with a 96 × 96 matrix resulting in an effective resolution of 2.5 mm^3 isotropic voxels. The entire scanning session, which included three functional runs with a different task (data not reported here), took approximately 45 min.

1.2.2. Alternating serial reaction time task (ASRT)

Participants viewed four black outlined open circles presented in a row on a 17 in. PC computer monitor. On each trial, one circle filled in black and participants were instructed to press one of four buttons that corresponded to the target locations using their dominant hand. If the first response was incorrect, the target remained on the screen until the correct response was made. Following a correct response, the target disappeared for a 120-ms interval until presentation of the target on the following trial. Participants completed 45 88-trial blocks, with each block containing eight practice trials followed by 10 repetitions of the 8-element sequence. Short breaks were offered after each block.

Unbeknownst to participants, this ASRT task contained second order sequential structure, such that the location of the target on every other trial was determined by a repeating sequence, and intervening trials were randomly determined. The six possible repeating sequences can be presented as 1r2r3r4r, 1r2r4r3r, 1r3r2r4r, 1r3r4r2r, 1r4r2r3r, and 1r4r3r2r; where the number refers to the location of the target from left to right, and "r" refers to a random trial where the target could occur at any of the four locations. Each participant was randomly assigned one of these repeating sequences.

Participants were instructed to respond to each target as quickly as possible. As older adults tend to make fewer errors than younger adults, which complicates age comparisons, differential feedback previously shown to match the groups on overall accuracy (Bennett et al., 2007; Negash, 2003) was used to maintain performance at approximately 92% accuracy. The feedback display included mean reaction time and accuracy scores for a given block for younger adults, but only mean reaction time scores for older adults. Depending on their mean accuracy for a given block, a statement also prompted participants to "focus more on speed" (> 93% for younger and 90% for older adults) or "focus more on accuracy" (< 91% for younger and 80% for older adults). Feedback stated "speed and accuracy are about right" when mean accuracy scores were between 91% and 93% for younger adults and 80–90% for older adults. As noted in the Results section, this procedure was successful in equating the age groups for accuracy.

1.2.3. Explicit awareness

Two tests of explicit awareness were used. First, a brief postexperiment interview assessed explicit knowledge of the repeating sequence with questions ranging from general ("Do you have anything to report about the task or stimuli?") to specific ("Did you notice a pattern, and if so, can you describe it to me?"). Participants then completed a card sort task in which they sorted cards depicting all possible combinations of three consecutive trials (three-rows of four circles, with one filled in on each row representing the target for that trial) into piles indicating whether that triplet occurred more often or less often.

1.3. Alternating serial reaction time data analysis

Sequence learning involves learning the relative frequency of the smallest repeating units of a sequence rather than the alternating pattern-random structure (Howard et al., 2004; Perruchet et al., 1990). Thus, for ASRT sequences with second-order structure, participants learn that certain three-element sequence chunks, or runs of three consecutive trials referred to as triplets, occur more frequently than others. High frequency triplets are those in which the first and third elements are consistent with the repeating pattern (e.g., 112, 122, 132, 142 for the sequence 1r2r3r4r), whereas low frequency triplets occur with lower probability because they never fall on pattern trials (e.g., 113 and 124). Sequence learning is therefore defined by triplet type effects, calculated as the difference in performance between high and low frequency triplets for reaction time and accuracy measures. As in previous research, certain low frequency triplets, repetitions (e.g., 111 and 222) and trills (e.g., 121 and 232), were omitted because responses to them are influenced by preexisting response biases (Howard et al., 2004).

1.4. Diffusion data analysis

1.4.1. Pre-processing

Diffusion-weighted data were separately processed for each participant using the University of Oxford's Center for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) release 4.0 (www.fmrib.ox.ac.uk/fsl). First, the two diffusion acquisitions were concatenated in time to increase the signal-to-noise ratio. The first volume within this merged data file that did not have gradient applied (i.e., the first b = 0 image) was used to generate a binary brain mask with the Brain Extraction Tool. All the volumes were then aligned to one another using Eddycorrect to minimize distortions due to head movement and eddy currents. Finally, DTIfit was used to independently fit diffusion tensors to each voxel, with the brain mask limiting the fitting of tensors to brain space. The output of DTIfit yielded voxelwise maps of FA for each participant.

