Limbic Tract Integrity Contributes to Pattern Separation Performance Across the Lifespan

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Accurate memory for discrete events is thought to rely on pattern separation to orthogonalize the representations of similar events. Previously, we reported that a behavioral index of pattern separation was correlated with activity in the hippocampus (dentate gyrus, CA3) and with integrity of the perforant path, which provides input to the hippocampus. If the hippocampus operates as part of a broader neural network, however, pattern separation would likely also relate to integrity of limbic tracts (fornix, cingulum bundle, and uncinate fasciculus) that connect the hippocampus to distributed brain regions. In this study, healthy adults (20–89 years) underwent diffusion tensor imaging and completed the Behavioral Pattern Separation Task-Object Version (BPS-O) and Rey Auditory Verbal Learning Test (RAVLT). After controlling for global effects of brain aging, exploratory skeleton-wise and targeted tractography analyses revealed that fornix integrity (fractional anisotropy, mean diffusivity, and radial diffusivity; but not mode) was significantly related to pattern separation (measured using BPS-O and RAVLT tasks), but not to recognition memory. These data suggest that hippocampal disconnection, via individual- and age-related differences in limbic tract integrity, contributes to pattern separation performance. Extending our earlier work, these results also support the notion that pattern separation relies on broad neural networks interconnecting the hippocampus.

Keywords: aging, diffusion tensor imaging, episodic memory, fornix, pattern separation

Introduction

The limbic system refers to a collection of cortical (e.g., hippocampus and cingulate gyrus) and subcortical (e.g., amygdala, hypothalamus, thalamus, and septal nuclei) brain regions and their connections (MacLean 1949). Initially described as a network that mediates emotional response (Papez 1937), the limbic system is also recognized as having a central role in memory (Mega et al. 1997; Rajmohan and Mohandas 2007). This can largely be attributed to involvement of the hippocampus (and adjacent medial temporal lobe cortex), which mediates a host of mnemonic processes (Squire et al. 2004). Pattern separation, for example, enables newly encoded events to be dissociated from similar, previously stored events (Yassa and Stark 2011). In this way, pattern separation is thought to be a fundamental component of episodic memory (i.e., memory for discrete "episodes," or events; McClelland et al. 1995; Tulving 2002; Norman and O'Reilly 2003).

The hippocampus is connected to other brain regions via 2 major limbic tracts; the fornix and cingulum bundle (Mark et al. 1995; Schmahmann and Pandya 2009). White matter fibers from the alveus of the hippocampus and the subiculum converge on the medial edge of the hippocampus within each hemisphere to form the fimbria, which traverse the crus of the fornix before coalescing in the body of the fornix. Fornix fibers then project either to the hypothalamus and thalamus via the mammillary bodies, or to septal nuclei and medial and inferior prefrontal regions. The cingulum bundle consists of white matter fibers that extend from the hippocampus and entorhinal cortex to retrosplenial and posterior cingulate cortices and to the amygdala (hippocampal cingulum). Along the cingulate gyrus of each hemisphere (superior cingulum), long association fibers project into frontal, parietal, and temporal cortices, with additional fibers projecting into adjacent cingulate regions, striatum, and thalamus. A third limbic tract of interest is the uncinate fasciculus, which projects from parahippocampal and superior temporal gyri in the anterior temporal lobe to medial and inferior prefrontal regions. Importantly, these limbic tracts (especially the fornix and cingulum) are bidirectional such that the hippocampus sends efferent output to, and receives afferent input from, the distributed cortical and subcortical regions that it projects to (e.g., Swanson 1977; Wyss et al. 1979; Mark et al. 1995).

Disconnection of the hippocampus, as a result of damage to these limbic tracts, was initially associated with memory impairments in human patient and animal lesion studies. Amnesic patients with damage to the fornix and cingulum, for example, perform worse on recall and recognition memory tests (Valenstein et al. 1987; Tucker et al. 1988; Rudge and Warrington 1991; Aggleton and Brown 1999; Moudgil et al. 2000). Similarly, episodic-like memory deficits are seen following fornix lesions in rats (Olton et al. 1982) and monkeys (Gaffan 1994; Buckley et al. 2008). In contrast, damage to the uncinate fasciculus is associated with only modest impairments on similar memory tasks in both humans and animals (Von Der Heide et al. 2013). Taken together, these findings support the notion that episodic memory is mediated by limbic networks (especially the fornix and cingulum) that interconnect the hippocampus with distributed brain regions.

Extrapolating from these effects of pathology-induced limbic tract disconnection, individual differences in hippocampal connectivity may also account for memory differences in healthy populations (O'Sullivan et al. 2001; Bartzokis 2004). One technique that can assess hippocampal connectivity in intact populations is diffusion tensor imaging (DTI). DTI measures the rate of molecular water diffusion, or movement (Beaulieu 2002; Le Bihan 2003). In white matter, diffusion is guided along the length of axons by microstructures (e.g., cell membranes and myelin sheaths) that restrict diffusion perpendicular to the axons. Disruption to these white matter microstructures, such as age-related axonal degeneration and demyelination (Peters 2002), has been associated with decreases in the degree of restricted diffusion (fractional anisotropy, FA), increases in the rate of overall diffusion (mean diffusivity, MD), and differences in the rate of diffusion parallel

(axial diffusivity, AD) and/or perpendicular (radial diffusivity, RD) to the primary diffusion direction (i.e., along the length of axons; Bennett et al. 2010; Madden et al. 2012). Microstructural differences may also be reflected in the shape of anisotropic diffusion (mode, MO), which characterizes the degree to which diffusion is linear versus planar, as would be expected in intact, highly organized regions with a single fiber population. Importantly, these DTI-based diffusion indices are sensitive to individual- and age-related differences in a number of properties of underlying white matter structure (e.g., axonal size and density, degree of myelination, and coherence of fiber orientation). Thus, we use them to infer differences in white matter "integrity" more generally, rather than in a specific microstructural property.

DTI studies have largely replicated the effect of hippocampal disconnection on memory performance using diffusion indices of limbic tract integrity in healthy adults. Across a number of studies, decreased integrity of the fornix, cingulum bundle, and uncinate fasciculus (i.e., decreased FA and increased MD) has been associated with impaired recall and recognition memory performance in healthy individuals aged 9–93 years (Mabbott et al. 2009; Rudebeck et al. 2009; Sasson et al. 2010; Kantarci et al. 2011; Metzler-Baddeley et al. 2011; Lockhart et al. 2012; Sasson et al. 2012, 2013; Sato et al. 2012). For studies with older adults in their sample, these positive limbic integrity-memory performance relationships are observed even after statistically controlling for age (cf. Lockhart et al. 2012; Sato et al. 2012). One limitation of these studies, however, is that they used relatively global indices of memory, often involving composite measures of free recall and recognition that collapse across many different mnemonic processes (e.g., pattern separation and completion, familiarity and recollection). A richer understanding of episodic memory and its neural substrates could arise if we separately examine these component mnemonic processes.

Our lab has specifically targeted behavioral pattern separation using a modified recognition task, that is, the Behavioral Pattern Separation-Object (BPS-O) task (Kirwan and Stark 2007; Stark et al. 2013). The BPS-O task allows for a behavioral measure that taps into pattern separation by including highly similar lure trials. The ability to orthogonalize similar inputs into distinct, non-overlapping representations (i.e., pattern separation) is especially important on trials in which highly similar lure objects must be "separated" from objects that belong to the memory set, and correctly identified as "similar" rather than "old." The BPS-O task also provides a measure of traditional recognition memory (e.g., the proportion of items correctly [hits] minus incorrectly [false alarms] identified as belonging to the memory set), which places minimal demands on pattern separation.

Several sources have supported an association between behavioral measures of pattern separation and the hippocampus, in particular, with the dentate gyrus (DG). Using high-resolution functional magnetic resonance imaging (MRI), for example, we have observed activity consistent with behavioral pattern separation (i.e., activity to similar lures that is different from that to repeated items, and instead more comparable with activity for novel foils) in the DG/CA3 in younger adults (Kirwan and Stark 2007; Kirwan et al. 2007; Bakker et al. 2008; Yassa and Stark 2008; Lacy et al. 2011). A similar, but attenuated, response has also been found in healthy older adults (Yassa, Lacy, et al. 2011; Yassa, Mattfeld, et al. 2011) and individuals diagnosed with mild cognitive impairment (Yassa,

Stark, et al. 2010; Bakker et al. 2012). Using high-resolution DTI, we have also observed that increased integrity of the perforant path (i.e., increased FA), which provides input to the DG and CA3 subfields of the hippocampus from entorhinal cortex, is related to better behavioral pattern separation as measured with BPS-O (Yassa, Mattfeld, et al. 2011) and Rey Auditory Verbal Learning Test (RAVLT) delayed recall (Yassa, Muftuler, et al. 2010) performance in healthy older adults. Though not specifically designed to assess pattern separation, the RAVLT task has many hallmarks of tasks that tap pattern separation in that distinct memory representations must be created for items on 2 separate, interfering word lists (Norman and O'Reilly 2003; Yassa and Stark 2011).

