Free water-corrected fractional anisotropy of the fornix and parahippocampal cingulum predicts longitudinal memory change in cognitively healthy older adults

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Abbreviations: AD: Alzheimer's Disease; AIC: Akaike Information Criterion; allbut-cingulum: all tracts other than the cingulum; allbut-fornix: all tracts other than the fornix; aMCI: amnesic Mild Cognitive Impairment; ANTs: Advanced Normalization Tools; BET: Brain Extraction Tool; CVLT: California Verbal Learning Test; DTI: Diffusion Tensor Imaging; EPI: Echoplanar Imaging; FA: Fractional Anisotropy; FAS: Letter and Category Fluency Tests; FSL: FMRIB Software Library; IQ: Intelligence Quotient; JHU atlas: John Hopkins University ICBM-DTI-81 white matter atlas; MMSE: Mini-Mental State Examination; MNI: Montreal Neurosciences Institute; $M_{oth\text{-}cog}$: other cognitive domains; MRI: Magnetic Resonance Imaging; PCA: Principal Component Analysis; ROI: Region of Interest; SDMT: Symbol/Digit Coding Test; SyN: Symmetric Image Normalization; TR: Repetition Time; WAIS-R: Wechsler Adult Intelligence Scale Revised; WTAR: Wechsler Test of Adult Reading.

Abstract

Prior studies have reported inconsistent results regarding the relationships between the integrity of the fornix and parahippocampal cingulum and both memory performance and longitudinal change in performance. In the present study, we examined associations in a sample of cognitively healthy older adults between free water-corrected fractional anisotropy (FA) metrics derived from the fornix and cingulum, baseline memory performance, and 3-year memory change. Neither fornix nor cingulum FA correlated with memory performance at baseline. By contrast, FA of each tract was predictive of memory change, such that greater FA was associated with less longitudinal decline. These associations remained significant after controlling for FA of other white matter tracts and for performance in other cognitive domains. Furthermore, fornix and cingulum FA explained unique variance in memory change. These results suggest that free water-corrected measures of fornix and parahippocampal cingulum integrity are reliable predictors of future memory change in cognitively healthy older adults. The findings for the fornix in particular highlight the utility of correcting for free water when estimating diffusion tensor imaging metrics of white matter integrity.

Keywords: cognitive performance, diffusion tensor imaging, aging, hippocampus, cognitive change, structural connectivity

1. Introduction

Compared to young adults, older adults consistently demonstrate reduced episodic memory performance, that is, poorer memory for unique events (Tulving, 1983). As we discuss below, age-related episodic memory decline has been linked to age differences in the integrity of the brain's white matter (i.e., structural connectivity), differences that likely impact the efficiency of communication between different neural regions.

Contemporary magnetic resonance imaging (MRI) studies of white matter microstructure typically employ diffusion tensor imaging (DTI) methods. These methods allow non-invasive investigation of inter-regional structural connectivity (for reviews, see Sporns, 2013; Yeh et al., 2020), providing estimates of microstructural integrity by measuring water diffusivity (Assaf and Pasternak, 2008). The most commonly employed DTI metric - fractional anisotropy (FA) – is a measure of the relative rate of water diffusion along as opposed to across the longitudinal axis of an axon. Relatively higher FA is widely assumed to indicate relatively greater white matter integrity (Madden et al., 2009).

Although widely employed, conventional DTI metrics are susceptible to contamination by partial volume effects caused by the juxtaposition of extracellular 'free water' and white matter. DTI metrics are more likely to be biased for white matter tracts adjacent to ventricles, and such effects are likely to be exaggerated in older adults because of age-related white matter atrophy and a corresponding partial volume effect (Alexander et al., 2007; Giorgio et al., 2010; Vos et al., 2011). Of importance, partial volume effects can lead to an underestimation of FA (Metzler-Baddeley et al., 2012; Pfefferbaum et al., 2003) and an overestimation of the effects of age on white matter integrity (Chad et al., 2018). It is, however, possible to correct for the contribution of extracellular free water to DTI metrics (Pasternak et al., 2009). Studies employing this correction procedure have reported that, compared with uncorrected FA estimates, the procedure increases estimates of FA both in individual tracts and across the whole brain (Albi et al., 2017; Bergamino et al., 2017; Edde et al., 2020). Moreover, Chad et al. (2018) reported that the relationship between age and whole brain mean FA was substantially reduced after free water correction (r = - 0.51 vs. r = - 0.25 before and after correction respectively) in a group of 212 participants aged between 39 and 92 years old. In light of the evidence reviewed above, we applied a free water correction procedure to the DTI data acquired in the current study.