1.4.2. Tractography

Probabilistic fiber tracking was conducted separately in each participant using FMRIB's Diffusion Toolbox (FDT) (Behrens et al., 2003). First, diffusion parameters were estimated at each voxel using BEDPOSTX, in which Markov chain Monte Carlo sampling generates a probability distribution function of the primary diffusion direction. ProbtrackX was then used to estimate the distribution of connections between seed and target regions (*see below*). To generate this connectivity distribution, 10,000 streamline samples were initiated from all voxels within the seed regions, traveling along the probability distribution functions of local voxels (step length = 0.5 mm, curvature threshold = 0.2), until they terminated in voxels within the target regions. The output contains a connectivity value for each voxel that represents the number of streamline samples that passed through that voxel.

Three bilateral tracts of interest were generated: caudate-DLPFC, hippocampus-DLPFC, and putamen-SMA. Seed and target regions for these tracts were masked in standard space (MNI152 2 mm^3 standard brain). Seed regions were created in bilateral caudate, hippocampus, and putamen by separately tracing voxels from the corresponding left and right hemisphere probability maps (threshold at 50%) in the Harvard-Oxford Subcortical Structural Atlas. Target regions were created in bilateral DLPFC and SMA. For the DLPFC, diagonal lines were traced separately for each hemisphere in the axial plane ($x = \pm 24$ to ± 30, $y = 30$ to 38, $z = 10$ to 20 in Montreal Neurological Institute space), adjacent to gray matter coordinates published in functional imaging studies of implicit sequence learning (e.g., Daselaar et al., 2003; Schendan et al., 2003). For the SMA, rectangular regions ($x = \pm 14$ to ± 26; $y = -6$ to -18) were traced separately for each hemisphere on adjacent axial slices ($z = 40$ and 42), adjacent to medial aspects of the GM premotor cortex BA6 probability maps (threshold at 25%) in the Juelich Histological Atlas (for SMA). A midline exclusion mask (at $x = 0$) was used to restrict tracking to the left or right hemisphere. To conduct tractography in diffusion space, Flirt was used to register the masks from standard space to each individual's diffusion space using a transformation with 12 degrees of freedom.

Resulting tracts were thresholded at 15% of the maximum connectivity value, leaving only those voxels with a high likelihood of being connected to the seed regions. In contrast to previous studies that have used a constant proportion of the total number of permutations for all participants (e.g., 25/25,000 in Johansen-Berg et al., 2007), the proportional threshold used here accommodates for individual differences in connectivity values for each tract, maximizing tract size and quality. To illustrate these tracts (Fig. 1), population maps were created by using the reverse transformation to convert thresholded tracts from diffusion space into standard space, and then overlaying tracts from each individual onto the standard MNI152 2 mm^3 brain.

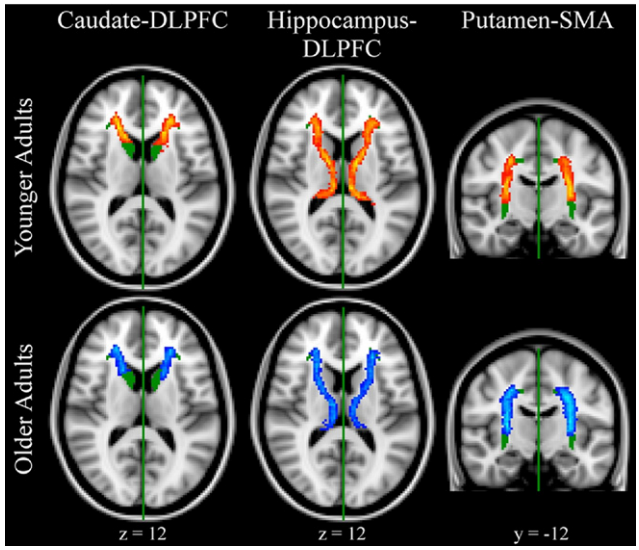

Figure 1. Population maps illustrate locations of the three bilateral tracts of interest in younger (upper) and older (lower) adults. Individual tracts in diffusion space were transformed into standard space and overlaid on the standard MNI152 2 mm^3 brain. To remove spurious tracts, images were thresholded to show only voxels common to at least two younger (red-yellow) or older (blue-light blue) adults. Axial slices are presented in radiological orientation (right = left). Note that portions of anterior corona radiata (caudate-DLPFC), anterior thalamic radiata (caudate-DLPFC, hippocampus-DLPFC), and superior thalamic and superior corona radiata (putamen-SMA) are included in these tracts.