What remains unknown, however, is whether behavioral pattern separation is mediated by neural networks that extend beyond the hippocampus. To address this gap, the current study extended our previous work by examining whether pattern separation performance is related to integrity of limbic tracts that connect the hippocampus and adjacent medial temporal lobe structures to distributed brain regions (i.e., fornix, cingulum bundle, and uncinate fasciculus). Behavioral pattern separation was measured with the BPS-O and RAVLT tasks, which are thought to place different demands on pattern separation. We further extended previous work by assessing these pattern separation-limbic integrity relationships in healthy adults across the lifespan (20–89 years) and by using multiple diffusion indices (i.e., FA, MD, AD, RD, and MO). Both whole-brain, skeleton-wise, and targeted limbic tractography analyses were used to provide converging evidence regarding the contribution of limbic tract integrity to pattern separation performance, controlling for the effect of age to isolate the contribution of white matter integrity to performance. Finally, we tested the hypothesis that age-related declines in pattern separation are mediated by differences in limbic tract integrity, consistent with the notion that age-related cortical disconnection accounts for cognitive aging (O' Sullivan et al. 2001; Bartzokis 2004).

Materials and Methods

Participants
A lifespan sample of 110 healthy adults aged 20–89 years (51.8 ± 18.9 years, 68 females) were recruited from the University of California, Irvine and nearby Orange County communities. Informed consent was obtained from each participant, and the University of California, Irvine Institutional Review Board, approved the experimental procedures. Participants were compensated for their time.

Prior to participation, all individuals were screened for health conditions that may interact with their neurological status (e.g., dementia, stroke, diabetes, etc.), use of psychoactive medication (e.g., neuroleptics, sedatives, etc.), and contraindications for MRI scanning (e.g., being pregnant, having ferrous metal implants, or being claustrophobic).

All participants were also screened to score within the age-expected range [i.e., within 1.5 standard deviations (SDs) of the mean for their age group] on a comprehensive neuropsychological test battery that assessed general cognition using the Mini-Mental State Exam (Folstein et al. 1975); memory using the RAVLT (Rey 1941) and Wechsler Memory Scale Logical Memory (Wechsler 1997b); executive functioning using Trails A and B (Reitan and Wolfson 1985), Verbal Fluency (Spreen and Benton 1977), and Letter Number Sequencing (Wechsler 1997a); working memory using Digit Span (Wechsler 1997a); and general intelligence using Wechsler Adult Intelligence Score III (Wechsler 1997a). Demographic and neuropsychological data are presented in Table 1.

Table 1
Demographic and neuropsychological test data

	20–39	40–59	60–74	75–89
Demographic variables				
Mean age	28.7 ± 5.6	49.5 ± 5.7	67.3 ± 4.2	78.9 ± 4.3
N	34	31	31	14
Male/female	11/23	13/18	11/20	7/7
Neuropsychological tests				
MMSE	29.5 ± 0.7	29.1 ± 1.1	28.7 ± 1.3	28.4 ± 1.4
RAVLT Immediate	12.3 ± 1.9	11.7 ± 2.9	10.9 ± 2.6	10.4 ± 2.5
RAVLT Delay	12.4 ± 2.1	11.5 ± 2.8	10.9 ± 2.7	10.4 ± 2.5
WMS LM Immediate	51.4 ± 7.9	43.9 ± 7.9	46.3 ± 7.9	45.6 ± 7.9
WMS LM Delay	35.0 ± 5.3	29.1 ± 6.7	29.4 ± 6.8	28.9 ± 5.0
Trails A	19.9 ± 6.0	23.5 ± 7.0	26.1 ± 6.7	29.5 ± 8.4
Trails B	45.6 ± 14.7	56.9 ± 20.0	62.5 ± 17.0	69.6 ± 19.6
Verbal Fluency	45.8 ± 10.3	44.6 ± 9.9	44.6 ± 12.4	47.5 ± 9.9
Category fluency	22.6 ± 4.7	22.2 ± 4.4	20.9 ± 4.3	19.6 ± 4.8
LN Sequencing	11.1 ± 2.3	10.6 ± 2.3	10.0 ± 1.9	9.1 ± 2.2
Digit Span	18.4 ± 3.6	18.1 ± 3.7	16.9 ± 3.9	18.1 ± 3.9
WAIS III IQ	114.6 ± 8.6	115.7 ± 8.6	117.5 ± 11.0	116.8 ± 9.4

Notes: Demographic and neuropsychological test scores (mean ± SD) are presented separately for 4 age groups (20–39, 40–59, 60–74, and 75–89 years) within our lifespan sample. MMSE: Mini-Mental State Exam; RAVLT: Rey Auditory Verbal Learning Test; WMS LM: Wechsler Memory Scale Logical Memory; LN Sequencing: Letter Number Sequencing; WAIS III: Wechsler Adult Intelligence Score third Edition; IQ: Intelligence Quotient.

Scanning Protocol

Participants were scanned using a Philips Achieva 3.0-T MRI system fitted with an 8-channel SENSE receiver head coil. A mirror mounted on the head coil allowed them to view the computer screen during scanning. Fitted padding was used to minimize head movements.

A single diffusion-weighted echo-planar imaging sequence was acquired using the following parameters: time repetition (TR)/time echo (TE) = 12 883/49 ms, field of view (FOV) = 230 × 230 mm, flip = 90°, 60 axial slices, and 2 mm^3 spatial resolution. Gradients (b = 800 s/mm^2) were applied in 32 orthogonal directions, with one image having no diffusion weighting (b = 0). A high-resolution T_1-weighted MPRAGE was also acquired using the following parameters: TR/TE = 11/4.6 ms, FOV = 240 × 231 mm, flip = 18°, 200 sagittal slices, and 0.75 mm^3 spatial resolution.

Measures of Behavioral Pattern Separation

The BPS-O task consists of separate encoding and test phases. During the encoding phase, participants viewed 128 common objects (i.e., the memory set) and indicated whether they were "indoor" or "outdoor" objects using a button press. During the test phase, participants were shown 192 objects that consisted of exact repetitions of memory set objects (64 targets), objects similar to those in the memory set (64 lures), and new objects not previously seen (64 foils). Participants judged whether each object was "old," "similar," or "new" using a keyboard press. For both task phases, each object was presented as a color photograph on a white background for 2 s with a 0.5 s interstimulus interval (see Stark et al. 2013 for additional details).

For the BPS-O task, a behavioral correlate of pattern separation is quantified by a behavioral pattern separation (BPS) score that estimates the probability of correctly responding "similar" to highly similar lure objects minus the probability of incorrectly responding "similar" to novel foil objects (to correct for any bias in responding "similar" overall). Note that correct "similar" responses are strongly correlated with incorrect "old" responses to lure objects (r = −0.93, P < 0.001), which in addition to the low rate of "new" responses indicates that participants tradeoff between these responses. Thus, higher BPS scores reflect better pattern separation performance, as measured by an increase in correct "similar" responses, and a corresponding decrease in incorrect "old" responses, to lure trials. Furthermore, whereas correct "similar" responses to similar lure objects likely reflect accurate behavioral pattern separation, incorrect "old" responses to similar lure objects may reflect inaccurate pattern completion, insufficient pattern separation, or both. Thus, while no behavioral metric provides a process-pure estimate of the cognitive process of interest, our BPS measure provides a better index of correct behavioral pattern separation than incorrect pattern completion.

The BPS-O task also provides a measure of traditional recognition memory (Recognition), calculated as the probability of correctly responding "old" to target objects (hits) minus the probability of incorrectly responding "old" to novel foil objects (false alarms). In contrast to the BPS score, the Recognition measure places minimal demands on pattern separation. Instead, simple familiarity can be used to dissociate repeated targets and novel foils from other dissimilar objects in the test phase.

A second task previously used as a behavioral correlate of pattern separation (Stark et al. 2010, 2013) is the RAVLT (Rey 1941). In this task, participants listen to and then recall a list of 15 words (List A). List A is repeated across 5 trials, after which a second list of words (List B) is presented and recalled for a single trial. Participants are then asked to recall List A immediately after the List B trial (RAVLT Immediate) and again after a 30-min delay (RAVLT Delay). The RAVLT has hallmarks of memory tasks that tap pattern separation, including the use of recall (and not recognition) and the presence of the interfering List B that places high demands on arbitrary associative learning (Norman and O'Reilly 2003; Norman 2010). Thus, demands on pattern separation are likely higher for RAVLT Delay relative to RAVLT Immediate performance.

Diffusion Data Analysis

Preprocessing

Diffusion-weighted data were separately processed for each participant using a combination of FSL (FMRIB Software Library; Behrens et al. 2003), AFNI (Analysis of Functional NeuroImages; Cox 1996), and ANTs (Advanced Normalization Tools; Avants et al. 2009). To correct for head movement, all volumes were aligned to the b = 0 image (ANTs). Diffusion tensors were independently fit to each voxel (DTIfit), using a binary mask to limit tensor fitting of brain space (3dSkullStrip). The output yielded voxel-wise diffusion maps of FA, MD, AD (λ_1), RD (average of λ_2 and λ_3), and MO.