Here, we focus on the fornix and parahippocampal cingulum, two limbic tracts that connect the hippocampus - long known to play a crucial role in episodic memory (Eichenbaum, 2017) - with a diverse range of other brain regions (Bubb et al., 2018; Mark et al. 1995; Schmahmann and Pandya 2009). The fornix is the major tract connecting the hippocampus with a number of sub-cortical structures, including the septum and, via the mammillary bodies, the anterior thalamus (Amaral and Lavenex, 2007; Senova et al., 2020). By contrast, the parahippocampal cingulum (hereafter, cingulum), which forms part of the cingulum bundle, has widespread connections with frontal, parietal, occipital and temporal cortical regions (Jones et al., 2013; Maldonado et al., 2020). Damage to either tract has been reported to lead to memory impairment, albeit less consistently in the case of the cingulum (for reviews, see Bubb et al., 2018; Douet and Change, 2015; Benear et al., 2020).

The great majority of studies investigating associations between memory performance and the integrity of the fornix and cingulum have employed conventional (i.e. uncorrected for free water) DTI metrics. In middle-aged and older adults, higher fornix FA has been reported to predict better performance on tests of face recognition (Ly et al., 2016), verbal recall (Hartopp et al., 2019; Metzler-Baddeley et al., 2011), visual recognition (Hartopp et al., 2019), visual recall (Metzler-Baddeley et al., 2011) and autobiographical memory (Memel et al., 2020). Higher fornix FA has also been linked to lower rates of false recollection in older adults (Chamberlain et al., 2021). Several studies have examined the relationship between fornix integrity and memory across the adult lifespan (Alm et al., 2020; Bennett et al., 2015; Bennett and Stark, 2016; Sasson et al., 2013; Henson et al., 2016). In four of these studies, no relationship between fornix integrity and memory performance was evident after controlling for age (Ly et al., 2016; Metzler-Baddeley et al., 2011; Bennett et al., 2015; Henson et al., 2016). By contrast, a positive correlation between fornix FA and measures of autobiographical memory and visual recognition was reported by Memel et al. (2020) and Hartopp et al. (2019) respectively.

Findings regarding the relationship between cingulum integrity and memory performance are also inconsistent (for review, see Bubb et al., 2018). Whereas two studies reported a positive relationship (after controlling for age) between cingulum FA and memory performance in middle-aged and older adults (Ezzati et al., 2016; Li et al., 2020), no significant relationship between these variables was reported in three other studies (Bennett et al., 2015; Hartopp et al., 2019; Memel et al., 2020).

Unlike the cross-sectional studies reviewed above, studies employing longitudinal designs allow identification of relationships between neural metrics and within-person, age-related changes in cognitive performance. Hence, such studies provide an opportunity to establish whether a given metric is a predictor of age-related cognitive decline. To date, however, only a handful of studies have examined whether fornix or cingulum integrity is predictive of longitudinal memory change in cognitively healthy older adults (Lancaster et al., 2016; Rabin et al., 2019a, b; Song et al., 2018). In the case of the fornix, Rabin et al. (2019b) reported that higher FA at baseline was associated with a smaller decline in verbal memory recall over 4 years, whereas no such relationship was identified in Lancaster et al. (2016). Using radial diffusivity as the DTI metric, Song et al. (2018) reported a nonsignificant association between fornix integrity and 4-year memory change. In a similar vein, of the two studies that examined the cingulum (Lancaster et al., 2016; Rabin et al., 2019a), only Lancaster et al. (2016) reported a significant relationship between cingulum FA and memory change over a 3-year follow-up period.

To our knowledge, only two studies have examined associations between free water-corrected DTI metrics derived from the fornix and cingulum and either memory performance or memory change in older adults (Ji et al., 2019; Archer et al., 2020). Ji et al. (2019) reported a significant correlation between water-corrected fornix FA and memory performance in patients with Alzheimer's Disease (AD) or amnesic mild cognitive impairment (aMCI). By contrast, Archer et al (2020) reported that neither fornix or cingulum water-corrected FA metrics were associated with memory performance in a sample comprising a mixture of cognitively healthy older adults and older adults with aMCI. However, water-corrected cingulum FA, but not fornix FA, was predictive of memory decline over 5 years. Given that both of these studies included cognitively diverse samples, it is unclear whether these DTI metrics are sensitive to memory performance or memory change in older samples comprising only cognitively healthy participants.