1.4.3. Tract-based measures of integrity

Mean FA values were calculated for all six tracts for each participant. Thresholded tracts were binarized and multiplied by the individual's FA map, leaving just the FA values for voxels within that tract, which were then averaged. All tracts were visually checked for quality. Due to significant distortions (e.g., pixilated, noncontinuous tracts; branching to nontarget regions) some individuals' tracts were excluded from analyses of the left caudate-DLPFC (two younger, one older), right caudate-DLPFC (two older), left and right hippocampus-DLPFC (one younger), and right putamen-SMA (one younger, two older) tracts.

2. Results

2.1. Neuropsychological profiles

The neuropsychological test results in Table 1 revealed the typical pattern of age effects, with all participants performing within the age-expected range. That is, older adults performed significantly worse than younger adults on measures of processing speed, cued recall, and free recall, but not vocabulary or reading ability. All participants had Mini-Mental State Examination scores (Folstein et al., 1975) that were ≥ 28.

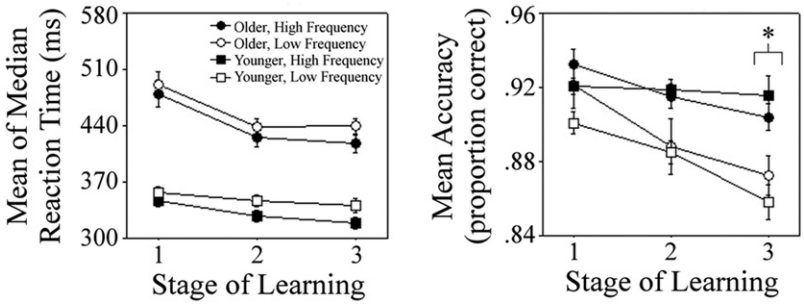

Figure 2. Learning data for mean of median reaction times for correct trials (left) and proportion of correct responses (right) are presented. Responses to high frequency (black shapes) and low frequency (white shapes) triplets are plotted separately across the three stages of learning in younger (squares) and older (circles) adults. * = Significant age group × triplet type interaction (from an analysis of Stage 3 alone) indicating greater learning in younger versus older adults.

2.2. Alternating serial reaction time performance

2.2.1. Sequence learning

For each participant, median reaction times for correct trials and percentage of correct responses were calculated separately for each triplet type for each block, and then averaged into three, 15-block stages of learning. These mean of median reaction times and mean accuracy scores were analyzed separately in age group (young, old) × triplet type (high frequency, low frequency) × learning stage (one, two, three) repeated measures ANOVAs, with age group as a between-subject variable, and triplet type and learning stage as within-subject variables. These data are presented in Fig. 2.

For reaction time, skill learning was seen as a significant main effect of learning stage, $F_{2,52} = 49.38$, $p < .001$, in which responses became faster with practice. Sequence learning was revealed by significant effects of triplet type, $F_{1,26} = 121.0$, $p < .001$, and triplet type × learning stage, $F_{2,52} = 12.6$, $p < .001$, showing that participants responded faster to high versus low frequency triplets, and this difference increased with practice. A significant main effect of age group, $F_{1,26} = 62.7$, $p < .001$, and an age group × learning stage interaction, $F_{2,52} = 11.7$, $p < .001$, indicated that reaction times of older adults were slower and decreased more with practice compared with younger adults. However, there were no group differences in sequence learning as seen by nonsignificant age group × triplet type and age group × triplet type × learning stage interactions, p's $> .25$.

Similar results were seen for accuracy. There was a significant main effect of learning stage, $F_{2,52} = 16.8$, $p < .001$, reflecting a decrease in accuracy with practice, which is typical in probabilistic sequence learning tasks as participants make increasingly more errors on pattern-inconsistent trials as they learn the regularity. Sequence learning was again observed as significant triplet type, $F_{1,26} = 48.8$, $p < .001$, and triplet type × learning stage effects, $F_{2,52} = 12.9$, $p < .001$, with participants responding more accurately to high versus low frequency triplets, and this difference increasing across learning stages. There were no significant effects with age group, p's $> .12$, indicating that the differential feedback was successful in equating the age groups on overall accuracy, and that there were no significant age group differences in the overall pattern of sequence learning.