Skeleton-Wise Correlations

Exploratory skeleton-wise correlations between diffusion indices (FA, MD, AD, RD, and MO) and demographic (age) and mnemonic (BPS, Recognition, RAVLT Immediate, and RAVLT Delay) measures were performed using Tract-Based Spatial Statistics (Smith et al. 2006). Each individual's FA map was nonlinearly aligned to the FMRIB58_FA_1-mm template in MNI152 standard space. Aligned images were averaged across all participants to form a mean FA image, which was used to generate a "skeleton" of white matter voxels common to all participants. After thresholding at 0.2 to exclude nonwhite matter voxels, aligned FA images from each participant were projected onto the mean FA skeleton by searching for maximum FA values perpendicular to the skeleton (see Smith et al. 2006). For each individual's non-FA maps (MD, AD, RD, and MO), the same nonlinear registrations used for FA were applied. Aligned, non-FA images were then averaged across all participants to form a mean non-FA image, which was projected onto the mean FA skeleton.

For all diffusion indices, skeletonized data were subjected to skeleton-wise (i.e., voxel-wise analyses within the mean skeleton) correlations. Threshold-free cluster enhancement (TFCE) was employed with 2D optimization (height = 2, extent = 1, and connectivity = 26) to enhance cluster-like structures without prior definition of a cluster-forming threshold (Smith and Nichols 2009). A TFCE value was calculated for each voxel within the mean skeleton, representing the weighted sum of the entire local cluster signal (i.e., height × extent of the voxel neighborhood). The TFCE value of each voxel was then tested against a null distribution of maximum TFCE values across the skeleton, generated from 5000 random permutations using the Randomise tool. The output contained statistical maps FWE-corrected for multiple comparisons across space (TFCE, P < 0.05). Due to incomplete DTI data (significantly cropped field of view), 2 participants were excluded from these analyses.

Tractography

To complement the skeleton-wise analyses while focusing specifically on the limbic tracts of interest, probabilistic fiber tracking was conducted separately in each participant using FMRIB's Diffusion Toolbox (Behrens et al. 2003). A probability distribution function of the primary diffusion direction was first estimated for each voxel (BEDPOSTX). Then, for each tract of interest, connectivity distributions between seed, waypoint, and target regions (see below) were generated using 5000 streamline samples that traveled along the probability distribution functions of local voxels (ProbtrackX; steplength = 0.5 mm, curvature threshold = 0.2). The output contained a connectivity value for each voxel that represents the number of streamline samples that passed through that voxel.

One midline (fornix) and 3 bilateral (superior cingulum, hippocampal cingulum, and uncinate fasciculus) limbic tracts were generated using seed, waypoint, and target masks that were traced in standard space and then aligned to subject space (see Fig. 1; see also Concha et al. 2005). For bilateral tracts, these masks were traced separately for the left and right hemispheres. For the fornix tract, seed, waypoint, and target masks (20 axial × 20 sagittal × 6 coronal voxels) were traced to encompass the column/anterior body, commissure, and bilateral crus of the fornix, respectively. For superior cingulum tracts, these
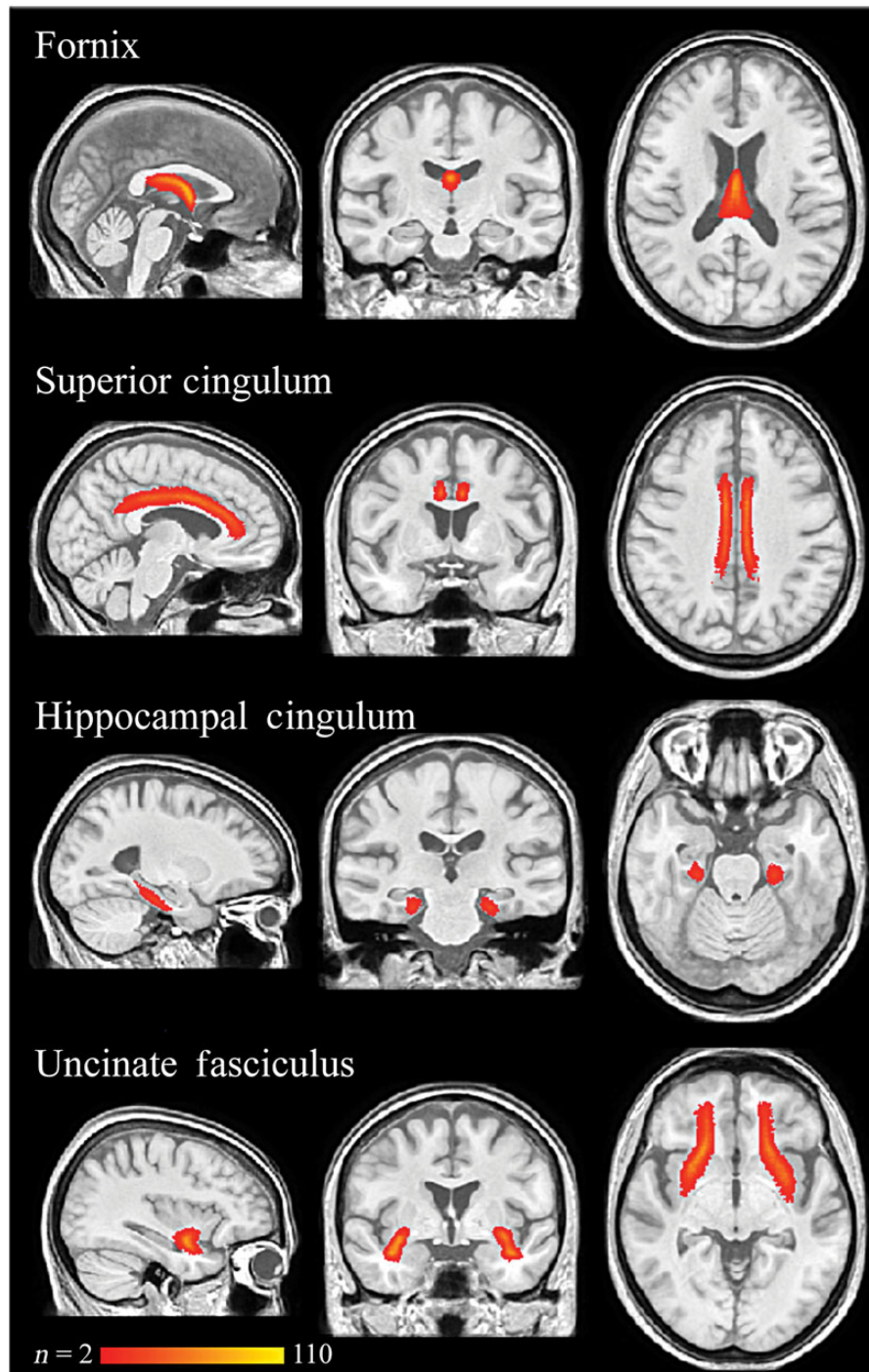

Figure 1. Heat maps show the 4 limbic tracts of interest: fornix, bilateral superior cingulum, bilateral hippocampal cingulum, and bilateral uncinate fasciculus. Yellow values indicate that a given voxel was part of the tract in more participants.

masks were traced in cingulate gyrus white matter adjacent to the genu (6 axial × 20 sagittal × 20 coronal voxels), body (20 axial × 20 sagittal × 6 coronal voxels), and splenium (6 axial × 20 sagittal × 20 coronal voxels) of the corpus callosum, respectively. For hippocampal cingulum tracts, seed masks were the target masks from the superior cingulum tracts (i.e., in cingulate gyrus white matter adjacent to the splenium of the corpus callosum), with waypoint and target masks (20 axial × 20 sagittal × 6 coronal voxels) centered around white matter at the head and tail of the hippocampus, respectively. For uncinate fasciculus tracts, seed, waypoint, and target masks were traced in temporal lobe white matter adjacent to the amygdala (6 axial × 20 sagittal × 20 coronal voxels), inferior frontal white matter below the putamen (20 axial × 20 sagittal × 6 coronal voxels), and the frontal pole (6 coronal slices), respectively. For all tracts, a white matter mask limited tracking to voxels with FA > 0.15.

Resulting tracts were thresholded at 20% of the maximum connectivity value, leaving only those voxels with a high likelihood of being connected to the seed and target regions. Mean diffusion (FA, MD, AD, RD, and MO) values were calculated for all 7 tracts for each participant by binarizing the thresholded tracts and multiplying them by the individual's diffusion maps. Due to incomplete DTI data (corrupt MPRAGE), one participant was excluded from these analyses.

Results

Age-Related Differences in Behavioral Pattern Separation

To assess the effect of aging on behavioral pattern separation, separate simple regression analyses were conducted between age and each of the behavioral mnemonic measures, using a Bonferroni correction for multiple comparisons ($P < 0.0125$ across 4 tasks). These data are presented in Figure 2. BPS-O and RAVLT performance are presented as a function of age in Tables 1 and 2, respectively. As expected (Toner et al. 2009; Yassa, Mattfeld, et al. 2011; Stark et al. 2013), results revealed that increasing age was associated with significantly impaired

BPS ($B = -0.006$, $t_{(108)} = -6.58$, $P < 0.001$), RAVLT Immediate ($B = -0.039$, $t_{(108)} = -3.18$, $P < 0.01$), and RAVLT Delay ($B = -0.042$, $t_{(108)} = -3.33$, $P < 0.01$), but there was no effect of age on Recognition ($P > 0.19$).