In the present study, we examined associations between free water-corrected FA (henceforth, FA) derived from the fornix and cingulum, memory performance, and 3-year longitudinal memory change in a sample of cognitively healthy older adults. Given that a tract-wise relationship between FA and memory might merely reflect a more general association between whole brain white matter integrity and cognition (Bennett and Madden, 2014; Rabin et al., 2019a), we also evaluated the specificity of the relationships between the two FA metrics and longitudinal memory change after controlling for FA in other white matter tracts as well as measures of performance in other cognitive domains.

2. Material and methods

Uncorrected FA metrics from a subsample of the present participants that were derived from hand-tracing of the anterior and posterior corpus callosum, along with their neuropsychological test data, were described in a prior report (de Chastelaine et al., 2016). The neuropsychological data and the associated cognitive component scores from the full sample were reported in Hou et al. (2020, 2021). Here, we describe DTI metrics derived from the fornix and parahippocampal cingulum, and their relationships with cognitive performance and longitudinal change. These data have not been reported previously.

2.1 Participants

Participants were 67 cognitively healthy older adults recruited from the greater Dallas community (see Table 1 for demographic details). They undertook the same neuropsychological test battery (see below) twice, separated by a one-month period. A subgroup (i.e. the longitudinal group) of 55 participants received the same neuropsychological test battery on a third occasion around 3 years after the second test session. Twelve older adults did not participate in session 3 due to death (N = 1), moving away from the Dallas area (N = 5), loss of contact (N = 5), or failure to attend (N = 1). Data from two additional older adults were excluded from all analyses because of abnormal anatomical scans.

All participants were right-handed, fluent in English by age 5, had no history of neurological or psychiatric disease and had normal or corrected to normal vision. They each gave informed consent according to procedures approved by the University of Texas at Dallas and University of Texas Southwestern Institutional Review Boards. They were compensated at the rate of $30 per hour for their participation.

2.2 Neuropsychological test battery

All participants completed the same neuropsychological test battery. The battery comprised the California Verbal Learning Test-II (CVLT; short and long delayed cued recall, free recall and delayed recognition, Delis et al., 2000), the immediate- and delayed Logical Memory tests of Wechsler Memory Scale (Wechsler, 2009), the Digit Span test (Forward and Backward tests) of the Wechsler Adult Intelligence Scale Revised (WAIS-R, Wechsler, 1981), the Symbol/Digit Coding test of the WAIS-R (SDMT, Smith, 1982), Trail Making Tests A and B (Reitan and Wolfson, 1985), letter and category fluency tests (FAS; Spreen and Benton, 1977), the Wechsler Test of Adult Reading (WTAR; Wechsler, 2001) and Raven's Progressive Matrices (short form, Raven et al., 1998). Potential participants were excluded from the MRI session if they had 1) scores > 1.5 SDs below the age-appropriate norm on any long-term memory sub-test (CVLT or Logical Memory) or on any two other tests; 2) an estimated full-scale intelligence quotient (IQ) < 100 as indexed by performance on the WTAR, or 3) a score on the Mini-Mental State Examination (MMSE) < 27. Since the short and long delay free and cued recall scores of the CVLT were highly correlated (rs > 0.79, ps < 0.001) we collapsed these into a single composite recall metric by averaging over the four scores. Similarly, a composite Logical Memory score was computed by averaging the scores of the immediate- and delayed Logical

Memory tests (correlation, $r = 0.89$, $p < 0.001$). These composite memory scores were used in all further analyses.

Missing values from one participant at session 3 for the SDMT, Trails A and Trails B tests were replaced by the mean performance of the remaining participants for that session. We excluded test scores for Trails A and Trails B from further analyses because of their low across-session reliability (correlations between session 1 and session 2 scores, $r = 0.45$ and 0.40 for Trails A and B, respectively; the equivalent correlations for the other tests ranged between 0.47 and 0.88).