2.2.2. Age group differences in later learning

Earlier behavioral research indicated that age group differences in sequence learning are largest at later stages (e.g., Howard et al., 2004), and Fig. 2 suggests an group difference in the last learning stage for accuracy. Therefore, separate age group × triplet type repeated measures ANOVAs were conducted for each behavioral measure in learning Stage 3 alone.

In line with the findings reported above, there was a main effect of age group for reaction time, $F_{1,26} = 60.4$, $p < .001$, and significant triplet type effects for both reaction time, $F_{1,26} = 80.6$, $p < .001$, and accuracy, $F_{1,26} = 82.3$, $p < .001$. More importantly, there was a significant age group × triplet type interaction for accuracy, $F_{1,26} = 7.0$, $p < .02$, indicating that younger adults (mean accuracy on high minus low frequency triplets: 0.17 ± 0.07) showed greater learning than older adults (0.09 ± 0.09) late in practice. This age group difference cannot be attributed to older adults being more cautious (i.e., making fewer errors) with practice, because, as mentioned previously, there were no age group differences in overall accuracy.

2.2.3. Implicitness

Self-reports from the postexperiment interview did not reveal any explicit awareness, consistent with previous results using this task (Howard et al., 2004). Five younger adults and five older adults reported noticing a regularity in the location of target presentations, but no individual was able to accurately describe the nature of the regularity. Similarly, participants did not recognize that high frequency triplets occurred more often than low frequency triplets. Recognition ratings were calculated for each participant as

the proportion of times each triplet type was reported as having occurred "frequently" during the recognition task. An age group × triplet type mixed design ANOVA revealed no significant effects, p's $> .34$, indicating that high and low frequency triplets were rated as occurring equally often in both younger (0.53 ± 0.16 and 0.55 ± 0.12, respectively) and older (0.58 ± 0.11 and 0.55 ± 0.12, respectively) adults.

Taken together, these behavioral data revealed implicit sequence learning in younger and older adults as faster and more accurate responses to high frequency versus low frequency triplets, with no explicit awareness of this regularity in either age group. Consistent with our predictions based on earlier work (Curran, 1997; Howard and Howard, 1997; Howard et al., 2004), there were significant age group differences in sequence learning late in practice, with more learning in younger adults compared with older adults.

2.3. Age group differences in white matter integrity

Separate between-group t-tests compared FA in younger and older adults for each tract of interest. In line with expectations, significant age group differences were seen in the left, $t_{(23)} = -2.82$, $p < .01$, and right, $t_{(24)} = -3.71$, $p < .01$, caudate-DLPFC tracts, with higher FA in younger adults compared with older adults. FA did not differ across groups in bilateral hippocampus-DLPFC or putamen-SMA tracts, p's $> .13$. These data are presented in Fig. 3. Similar group comparisons for additional measures of white matter integrity (axial and radial diffusivity, AD and RD) are presented in Supplemental Table 1.

2.4. White matter integrity correlates of sequence learning

Correlations were performed between FA from the tracts of interest and triplet type learning scores for reaction time and accuracy at each learning stage. These learning scores were calculated as the difference in performance on each triplet type (low minus high for reaction time, high minus low for accuracy) summed across the 15 blocks of each learning stage, with more positive scores representing larger magnitudes of learning. To maximize power for detecting these effects, all participants were included regardless of age group. Significant effects are presented in Fig. 4. Additional correlations between diffusivity measures (AD and RD) and sequence learning scores are presented in Supplemental Table 1.

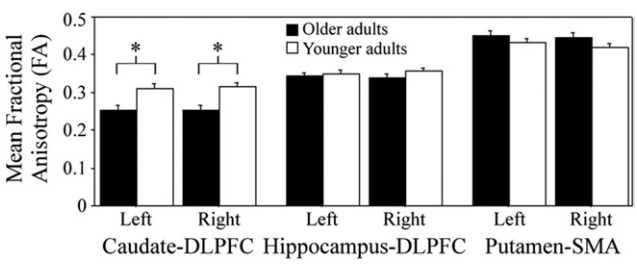

Figure 3. Bar graph shows mean FA values for each tract in younger (white) and older (black) adults. * = Significant age group differences in FA are seen in the bilateral caudate-DLPFC tracts.