While both BPS and the RAVLT measures were sensitive to age-related declines relative to Recognition, the BPS score accounted for approximately three times the amount of age-related variance in behavioral pattern separation performance (i.e., 28.6 vs. 8.6%). A comparison of their (standardized) regression coefficients (Meng et al. 1992) further revealed that the relationship between age and BPS was significantly greater than the effect of age on RAVLT Immediate ($z = -3.10$, $P < 0.01$), RAVLT Delay ($z = -3.09$, $P < 0.01$), and Recognition ($z = -4.31$, $P < 0.001$).

Skeleton-Wise Analyses

Age-Related Differences in White Matter Integrity

To assess the effect of aging on whole-brain white matter integrity, skeleton-wise correlations were conducted between age and each diffusion index (FA, MD, AD, RD, and MO). These data are presented in Figure 3. Consistent with previous reports (see Madden et al. 2009 , 2012; Bennett and Madden 2014 for reviews), results revealed that older adults had significantly lower FA than younger adults in white matter throughout the brain. These age-related decreases in FA were primarily accompanied by age-related increases in MD and RD. Only a few white matter regions, such as the fornix and genu of the corpus callosum, also showed age-related increases in AD.

We also observed, for the first time, significant age-related differences in MO (see Fig. 3, bottom). Increases in MO with age were found in bilateral superior corona radiata and anterior thalamic radiations. In contrast, age-related decreases in MO were primarily seen in white matter regions that also
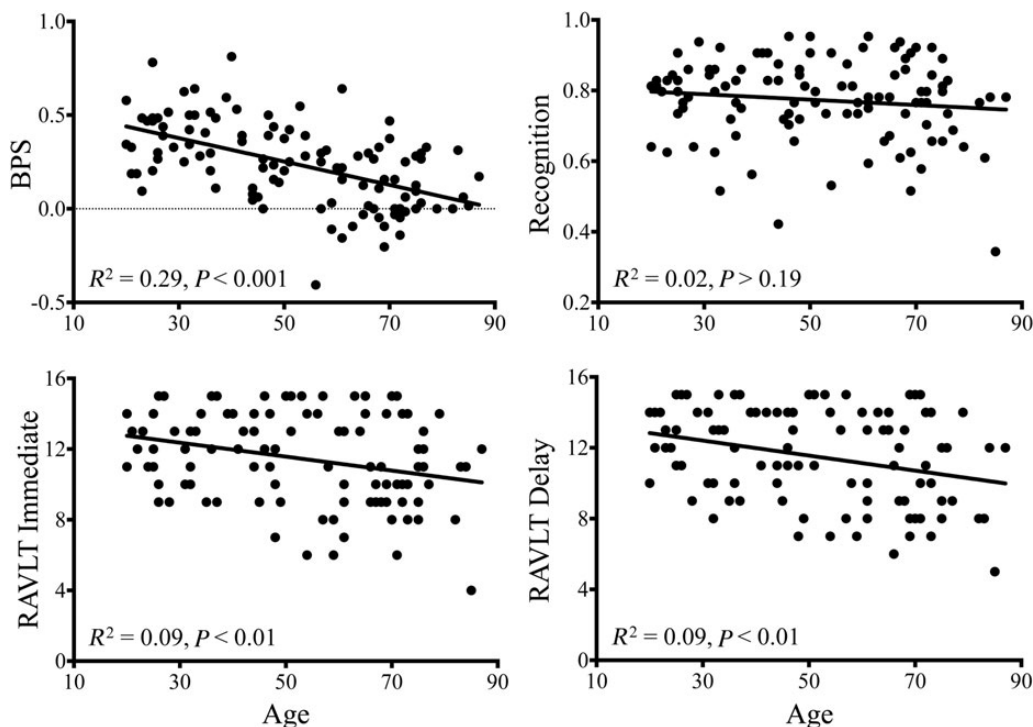

Figure 2. Scatterplots show relationships between age and performance on the BPS-O and RAVLT tasks. Results revealed that increased age was significantly associated with decreased BPS, RAVLT Immediate, and RAVLT Delay ($P < 0.0125$, Bonferroni corrected for 4 comparisons). However, there was no significant effect of age on Recognition.

Age group	BPS	Recognition	Targets			Lures			Foils		
			"Old"	"Similar"	"New"	"Old"	"Similar"	"New"	"Old"	"Similar"	"New"
20–39	0.41 ± 0.16	0.78 ± 0.10	0.82 ± 0.11	0.14 ± 0.10	0.04 ± 0.04	0.40 ± 0.16	0.52 ± 0.17	0.08 ± 0.08	0.03 ± 0.03	0.11 ± 0.07	0.86 ± 0.08
40–59	0.26 ± 0.23	0.82 ± 0.10	0.84 ± 0.09	0.11 ± 0.07	0.04 ± 0.05	0.49 ± 0.16	0.42 ± 0.18	0.09 ± 0.06	0.03 ± 0.04	0.17 ± 0.11	0.80 ± 0.13
60–74	0.12 ± 0.21	0.82 ± 0.10	0.87 ± 0.10	0.08 ± 0.08	0.05 ± 0.05	0.62 ± 0.18	0.25 ± 0.17	0.13 ± 0.09	0.05 ± 0.05	0.13 ± 0.11	0.82 ± 0.13
75–89	0.16 ± 0.13	0.76 ± 0.12	0.84 ± 0.08	0.09 ± 0.06	0.07 ± 0.04	0.62 ± 0.17	0.26 ± 0.16	0.12 ± 0.07	0.08 ± 0.10	0.11 ± 0.10	0.81 ± 0.12

Notes: In addition to BPS and Recognition scores, the proportion (mean ± SD) of each response type ("old," "similar," and "new") is presented for each trial type (target, lure, and foil) separately for 4 age groups (20–39, 40–59, 60–74, and 75–89 years) within our lifespan sample. These data reveal an age-related decline in BPS, but not recognition. Increasing age is also associated with an increase in incorrect "old" responses and a decrease in correct "similar" responses to lure items.

exhibited an age-related decrease in FA, such as the fornix, indicating a change in both the shape and degree of anisotropic diffusion as a function of age. Whereas MO has been assessed in individuals diagnosed with mild cognitive impairment and Alzheimer's disease (Douaud et al. 2011), to our knowledge this is the first examination of MO in healthy adults.

White Matter Integrity Correlates of Behavioral Pattern Separation

Relationships between white matter integrity and behavioral pattern separation were first assessed using a skeleton-wise correlation between FA and BPS. Results revealed that better performance was significantly associated with higher FA in the fornix (body, commissure, and crus), frontal white matter (anterior pericallosal and forceps minor), and superior white matter (superior corona radiata and anterior superior longitudinal fasciculus). However, given the widespread effects of aging on FA discussed in the previous section, we were further interested in separating global effects of aging on white matter integrity from specific relationships between white matter integrity and mnemonic performance. Thus, we reassessed the relationship between FA and BPS after controlling for mean FA (calculated as average FA within the white matter skeleton). Results revealed that only the relationship between fornix FA and BPS remained significant, indicating that integrity of the fornix contributes to pattern separation above and beyond the effect of age. These data are presented in Figure 4. Separate skeleton-wise correlations between BPS and each non-FA diffusion index (MD, AD, and RD) further supported this observation, revealing that increased fornix integrity (i.e., lower diffusivity) was associated with better BPS, after controlling for the corresponding mean diffusion index.

Skeleton-wise correlations between each diffusion index and each RAVLT measure revealed a similar pattern of results. After controlling for the corresponding mean diffusion index, better RAVLT Immediate performance was associated with higher FA in the fornix and better performance on both RAVLT measures was associated with lower diffusivity (MD, AD, and RD) in the fornix.

Aside from the fornix, higher BPS scores were associated with decreased integrity (i.e., increased MD, AD, and RD) in white matter adjacent to the right globus pallidus, and better RAVLT Immediate and RAVLT Delay performance were related to decreased FA in white matter adjacent to nucleus accumbens. There were no significant relationships between Recognition and any diffusion index, or between MO and any mnemonic measure.

Tractography Analyses

Age-Related Differences in Limbic Tract Integrity

To assess the specific effect of aging on limbic tract integrity, separate simple regression analyses were conducted between age and FA from each limbic tract. Reported effects for FA survived Bonferroni correction for multiple comparisons ($P < 0.007$ across 7 tracts). Results revealed that older adults had significantly lower FA than younger adults in the fornix ($B = -1.90 \times 10^{-3}$, $t_{(107)} = -9.76$, $P < 0.001$), left ($B = -0.80 \times 10^{-3}$, $t_{(106)} = -3.58$, $P < 0.005$) and right ($B = -0.64 \times 10^{-3}$, $t_{(107)} = -2.98$, $P < 0.004$) superior cingulum, and left ($B = -0.45 \times 10^{-3}$, $t_{(106)} = -3.60$, $P < 0.005$) and right ($B = -0.44 \times 10^{-3}$, $t_{(106)} = -2.76$, $P < 0.007$) uncinate fasciculi, but not the hippocampal cingulum tracts, $P > 0.69$. Finding that fornix FA accounted for approximately six times the amount of age-related variance relative to other limbic tracts is consistent with previous DTI studies that observed age-related declines in integrity of these limbic tracts that were especially prominent for the fornix (Stadlbauer et al. 2008; Michielse et al. 2010; Jang et al. 2011).