A principal component analysis (PCA) was conducted on the neuropsychological test scores from the first session. This analysis gave rise to three latent cognitive components, here labelled ‘memory’, ‘fluency’ and ‘crystalized IQ’. Loadings for each component were reported in Hou et al. (2021) and are re-described here in Supplemental Table 1. To compute component scores for each session, the loadings were applied to each participants’ test scores from each session after the scores had been standardized across sessions (sessions 1-2 for the full group and sessions 1-3 for the longitudinal group).

The means of the component scores from sessions 1 and 2 provided a single score that was used to characterize memory performance in the full sample and as the baseline from which to assess memory change in the longitudinal sub-sample. The same procedure was adopted with component scores for the remaining cognitive domains. As was discussed at length in Hou et al. (2021), averaging scores across sessions 1 and 2 has the advantages of providing more reliable estimates of baseline performance than those provided by a single test session and attenuating both the effects of regression to the mean (Bland and Altman, 1994) as well as session 3 re-test effects. To assess whether any relationships between FA metrics and memory performance could be accounted for by general cognitive performance (see Introduction), we computed an across-domain component score by averaging the scores across the two non-memory cognitive domains ($M_{oth\text{-}cog}$).

2.3 MRI acquisition and processing

MRI scanning took place during the interval between the first 2 neuropsychological testing sessions (average of 22 days after session 1). Structural brain images were acquired with a Philips Achieva 3T MR scanner (Philips Medical System, Andover, MA USA) equipped with a 32-channel head coil. Diffusion weighted images and high-resolution T1-weighted images were acquired following a functional scanning session. DTI acquisition involved a single-shot EPI sequence [30 directions, 50 transverse slices, 2-mm thick, 1-mm gap, matrix size 112 ×110, repetition time (TR) 4410 ms, echo time 51 ms; flip angle 90 degrees, b 1000 s/mm2, plus a b0 non-diffusion weighted image].

The diffusion weighted data were preprocessed using the FMRIB software library (FSL)/v6.0.4 (Smith et al., 2004). Images were skull-stripped using the Brain Extraction Tool (BET) and corrected for motion and eddy current-induced distortions and slice-wise outliers in FSL eddy. The resulting images were resampled in MRtrix3 (Tournier et al., 2019) to a voxel size of $1.25 \times 1.25 \times 1.25$ mm^3. A brain mask was defined for each participant by applying BET to the b0 images. Free water-corrected diffusion maps were calculated using an in-house MATLAB script. The maps were computed by fitting a bi-tensor model predicting a voxel-wise signal attenuation factor for free water contamination (Pasternak et al., 2009). The model was specified as:

$$A_g(D,f) = f[\exp(-bg^T Dg)] + (1-f)\exp(-bD_{water})$$

Where, A_g is the modeled attenuated signal (normalized by b0) for the applied diffusion gradient g, and b is the b-value (1000 s/mm^2). The first term reflects the tissue compartment, where D is the diffusion tensor of this compartment, f is the fractional volume of the compartment, and g^T is the transpose of the vector g. The second term reflects an isotropic free-water compartment with a fractional volume of (1-f), where the diffusion coefficient D_{water} is set to the diffusivity of water at body temperature (3×10^{-3} mm^2/s).

DTI-derived metrics were co-registered to MNI152 space through two steps using Advanced Normalization Tools (ANTs) (Avants et al., 2011). First, a b0 group template image was created by applying *buildtemplateparallel.sh* to b0 images acquired from a lifespan sample of healthy adults that included most of the present sample (de Chastelaine et al., 2019). The template image was then normalized to the *MNI_1mm_mean_b0* image by the symmetric image normalization (SyN) algorithm (Avants et al., 2008). The warp fields generated from these two steps were employed to normalize the diffusion maps to the MNI standard space.

The John Hopkins University ICBM-DTI-81 white matter atlas (JHU atlas, Mori et al., 2008) was employed to define regions of interest (ROI), which were labeled in the atlas as fornix (body and column), and left and right parahippocampal cingulum. Minor edits were made to the cingulum ROIs to ensure that they overlapped with the across-participants mean FA map. The fornix and cingulum ROIs are illustrated in Fig 1. Participant-specific mean FA values from the normalized white matter images were extracted from each of these ROIs. Because we had no *a priori* predictions of lateralization effects on cingulum FA, DTI metrics from the left and right cingulum were averaged prior to statistical analysis. Mean FA values averaged across all tracts labeled in the atlas other than the fornix (allbut-fornix) or the cingulum (allbut-cingulum) were also computed.