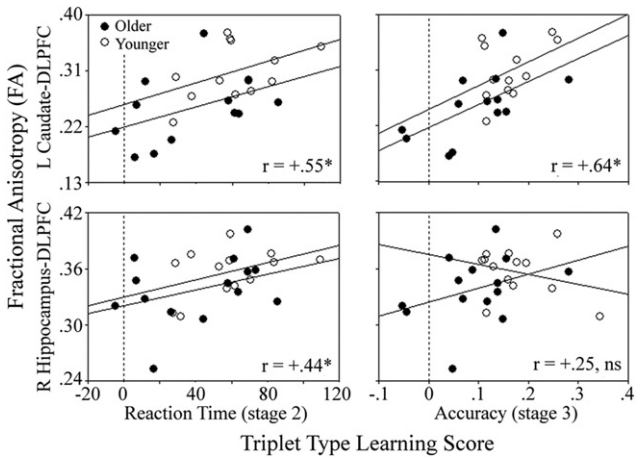

Figure 4. Scatter plots show correlations between white matter integrity (fractional anisotropy) in the left caudate-DLPFC (upper) and right hippocampus-DLPFC (lower) tracts and triplet type learning scores for Stage 2 mean of median reaction time (left) and Stage 3 proportion of correct responses (right). * = Significant FA-sequence learning relationships are shown, with separate regression lines for younger (white circles) and older (black circles) adults illustrating how similar these slopes are across age groups. ns = not significant. Note that relationships between left caudate-DLPFC tract FA and sequence learning (Stage 3 for accuracy and Stage 2 for reaction time) survived Bonferroni correction for six comparisons per tract (i.e., three learning stages × two behavioral measures per tract; $p < .0083$).

Results revealed that, for reaction time, learning in Stage 2 was positively correlated with FA in the left caudate-DLPFC, $r = +0.44$, $p < .01$, and right hippocampus-DLPFC, $r = +0.44$, $p < .03$, tracts. For accuracy, learning in Stage 3 was significantly related to FA in the left caudate-DLPFC tract, $r = +0.62$, $p < .01$, and marginally significant in the right caudate-DLPFC tract, $r = +0.38$, $p < .06$. These findings are generally consistent with predictions that integrity of the hippocampus-DLPFC tract would relate to earlier stages of learning, caudate-DLPFC tract would relate to all stages of learning, and putamen-SMA tract FA would not be related to sequence learning. The fact that no correlations with sequence learning were detected in learning Stage 1 may be due to the triplet type learning scores being significantly, p's $< .02$, lower in stage 1 versus 2 and three for both reaction time (32.8 vs. 49.0 and 65.0) and accuracy (0.05 vs. 0.09 and 0.13).

The reasons for the laterality of these FA-sequence learning effects, and their being related to different behavioral measures at different stages of learning (e.g., caudate-DLPFC FA relating to Stage 2 reaction time and Stage 3 accuracy learning measures), is unclear. Previous functional imaging studies have observed both left and right hemisphere learning-related activity in these gray matter regions (e.g., Rauch et al., 1997; Schendan et al., 2003), suggesting that the lateralized effects reported here are not likely due to

hemispheric dominance of the underlying processes. Interhemispheric between-subject variability can also not explain why left versus right caudate-DLPFC (SD = 0.058 vs. 0.053) and right vs. left hippocampus-DLPFC (SD = 0.032 vs. 0.038) tracts show significant effects with sequence learning scores for reaction time. Thus, the neural underpinnings of these laterality differences, and their relation to the different behavioral measures, warrants further investigation.

2.4.1. Age group does not moderate FA-sequence learning relationships

Separate regression analyses were conducted using triplet type learning scores for reaction time (Stage 2) and accuracy (Stage 3) as outcome variables. For both models, predictor variables included age group, FA from tracts significantly related to sequence learning (left caudate-DLPFC and right hippocampus-DLPFC for reaction time, left caudate-DLPFC for accuracy), and the interactions between age group and FA. Results revealed that the interaction term was not a significant predictor of sequence learning for either outcome measure, p's $> .37$, indicating that we did not detect a significant age group difference in the FA-sequence learning relationships (see Fig. 4).

2.4.2.Controlling for overall reaction time

The impact of response speed on FA-sequence learning relationships was assessed using a measure of overall reaction time in the ASRT (mean reaction time on all trials). Multiple regression analyses revealed that, after controlling for overall reaction time, FA in the right hippocampus-DLPFC and/or left caudate-DLPFC tracts remained at least marginally significant predictors of sequence learning for the Stage 2 reaction time measure, p's $< .07$, and Stage 3 accuracy measure, $p < .02$, indicating that these learning effects are not due solely to higher FA influencing overall speed. Similar effects were also observed after controlling for skill learning.