Follow-up analyses conducted between age and non-FA diffusion indices (MD, AD, RD, and MO) for each tract revealed a pattern of results that is consistent with the skeleton-wise analyses. That is, for each tract that showed an age-related decrease in FA, there was a corresponding increase in MD ($B > 0.75 \times 10^{-6}$, $P < 0.001$) and RD ($B > 0.86 \times 10^{-6}$, $P < 0.001$). There was also an age-related increase in AD in the fornix tract ($B = 6.92 \times 10^{-6}$, $t_{(107)} = 6.66$, $P < 0.001$) and a decrease in MO in the right superior cingulum tract ($B = -0.002$, $t_{(107)} = -4.22$, $P < 0.001$).

Limbic Tract Integrity Correlates of Behavioral Pattern Separation

A more targeted assessment of relationships between limbic tract integrity and behavioral pattern separation was conducted using separate multiple regression analyses. For each tract, separate models tested whether each diffusion index (FA, MD, AD, RD, and MO) predicts each behavioral mnemonic measure (BPS, Recognition, RAVLT Immediate, and RAVLT Delay). As with the skeleton-wise correlations, these analyses controlled for mean diffusion indices (calculated separately for each measure as the average diffusion index within each individual's white matter mask). Reported effects survived Bonferroni correction for multiple comparisons ($P < 0.0025$ across 5 integrity and 4 mnemonic measures).

Overall, results further strengthened the pattern observed in the skeleton-wise analyses, with increased fornix tract integrity relating to better behavioral pattern separation. Increased
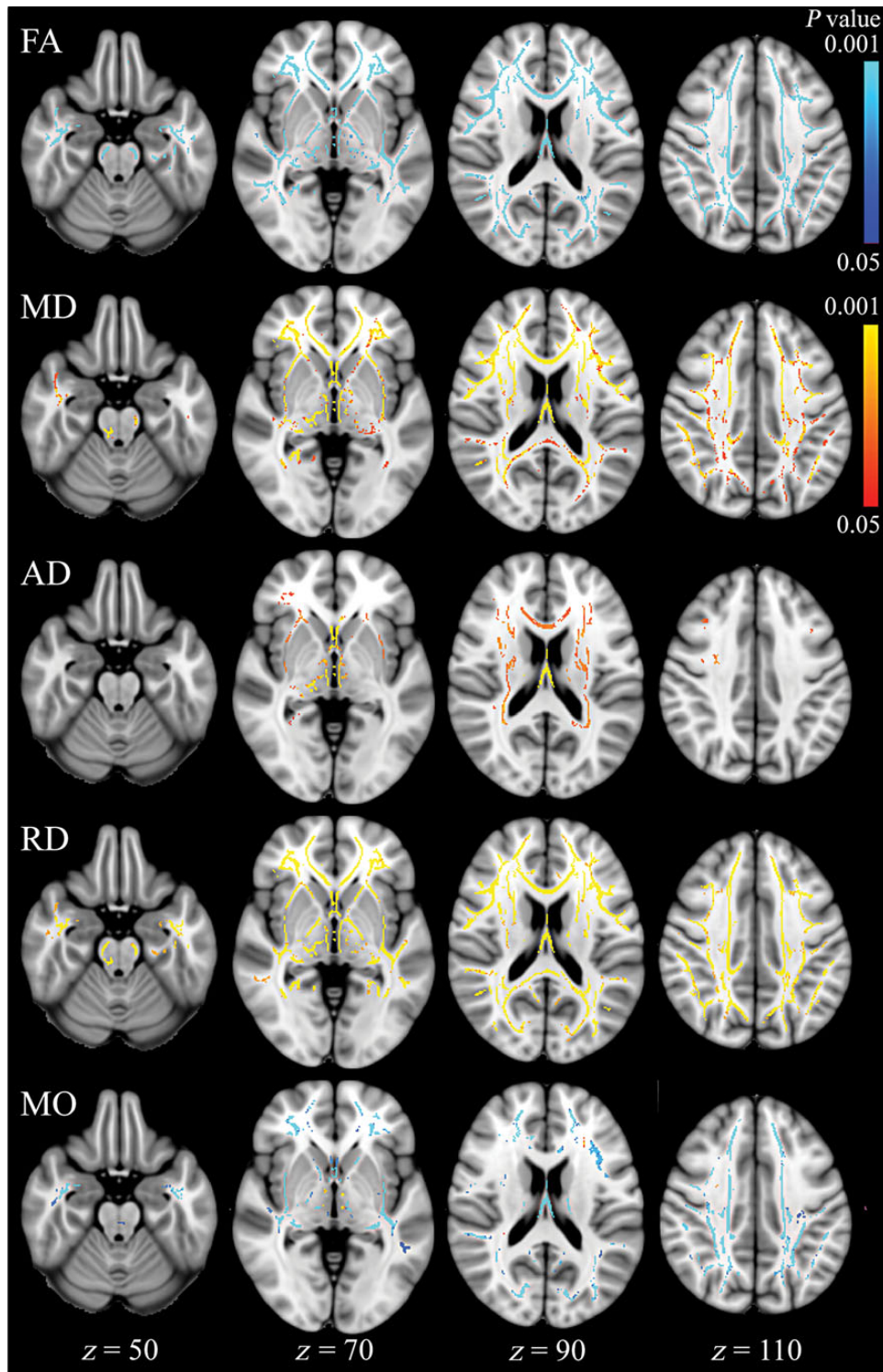
Figure 3. Statistical maps show white matter clusters in which 5 complementary measures of white matter integrity significantly decreased (blue-light blue) or increased (red-yellow) with age (TFCE, $P < 0.05$). Skeleton-wise correlations revealed that older adults had significantly decreased FA, and increased MD, AD, and RD compared with younger adults. MO, on the other hand, revealed both age-related increases and decreases. The lack of age effects in occipital and cerebellar cortices is due to cropped field of view. Axial slices are presented in radiological orientation (right = left).

fornix tract FA was significantly related to better BPS ($B = 1.89$, $t_{(106)} = 4.35$, $P < 0.001$), with marginally significant relationships for fornix tract MD ($B = -290.56$, $t_{(106)} = -2.72$, $P < 0.008$) and RD ($B = -331.28$, $t_{(106)} = -2.96$, $P < 0.004$). Marginally significant positive relationships were also observed between fornix tract FA and both RAVLT Immediate ($B = 15.05$, $t_{(106)} = 2.86$, $P < 0.006$) and RAVLT Delay ($B = 15.25$, $t_{(106)} = 2.80$, $P < 0.007$). However, although fornix tract FA accounted for more than twice the amount of variance in BPS versus RAVLT performance (i.e., 15.9 vs. 6.9%), a comparison
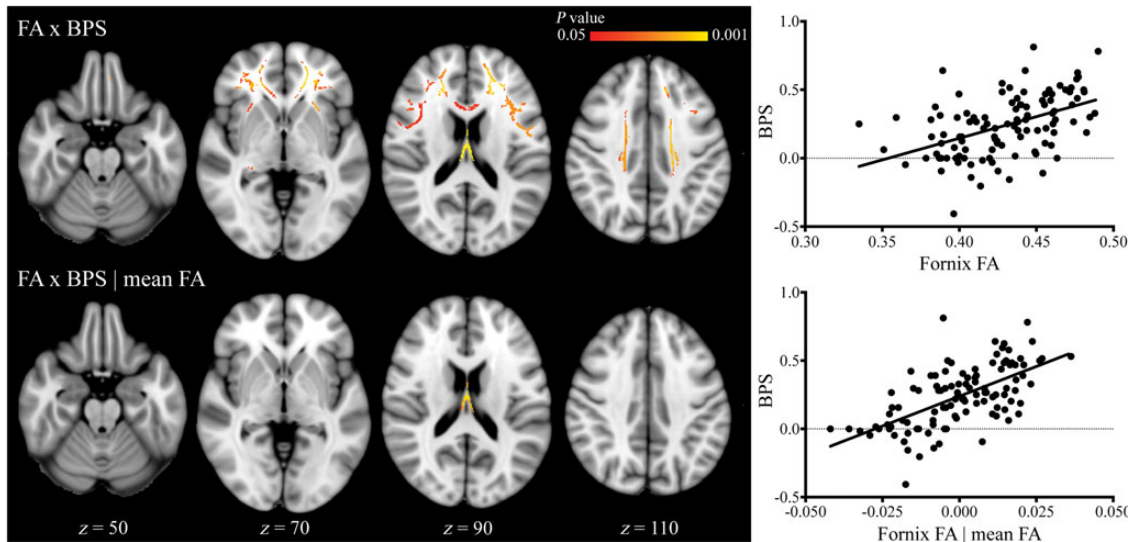
Figure 4. Statistical maps show white matter clusters that had a significant positive relationship (red-yellow) between FA and BPS before (top) and after (bottom) controlling for the effect of aging on FA (denoted by "| mean FA"; TFCE, $P < 0.05$). Skeleton-wise correlations revealed that, after controlling for mean FA, increased fornix FA remained significantly related to better BPS performance. Scatterplots show the relationships between fornix tract FA and BPS before (top) and after (bottom) controlling for mean FA for significant voxels common to both skeleton-wise analyses. Axial slices are presented in radiological orientation (right = left).
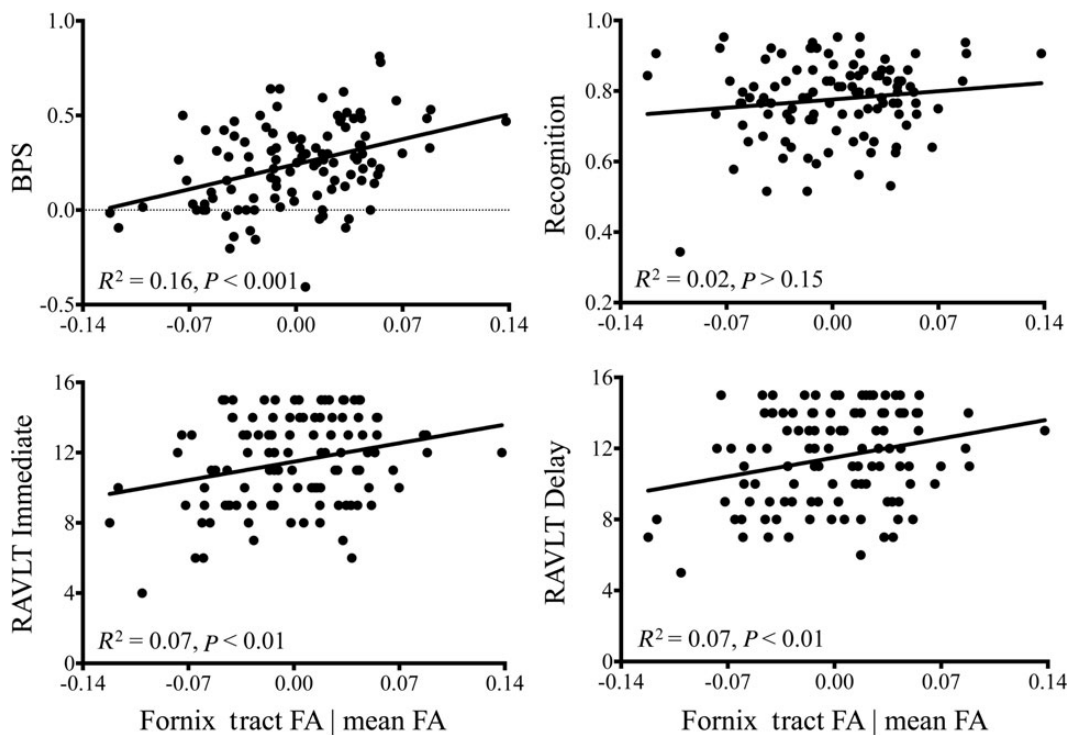

Figure 5. Scatterplots show relationships between fornix tract FA and performance on the BPS-O and RAVLT tasks, after controlling for mean FA (denoted by "| mean FA"; i.e., fornix tract FA values represent residuals from the relationship with mean FA). Results revealed that increased fornix tract FA was significantly associated with better BPS, and marginally associated with better RAVLT Immediate and RAVLT Delay performance ($P < 0.0025$, Bonferroni corrected for 20 comparisons). However, there was no significant effect of fornix tract FA on Recognition.

of their (standardized) regression coefficients (Meng et al. 1992) revealed that the effect of fornix tract FA on BPS was not significantly different than that of fornix tract FA on RAVLT Immediate, $z = 1.39$, $P > 0.16$, or RAVLT Delay, $z = 1.41$, $P > 0.15$, performance. However, the effect of fornix tract FA on BPS was significantly different than that of fornix tract FA on Recognition, $z = 1.93$, $P < 0.05$. No other relationship between limbic

tract diffusion indices and the behavioral mnemonic measures attained significance. The data for fornix tract FA are presented in Figure 5.

Finally, given that we observed significant effects of age on fornix tract FA and BPS, we assessed whether fornix tract FA mediated the effect of age on BPS (see Baron and Kenny 1986). When age was the sole predictor of BPS, 28.6% of the

variance in performance was age-related. When entering fornix FA into the model before age, only age remained a significant predictor, accounting for 29.0% of the variance. Thus, fornix tract FA did not significantly mediate the effect of age on BPS. The mediation analysis further revealed that fornix tract FA did not exhibit an age-independent relation to BPS. This is potentially due to chronological age being such a strong predictor variable, accounting for large portions of the variance in both white matter integrity and cognitive performance. In this way, chronological age can overshadow the contribution of neurobiological substrates, such as white matter tract integrity, to cognitive performance. Importantly, when controlling for the specific effect of age on white matter integrity (i.e., mean FA) in both the skeleton-wise and tractography analyses, we observed a significant, albeit smaller, contribution of fornix tract FA to behavioral pattern separation, with fornix tract FA accounting for 16.0% of the variance in behavioral pattern separation across our lifespan sample. The relationship between fornix tract FA and BPS showed a similar trend in both younger (20–59 years; $R^2 = 0.04$, $P < 0.11$) and older (60–89 years; $R^2 = 0.07$, $P < 0.09$) adults.

Discussion

Whereas our earlier work demonstrated that behavioral pattern separation was associated with integrity of an intrinsic hippocampal connection (i.e., the perforant path; Yassa, Muftuler, et al. 2010; Yassa, Stark, et al. 2010), the current study tested the hypothesis that pattern separation also relies on neural networks that extend beyond the hippocampus. Integrity of limbic tracts that connect the hippocampus to distributed brain regions (fornix, cingulum bundle, and uncinate fasciculus) were examined in relation to pattern separation performance (measured directly using BPS-O and indirectly using RAVLT tasks) in a lifespan sample of healthy adults (20–89 years). Our primary result revealed that increased integrity of the fornix (i.e., increased FA; decreased MD, AD, and RD) was significantly related to better BPS scores. These fornix integrity-pattern separation relationships were observed using both standard-space, skeleton-wise analyses and subject-space, tractography analyses, indicating that the effect of fornix integrity on pattern separation performance cannot be attributed to potential alignment issues across participants. The potential influence of partial volume effects was also mitigated by thresholding procedures in both the skeleton-wise and tractography analyses that resulted in mean fornix FA values within the expected range for normal appearing white matter (FA >0.30).

Similar relationships between fornix integrity and behavioral pattern separation were also observed when using the RAVLT task; a widely used mnemonic task with features thought to rely on pattern separation (e.g., recall and interference; Norman and O'Reilly 2003; Norman 2010). That is, as with the BPS measure, results revealed that increased fornix integrity (i.e., increased FA and/or decreased MD, AD, and RD) was related to better RAVLT Immediate and RAVLT Delay performance. However, whereas these fornix integrity-pattern separation relationships were significant in the skeleton-wise analyses, they did not attain significance in the tractography analyses. Thus, to the extent that fornix integrity reflects a neural substrate of pattern separation, the BPS score, which was significantly related to fornix integrity in both analyses, may be a more sensitive index of behavioral pattern separation

than RAVLT performance. Because the RAVLT Immediate and RAVLT Delay measures were highly correlated ($r = 0.90$, $P < 0.0001$), we were not able to detect potential differences in these measures as a function of the degree to which they place different demands on pattern separation. Future research will be necessary to replicate and extend these examinations of fornix integrity in relation to tasks that differ in their demands on pattern separation, after controlling for task difficulty. Disambiguating task difficulty and behavioral pattern separation is inherently complicated because the degree of demands on pattern separation is likely a substantial contributor to the overall difficulty of a task. Thus, we believe that our measures of BPS, RAVLT Immediate, and RAVLT Delay are a good first step toward this goal.

Taken together, finding relationships between fornix integrity and the BPS and RAVLT measures support the notion that behavioral pattern separation is mediated by broader neural networks that extend beyond the hippocampus. Whereas the fornix is predominantly thought of as a hippocampal efferent, projecting either to the hypothalamus and thalamus via the mammillary bodies or to septal nuclei and medial and inferior prefrontal regions, it has also been found to contain afferent fibers that terminate in the hippocampal formation (Swanson 1977; Mark et al. 1995; Schmahmann and Pandya 2009). Thus, it is possible that the fornix transmits hippocampal outputs to these subcortical and cortical regions for further processing in relation to the task demands (e.g., recollection and response selection) and/or that outputs from these regions inform the pattern separation computations mediated by the hippocampal DG/CA3. DTI data unfortunately lack the directional information necessary to adjudicate between these possibilities. Nonetheless, our findings suggest that behavioral pattern separation is mediated by a distributed network of brain regions that connect to the hippocampus via the fornix, supporting earlier work that has emphasized the role of broad neural networks in mnemonic processes that include, but are not limited to, the hippocampus (e.g., Zola-Morgan and Squire 1993; Gaffan 2002).