2.4.3. Age group differences in later learning are mediated by individual differences in FA

The previously reported age-related difference in the last learning stage for accuracy, in combination with the age-related difference in FA in the left and right caudate-DLPFC tracts, suggest that individual differences in FA may mediate age group differences in ASRT sequence learning. To test this, hierarchical regression analyses were conducted separately for these two tracts in accordance with the procedures laid out by Baron and Kenny (Baron and Kenny, 1986).

When age group was the sole predictor of the Stage 3 learning score for accuracy, 21.3% of the variance in learning was age-related. When FA in the left caudate-DLPFC tract was entered before age group, FA was a significant predictor, accounting for 41.2% of the variance, and age group no longer accounted for a significant portion of the variance (1.5%). To calculate the degree to which FA attenuated the amount of variance in ASRT sequence learning that can be explained by age, the amount of variance uniquely associated with age group (after partialling out the effect of FA in the left caudate-DLPFC tract) was subtracted from the amount of variance associated with age group as the sole predictor, and then divided by the amount of variance associated with age group as the sole predictor (see Salthouse, 1991). This procedure revealed that FA attenuated age-related variance in sequence learning by 93.0%. In contrast, FA in the right caudate-DLPFC tract did not exhibit an age-independent relation to the Stage 3 learning score for accuracy, indicating that it did not qualify as a mediator of the age group-sequence learning relationship.

3. Discussion

The present study was the first to use DTI tractography to examine tract-based white matter integrity correlates of implicit sequence learning, and to assess whether these relationships were moderated by healthy aging. The main result regarding our first aim was that FA in the caudate-DLPFC and hippocampus-DLPFC tracts, but not the putamen-SMA tract, was significantly related to sequence learning in the ASRT. Hippocampus-DLPFC tract FA was significantly related only to earlier learning (Stage 2), whereas FA in the caudate-DLPFC tract was related to both earlier and later learning (Stages 2 and 3). Importantly, these FA-sequence learning relationships did not differ for younger and older adults. For our second aim of assessing age group differences in white matter integrity and implicit sequence learning, results revealed significant age group differences in FA in the caudate-DLPFC, but not hippocampus-DLPFC or putamen-SMA tracts. In addition, individual differences in caudate-DLPFC tract FA mediated a significant age-related difference in late learning (Stage 3 for accuracy).

Of central importance here was whether sequence learning in the ASRT was related to white matter integrity from tracts connecting discrete gray matter regions that were previously identified as being involved in implicit sequence learning. Using a different measure of white matter integrity, an earlier structural imaging study had found that one measure of implicit learning (skill learning) was related to the degree of white matter hyperintensity burden in older adults (Aizenstein et al., 2002). The present study expanded these previous findings by showing relationships between sequence-specific learning and integrity of normal-appearing white matter in younger and older adults. Unlike the previous report, white matter hyperintensity burden cannot explain the current findings because the integrity-sequence learning relationships were also observed in younger adults with negligible white matter hyperintensities. In addition, we used tract-based measures of integrity that are more sensitive to specific white matter pathways relevant to task performance (compared with periventricular and deep white

matter), and a high-order sequence learning task that minimizes explicit awareness.

Results revealed that hippocampus-DLPFC tract integrity was significantly related to earlier learning (Stage 2), whereas caudate-DLPFC integrity was related to both earlier and later learning (Stages 2 and 3). For both tracts, higher integrity was related to greater sequence learning. These findings are consistent with previous evidence that the hippocampus is active early in learning and the caudate is engaged throughout learning (Aizenstein et al., 2006; Schendan et al., 2003), which may be due to the common role of the caudate and hippocampus in forming learning-related associations (Curran, 1995), such as associations among the locations of stimuli in frequently occurring triplets. When two learning systems perform the same function, they may act cooperatively, with learning mediated by the hippocampus and caudate at earlier and later stages of training, respectively (Poldrack and Packard, 2003). Alternatively, the hippocampus-DLPFC tract may be responsible for forming these high-order associations, whereas the caudate-DLPFC tract may use them to guide behavior (e.g., execute relevant motor plans) (Curran, 1995) or for cognitive control processes such as strengthening relevant associations (i.e. high frequency triplets) and suppressing irrelevant ones (i.e. low frequency triplets) (Liston et al., 2006). In contrast, the absence of a significant relationship between sequence learning and FA in the putamen-SMA tract may reflect this network being involved in response selection and execution (Curran, 1995), which may not depend on sequence-specific implicit knowledge.