As previously noted, the current results complement our earlier DTI work in which we showed that increased integrity of the perforant path was significantly associated with better BPS (Yassa, Mattfeld, et al. 2011) and RAVLT Delay (Yassa, Muftuler, et al. 2010) in healthy older adults. In contrast to the fornix and perforant path, however, integrity of the cingulum bundle and uncinate fasciculus tracts did not relate to behavioral pattern separation in this study. Whereas the fornix and perforant path consist of white matter fibers that directly project from hippocampal subfields (Witter 2007; Schmahmann and Pandya 2009), fibers of the cingulum bundle and uncinate fasciculus connect to the hippocampus indirectly via entorhinal cortex and parahippocampal regions (Sørensen 1985; Schmahmann and Pandya 2009). Thus, the present result may indicate that pattern separation relies on the integrity of networks that directly (i.e., perforant path and fornix) versus indirectly (i.e., cingulum bundle and uncinate fasciculus) connect the hippocampus to distributed cortical and subcortical regions.

In contrast to the results for behavioral pattern separation (measured using BPS-O and RAVLT tasks), Recognition did not relate to integrity from any white matter region. This finding is inconsistent with previous DTI studies that have shown significant relationships between fornix integrity and performance on recognition memory tasks (Rudebeck et al. 2009; Sasson

et al. 2013). However, relative to the measure of recognition used here (i.e., hits minus false alarms, using dissimilar novel foils in a speeded task), both prior studies employed recognition memory tasks that may have placed demands on behavioral pattern separation (e.g., emphasizing recollection vs. familiarity and involving associative learning with related conjunctions; Norman and O'Reilly 2003; Norman 2010). Specifically, Rudebeck et al. found that fornix integrity was preferentially related to their hippocampal-dependent recognition-based measure of recollection, but not familiarity. Sasson et al., on the other hand, used a factor score combining immediate and delayed verbal and nonverbal recognition of paired associate memory. Thus, to the extent that behavioral pattern separation was tapped by their more complex and demanding recognition memory measures, the current results suggest that fornix integrity is sensitive to behavioral pattern separation, but not to simple components of recognition memory (e.g., item-only familiarity). Furthermore, because our results for RAVLT performance replicated previously observed relationships between fornix integrity and recall memory (Metzler-Baddeley et al. 2011), we propose that pattern separation is a component process that accounts, at least in part, for previously observed relationships between fornix tract integrity and recall and recognition performance.

In addition to the novel finding that fornix integrity relates to behavioral pattern separation in our lifespan sample (as seen in the skeleton-wise and tractography analyses), the current study replicated and extended well-documented effects of cognitive and brain aging. For example, consistent with previous behavioral reports (Toner et al. 2009; Yassa, Mattfeld, et al. 2011; Stark et al. 2013), results revealed that increasing age was associated with significantly impaired pattern separation measured using BPS, RAVLT Immediate, and RAVLT Delay; but there was no effect of age on Recognition. The BPS score was found to account for approximately three times as much age-related variability in behavioral pattern separation than either RAVLT measure, further supporting the notion that it may be a more sensitive index of pattern separation performance.

Consistent with previous DTI studies (see Madden et al. 2009, 2012 for review), the present results also revealed that older adults had significantly lower white matter integrity than younger adults. Our skeleton-wise and tractography analyses tested for linear relationships between age and each diffusivity index, which fit our data better than nonlinear models. We observed age-related declines in FA throughout the brain that were primarily accompanied by age-related increases in MD and RD. This pattern of results is in line with the notion that healthy aging has widespread effects on white matter microstructure, including mild demyelination or axonal shrinkage (Peters 2002; Gunning-Dixon et al. 2009). However, a few white matter regions, including the fornix and genu of the corpus callosum, also showed age-related increases in AD and/or decreases in MO. Thus, select regions may be more susceptible to age-related declines in white matter microstructure, possibly resulting from severe demyelination and axonal degeneration, that lead to increased AD (e.g., genu of the corpus callosum) and ultimately decreased MO (e.g., fornix; Bennett et al. 2010).

To ensure that relationships between limbic tract integrity and behavioral pattern separation were not attributed to the previously described age effects, our analyses controlled for the effect of aging on white matter integrity. Importantly, the current results indicate that fornix integrity contributes to behavioral pattern separation above and beyond the global effect of age on white matter. Whereas initial skeleton-wise analyses showed that better BPS was related to increased FA in the fornix and in clusters of frontal and superior white matter, only fornix FA remained significantly positively related to BPS after controlling for mean FA. Within the DTI aging literature, a central question is whether age-related cognitive declines are due to localized effects of aging within specific white matter tracts thought to mediate the cognitive processes of interest or more global age-related changes shared across all white matter (Penke et al. 2010; Lövdén et al. 2012). By controlling for the effect of aging on white matter integrity, relationships between fornix tract integrity and behavioral pattern separation reported here can be attributed to local differences in white matter integrity across individuals rather than global effects of aging on integrity. Additional support for this conclusion comes from the finding that MO exhibited significant changes with aging, but MO did not relate to any behavioral measure as would be expected if the fornix integrity-pattern separation relationships were attributed to global effects of aging.

Finally, mediation analyses in the current study revealed that age-related declines in pattern separation performance were not significantly mediated by fornix tract integrity. One interpretation of the mediation results is that fornix tract FA and BPS are only related to each other because of their relationship to chronological age. However, as discussed in the Results section, we believe that this finding is due to the fact that chronological age accounts for such a large portion of the variance in both fornix tract FA and behavioral pattern separation that it overshadows the smaller, yet significant, relationship between these measures. The present data are not consistent with predictions of cortical disconnection theories, which propose that age-related differences in white matter connectivity between brain regions disrupt coordinated processing within those regions, ultimately leading to decreased cognitive functioning in older adults (O'Sullivan et al. 2001; Bartzokis 2004). However, the positive fornix integrity-pattern separation relationships observed in both the skeleton-wise and tractography analyses after controlling for the specific effect of aging on white matter integrity are consistent with the notion that individual differences in hippocampal connectivity mediate this component mnemonic process in healthy adults across the lifespan.

Funding

Notes

References

Aggleton JP, Brown MW. 1999. Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav Brain Sci. 22:425–489.
Avants B, Tustison N, Song G. 2009. Advanced Normalization Tools, ANTS 1.0. Sourceforge. http://sourceforge.net/projects/advants/.

Bakker A, Kirwan CB, Miller MI, Stark CEL. 2008. Pattern separation in the human hippocampal CA3 and dentate gyrus. Science. 319:1640–1642.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M. 2012. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron. 74:467–474.

Baron RM, Kenny DA. 1986. The moderator–mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol. 51:1173.

Bartzokis G. 2004. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiol Aging. 25:5–18.

Beaulieu C. 2002. The basis of anisotropic water diffusion in the nervous system—a technical review. NMR Biomed. 15:435–455.

Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, Matthews PM, Brady JM, Smith SM. 2003. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. Magn Reson Med. 50:1077–1088.

Bennett IJ, Madden DJ. 2014. Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience. 276:187–205.

Bennett IJ, Madden DJ, Vaidya CJ, Howard DV, Howard JHJ. 2010. Age-related differences in multiple measures of white matter integrity: a diffusion tensor imaging study of healthy aging. Hum Brain Mapp. 31:378–390.

Buckley MJ, Wilson CRE, Gaffan D. 2008. Fornix transection impairs visuospatial memory acquisition more than retrieval. Behav Neurosci. 122:44.

Concha L, Gross DW, Beaulieu C. 2005. Diffusion tensor tractography of the limbic system. Am J Neuroradiol. 26:2267–2274.

Cox RW. 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 29:162–173.

Douaud G, Jbabdi S, Behrens TEJ, Menke RA, Gass A, Monsch AU, Rao A, Whitcher B, Kindlmann G, Matthews PM. 2011. DTI measures in crossing-fibre areas: increased diffusion anisotropy reveals early white matter alteration in MCI and mild Alzheimer's disease. Neuroimage. 55:880–890.

Folstein M, Folstein SE, McHugh PR. 1975. Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. J Psychiatry Res. 12:189–198.

Gaffan D. 2002. Against memory systems. Philos Trans R Soc Lond B Biol Sci. 357:1111–1121.

Gaffan D. 1994. Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. J Cogn Neurosci. 6:305–320.

Gunning-Dixon FM, Brickman AM, Cheng JC, Alexopoulos GS. 2009. Aging of cerebral white matter: a review of MRI findings. Int J Geriatr Psychiatry. 24:109–117.

Jang SH, Cho S-H, Chang MC. 2011. Age-related degeneration of the fornix in the human brain: a diffusion tensor imaging study. Int J Neurosci. 121:94–100.

Kantarci K, Senjem ML, Avula R, Zhang B, Samikoglu AR, Weigand SD, Przybelski SA, Edmonson HA, Vemuri P, Knopman DS. 2011. Diffusion tensor imaging and cognitive function in older adults with no dementia. Neurology. 77:26–34.

Kirwan CB, Jones C, Miller MI, Stark CEL. 2007. High-resolution fMRI investigation of the medial temporal lobe. Hum Brain Mapp. 28:959–966.

Kirwan CB, Stark CEL. 2007. Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. Learn Mem. 14:625–633.

Lacy JW, Yassa MA, Stark SM, Muftuler LT, Stark CE. 2011. Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. Learn Mem. 18:15–18.

Le Bihan D. 2003. Looking into the functional architecture of the brain with diffusion MRI. Nat Rev Neurosci. 4:469–480.

Lockhart SN, Mayda AB, Roach AE, Fletcher E, Carmichael O, Maillard P, Schwarz CG, Yonelinas AP, Ranganath C, Decarli C. 2012. Episodic memory function is associated with multiple measures of white matter integrity in cognitive aging. Front Hum Neurosci. 6:56.

Lövdén M, Laukka EJ, Rieckmann A, Kalpouzos G, Li T, Jonsson T, Wahlund L, Fratiglioni L, Bäckman L. 2012. The dimensionality of between-person differences in white matter microstructure in old age. Hum Brain Mapp. 34:1386–1398.

Mabbott DJ, Rovet J, Noseworthy MD, Smith ML, Rockel C. 2009. The relations between white matter and declarative memory in older children and adolescents. Brain Res. 1294:80–90.

MacLean PD. 1949. Psychosomatic disease and the "visceral brain" recent developments bearing on the papez theory of emotion. Psychosom Med. 11:338–353.

Madden DJ, Bennett IJ, Burzynska A, Potter GG, Chen NK, Song AW. 2012. Diffusion tensor imaging of cerebral white matter integrity in cognitive aging. Biochim Biophys Acta. 1822:386–400.

Madden DJ, Bennett IJ, Song AW. 2009. Cerebral white matter integrity and cognitive aging: contributions from diffusion tensor imaging. Neuropsychol Rev. 19:415–435.

Mark LP, Daniels DL, Naidich TP, Hendrix LE. 1995. Limbic connections. Am J Neuroradiol. 16:1303–1306.

McClelland JL, McNaughton BL, O'Reilly RC. 1995. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol Rev. 102:419–457.

Mega MS, Cummings JL, Salloway S, Malloy P. 1997. The limbic system: an anatomic, phylogenetic, and clinical perspective. J Neuropsychiatry Clin Neurosci. 9:315.

Meng X-L, Rosenthal R, Rubin DB. 1992. Comparing correlated correlation coefficients. Psychol Bull. 111:172.

Metzler-Baddeley C, Jones DK, Belaroussi B, Aggleton JP, O'Sullivan MJ. 2011. Frontotemporal connections in episodic memory and aging: a diffusion MRI tractography study. J Neurosci. 31:13236–13245.

Michielse S, Coupland N, Camicioli R, Carter R, Seres P, Sabino J, Malykhin N. 2010. Selective effects of aging on brain white matter microstructure: a diffusion tensor imaging tractography study. Neuroimage. 52:1190–1201.

Moudgil SS, Azzouz M, Al-Azzaz A, Haut M, Gutmann L. 2000. Amnesia due to fornix infarction. Stroke. 31:1418–1419.

Norman KA. 2010. How hippocampus and cortex contribute to recognition memory: revisiting the complementary learning systems model. Hippocampus. 20:1217–1227.

Norman KA, O'Reilly RC. 2003. Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. Psychol Rev. 110:611–646.

Olton DS, Walker JA, Wolf WA. 1982. A disconnection analysis of hippocampal function. Brain Res. 233:241–253.

O'Sullivan M, Jones DK, Summers PE, Morris RG, Williams SC, Markus HS. 2001. Evidence for cortical disconnection as a mechanism of age-related cognitive decline. Neurology. 57:632–638.

Papez JW. 1937. A proposed mechanism of emotion. Arch Neurol Psychiatry. 38:725.

Penke L, Maniega SM, Murray C, Gow AJ, Hernández MCV, Clayden JD, Starr JM, Wardlaw JM, Bastin ME, Deary IJ. 2010. A general factor of brain white matter integrity predicts information processing speed in healthy older people. J Neurosci. 30:7569–7574.

Peters A. 2002. The effects of normal aging on myelin and nerve fibers: a review. J Neurocytol. 31:581–593.

Rajmohan V, Mohandas E. 2007. The limbic system. Indian J Psychiatry. 49:132.

Reitan RM, Wolfson D. 1985. The Halstead-Reitan Neuropsychological Test Battery. Tuscon: Neuropsychology Press.

Rey A. 1941. L'examen psychologique dans les cas d'encephalopathie traumatique. Arch Psychol. 28:286–340.

Rudebeck SR, Scholz J, Millington R, Rohenkohl G, Johansen-Berg H, Lee AC. 2009. Fornix microstructure correlates with recollection but not familiarity memory. J Neurosci. 29:14987–14992.

Rudge P, Warrington EK. 1991. Selective impairment of memory and visual perception in splenial tumours. Brain. 114:349–360.

Sasson E, Doniger GM, Pasternak O, Assaf Y. 2010. Structural correlates of memory performance with diffusion tensor imaging. Neuroimage. 50:1231–1242.

Sasson E, Doniger GM, Pasternak O, Tarrasch R, Assaf Y. 2012. Structural correlates of cognitive domains in normal aging with diffusion tensor imaging. Brain Struct Funct. 217:503–515.

Sasson E, Doniger GM, Pasternak O, Tarrasch R, Assaf Y. 2013. White matter correlates of cognitive domains in normal aging with diffusion tensor imaging. Front Neuroscience. 7:1–13.

Sato T, Maruyama N, Hoshida T, Minato K. 2012. Correlation between uncinate fasciculus and memory tasks in healthy individual using diffusion tensor tractography. Conf Proc IEEE Eng Med Biol Soc. 424–427.

Schmahmann JD, Pandya DN. 2009. Fiber pathways of the brain. New York: Oxford University Press.

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM et al. 2006. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage. 31:1487–1505.

Smith SM, Nichols TE. 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage. 44:83–98.

Sørensen KE. 1985. The connections of the hippocampal region new observations on efferent connections in the guinea pig, and their functional implications. Acta Neurol Scand. 72:550–560.

Spreen O, Benton AL. 1977. Neurosensory center comprehensive examination for aphasia: manual of instructions. Vicoria, BC: Neuropsychology Laboratory, University of Victoria.

Squire LR, Stark CEL, Clark RE. 2004. The medial temporal lobe. Ann Rev Neurosci. 27:279–306.

Stadlbauer A, Salomonowitz E, Strunk G, Hammen T, Ganslandt O. 2008. Quantitative diffusion tensor fiber tracking of age-related changes in the limbic system. Eur Radiol. 18:130–137.

Stark SM, Yassa MA, Lacy JW, Stark CEL. 2013. A task to assess behavioral pattern separation (BPS) in humans: data from healthy aging and mild cognitive impairment. Neuropsychologia. 51:2442–2449.

Stark SM, Yassa MA, Stark CEL. 2010. Individual differences in spatial pattern separation performance associated with healthy aging in humans. Learn Mem. 17:284–288.

Swanson LW. 1977. The anatomical organization of septo-hippocampal projections. Ciba Found Symp. 58:25–48.

Toner CK, Pirogovsky E, Kirwan CB, Gilbert PE. 2009. Visual object pattern separation deficits in nondemented older adults. Learn Mem. 16:338–342.

Tucker DM, Roeltgen DP, Tully R, Hartmann J, Boxell C. 1988. Memory dysfunction following unilateral transection of the fornix: a hippocampal disconnection syndrome. Cortex. 24:465–472.

Tulving E. 2002. Episodic memory: from mind to brain. Annu Rev Psychol. 53:1–25.

Valenstein E, Bowers D, Verfaellie M, Heilman KM, Day A, Watson RT. 1987. Retrosplenial amnesia. Brain. 110:1631–1646.

Von Der Heide RJ, Skipper LM, Klobusicky E, Olson IR. 2013. Dissecting the uncinate fasciculus: disorders, controversies and a hypothesis. Brain. 136:1692–1707.

Wechsler D. 1997a. Wechsler Adult Intelligence Scale (WAIS-III): administration and scoring manual. San Antonio (TX): The Psychological Corporation.

Wechsler D. 1997b. Wechsler Memory Scale—Third Edition (WMS-III). TX (USA): The Psychological Corporation.

Witter MP. 2007. The perforant path: projections from the entorhinal cortex to the dentate gyrus. Prog Brain Res. 163:43–61.

Wyss JM, Swanson LW, Cowan WM. 1979. A study of subcortical afferents to the hippocampal formation in the rat. Neuroscience. 4:463–476.

Yassa MA, Lacy JW, Stark SM, Albert MS, Gallagher M, Stark CEL. 2011. Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. Hippocampus. 21:968–979.

Yassa MA, Mattfeld AT, Stark SM, Stark CEL. 2011. Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. Proc Natl Acad Sci USA. 108:8873–8878.

Yassa MA, Muftuler LT, Stark CEL. 2010. Ultrahigh-resolution microstructural diffusion tensor imaging (msDTI) reveals perforant path degradation in aged humans in vivo. Proc Natl Acad Sci USA. 107:12687–12691.

Yassa MA, Stark CEL. 2008. Multiple signals of recognition memory in the medial temporal lobe. Hippocampus. 18:945–954.

Yassa MA, Stark CEL. 2011. Pattern separation in the hippocampus. Trends Neurosci. 34:515–525.

Yassa MA, Stark SM, Bakker A, Albert MS, Gallagher M, Stark CEL. 2010. High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic mild cognitive impairment. Neuroimage. 51:1242–1252.

Zola-Morgan S, Squire LR. 1993. Neuroanatomy of memory. Ann Rev Neurosci. 16:547–563.