Importantly, these white matter integrity correlates of sequence learning did not differ for younger and older adults. Finding that age group did not moderate the FA-sequence learning relationships may indicate that younger and older adults are calling on the same neural networks, which is consistent with one functional imaging study that found similar gray matter regions activated during implicit sequence learning performance in younger and older adults (Daselaar et al., 2003). However, another study found different degrees of activation across age groups (Aizenstein et al., 2006). Thus additional functional Imaging Research, especially in combination with DTI, will be necessary to clarify whether both age groups are calling on these networks to the same degree. Nonetheless, the present data provide the first evidence that sequence-specific learning is related to the integrity of frontostriatal (caudate) and MTL networks for both younger and older adults.

One of the most interesting findings from the present study was that caudate-DLPFC, but not hippocampus-DLPFC, tract integrity was significantly reduced in older adults compared with younger adults. This finding was somewhat unexpected given that previous DTI studies report that age group differences in FA are largest in frontal white matter (e.g., Bennett et al., 2010; Davis et al., 2009; Sullivan et al., 2010), indicating that both caudate-DLPFC and hippocampus-DLPFC tracts should be affected by healthy aging. Nonetheless, the reduction in caudate-DLPFC tract integrity is consistent with known changes in the aging brain that primarily affect frontal and striatal regions, including structural imaging studies that show robust age-related shrinkage of prefrontal and striatal gray matter, functional imaging studies that find hyper- and hypo-activation of frontal regions in aging, and neurochemical studies that report significant age-related disruption of neurotransmitter systems associated with the frontal-striatal network (see Hedden and Gabrieli, 2005 for review). More interestingly, the lack of an age group difference in FA for the hippocampus-DLPFC tract is consistent with findings that MTL structures are relatively unaffected in healthy aging (Head et al., 2005; Hedden and Gabrieli, 2005).

In line with previous reports that integrity of underlying white matter tracts mediates age group differences in performance on explicit cognitive tasks (Madden et al., 2009b; Perry et al., 2009; Zahr et al., 2009), the present results revealed that age group differences in FA from the left caudate-DLPFC tract mediated age-related differences in the ASRT late learning score for accuracy, with integrity of this tract attenuating the amount of variance in sequence learning that could be attributed to age group by 93%. One explanation for this mediating relationship is that age-related changes in integrity of the underlying frontostriatal tract may alter communication between the caudate and DLPFC, for example by disrupting the timing of neural signals, which in turn may affect the coordination of associative processes (e.g., binding) necessary for sequence learning (see Andrews-Hanna et al., 2007; Nordahl et al., 2006; Sullivan and Pfefferbaum, 2006).

3.1. Methodological considerations

White matter tractography can be conducted using probabilistic or deterministic algorithms, each of which has a different set of strengths and weaknesses (Jones, 2008; Madden et al., 2009a). One advantage of probabilistic algorithms is that they are better suited for tracking in regions with low FA (i.e. gray matter) compared with deterministic methods. Thus, we elected to use a probabilistic tractography program (FSL) because the tracts of interest in the present study originated from gray matter seed regions in the striatum (caudate, putamen) and hippocampus.

A second methodological decision was to use a highly reproducible technique to generate the tracts. Thus, well-defined seed and target regions were traced in standard space and then registered to each individual's diffusion space (Heiervang et al., 2006). One disadvantage of this method is that standard-to-diffusion transformations lead to individual differences in the exact locations of the seed and target masks, with additional between-group differences in mask locations because of age-related differences in brain structure (e.g., cortical atrophy). In the present study, poor registration may have contributed to the distortions seen in

tracts that were excluded from analyses (e.g., pixilated, noncontinuous tracts; branching to nontarget regions), though other factors that can also influence calculations of the diffusion tensor may underlie these excluded tracts (e.g., incomplete correction for eddy current distortion). Importantly, the probabilistic tractography algorithm used here may have minimized the effect of these registration issues on tract reconstruction because the probabilistic output (i.e., connectivity value) can be thresholded to confine the tract to only those voxels with a high likelihood of being connected to the seed regions, thus eliminating nontarget connection. This may explain why the tracts in the present study were remarkably similar both across individuals and age groups (see Fig. 1), despite the use of standard space masks.

3.2. Conclusions and future directions

In summary, the present results complement previous functional imaging and patient studies of gray matter substrates involved in implicit sequence learning, finding that the integrity of white matter tracts connecting these distributed regions (caudate-DLPFC, hippocampus-DLPFC) is significantly related to sequence learning in the ASRT in younger and healthy older adults. Future research will be necessary to replicate and expand this finding using a larger sample size, examining additional tracts, and characterizing these effects along the length of the tracts. Moreover, the present results revealed that individual differences in caudate-DLPFC tract integrity mediated age group differences in the later stage of learning. Future research combining DTI and functional imaging will be central to advancing our understanding of the interaction between white matter integrity and gray matter activity within these neural networks underlying implicit sequence learning. Such research would also improve interpretations of the mediating role of white matter integrity, identifying whether age-related declines in white matter integrity are associated with age-related differences in functional activity (e.g., inefficient or compensatory processing), which in turn affect behavioral performance in healthy aging.

Disclosure statements

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Supplemental Table 1
Statistics for age group differences in diffusivity measures and their relation to sequence learning

White matter tract	Age group differences						Relation to learning[1]	
	AD ($\times 10^{-3}$ mm^2/sec)			RD ($\times 10^{-4}$ mm^2/sec)			*r*	
	t	Younger	Older	*t*	Younger	Older	AD	RD
L caudate-DLPFC	3.0*	1.08 ± 0.02	1.33 ± 0.28	3.2*	6.87 ± 0.94	9.69 ± 2.96	−0.66*	−0.68*
R caudate-DLPFC	2.4†	1.07 ± 0.08	1.21 ± 0.21	3.3*	6.69 ± 0.97	8.76 ± 2.11	−0.41*	−0.47†
L putamen-SMA	3.0*	1.03 ± 0.03	1.10 ± 0.08	1.3	5.10 ± 0.31	5.38 ± 0.72	−0.46†	−0.81
R putamen-SMA	2.9†	1.02 ± 0.04	1.08 ± 0.06	0.4	5.17 ± 0.31	5.25 ± 0.69	−0.39	−0.10
L hippocampus-DLPFC	1.5	1.30 ± 0.08	1.35 ± 0.09	1.6	7.71 ± 0.82	8.25 ± 0.97	−0.23	−0.22
R hippocampus-DLPFC	3.9*	1.22 ± 0.06	1.32 ± 0.08	4.1*	7.07 ± 0.58	8.16 ± 0.78	−0.52*	−0.49†

Note. The *t* statistic and mean ± standard deviation (M ± SD) scores for younger and older adults are presented for each diffusivity measure and each white matter tract.

[1] = Diffusivity measures correlated with the stage 3 sequence learning score for accuracy. AD = axial diffusivity, RD = radial diffusivity, L = left hemisphere, R = right hemisphere, DLPFC = dorsolateral prefrontal cortex, SMA = supplementary motor area. Asterisk (*) denotes significant effects at $p < .008$ (Bonferroni-corrected for 6 comparisons per diffusivity measure). Cross (†) denotes significant effects at $p < .05$.

Age group differences in diffusivity measures. Between-group t-tests revealed that significant age-related decreases in caudate-DLPFC tract FA were accompanied by significant age-related decreases in AD and RD in both hemispheres. Age-related decreases in AD and RD were also observed for the right hippocampus-DLPFC tract. Based on earlier research (Budde et al., 2007; Song et al., 2003), these findings suggest that a combination of age-related microstructural differences, such as axonal damage or loss and demyelination, may contribute to white matter integrity decline in these tracts. The putamen-SMA tracts showed bilateral age-related decreases in AD, which may reflect age-related microstructural differences, especially axonal differences, though age group differences in macrostructural organization may also be a contributing factor (Bennett et al., 2010).

Diffusivity correlates of sequence learning. Correlations between AD and RD from the tracts of interest and sequence learning scores for reaction time and accuracy revealed a pattern of results that was similar to those found for correlations between sequence learning and FA. As shown above, the stage 3 learning score for accuracy was significantly related to RD and/or AD in bilateral caudate-DLPFC tracts, and AD in the right hippocampus-DLPFC tract. In addition, the stage 2 learning score for reaction time was significantly related to AD, $r = -0.62$, $p < .001$, and RD, $r = -0.63$, $p < .001$, in the left caudate-DLPFC tract; and to AD, $r = -0.40$, $p < .04$, and RD, $r = -0.53$, $p < .01$, in the right hippocampus-DLPFC tract.

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