2.4 Statistical analyses

For the full group, partial Pearson correlation coefficients were computed to examine relationships between FA metrics and memory performance while controlling for age, sex and years of education. To examine associations between the FA metrics and memory change in the longitudinal subgroup, we constructed a series of linear mixed effects regression models of the general form:

$$Memory_{ij} = B_0 + B_1 Age_i + B_2 Sex_i + B_3 Edu_i + B_4 Session_j + B_5 FA_i \\ + B_6 (FA_i \times Session_j) + b_{0i} + e_{ij}$$

where, $Memory_{ij}$ refers to participant i's memory performance at session j, and Age, Sex and Edu refer, respectively, to the participant age at baseline, their sex (male coded as 0, female coded as 1) and years of education. Session refers to test session (baseline coded as 0, session 3 coded as 1), FA refers to a tract-specific free water-corrected FA value, and FA × Session refers to the interaction between FA and test session. B denotes fixed effects estimates, b0 denotes estimates for participant-specific random-effects (i.e., memory or other cognitive performance), and e is residual error. As is evident from their coding, both session and sex were modeled as categorical variables. For each linear mixed regression model, we report the marginal R^2 and conditional R^2 estimated by method proposed by Nakagawa et al. (2017). Marginal R^2 refers to variance explained by the fixed effects, whereas conditional R^2 reflects variance accounted for by both the fixed and random effects. To complement results of the significance testing, we also report Akaike Information Criterion (AIC) score for the linear mixed effects models. AIC is a measure of how well a statistical model fits the data, with a lower value reflecting a better fit. AIC was compared between models employing the FA x session interaction term as predictors and models without the term, with all the other predictors held constant.

In both the correlational and regression analyses described below, additional covariates (e.g., cognitive performance in other cognitive domains, mean FA of other tracts, see above) were added to the model when the FA metrics demonstrated a significant association with memory performance or memory change.

Correlational analyses were conducted using SPSS v.25 (IBM Corp., Armonk, NY). Linear mixed effects models were estimated in R software (R core Team 2018) using the lmer function from the lme4 package (Bates et al., 2015). Significance levels for all tests were set at $p < 0.05$. Findings from each family of the principal statistical analyses (i.e. the correlational and the regression analyses) were family-wise corrected for multiple comparisons using the Holm-Bonferroni procedure. Unless noted, the reported findings remained unchanged after correction.

3 Results

3.1 Demographic information and FA statistics

Demographic information and summary FA statistics are given in Table 1. As is evident from the table, these measures are highly similar for the full group and the longitudinal subgroup.

3.2 Neuropsychological performance

Neuropsychological test scores were reported in Hou et al. (2020, 2021) but are redescribed here in Table 1 for the convenience of the reader. Briefly, the full group and the longitudinal subgroup were well matched in terms of performance on the first two sessions. Also, in both groups, test performance improved from session 1 to session 2. In the longitudinal subgroup, mean performance showed little evidence of change between baseline and session 3 (baseline: M = 0.06, SD = 2.37; session 3: M = -0.12, SD = 2.67; t(54) = 1.02, p = 0.313, Cohen's d = 0.14).

3.3 Associations between fornix and cingulum FA and memory performance

We examined the relationships between fornix and cingulum FA and memory performance in the full group. Neither FA metric was significantly correlated with the baseline memory score: for fornix, partial r = 0.05, p = 0.669; for cingulum, partial r = -0.02, p = 0.882. In the longitudinal subgroup, neither fornix FA nor cingulum FA correlated reliably with memory scores either at baseline or at session 3 (for session 3: respectively, partial r = 0.22, p = 0.117, partial r = 0.24, p = 0.082, for the findings associated with other cognitive domains, see Supplemental Table 3).

3.4 Associations between fornix and cingulum FA and memory change

Table 2 shows the results of the linear mixed effects regression models in which FA metrics were employed as predictors of memory change. As is evident from the table, sex was a significant predictor of memory performance, reflecting higher memory performance in female than in male participants. This result is consistent with the well-established finding that females out-perform males on tests of verbal memory (e.g., Bleecker et al., 1988; Herlitz et al., 1999; de Chastelaine et al., 2023; for recent reviews, see Asperholm et al., 2019; Hirnstein et al., 2022). FA in both fornix and cingulum was predictive of memory change as evidenced by the significant interaction terms in the models (see also Fig. 2). For both the fornix and cingulum, the model employing the FA x session interaction as a predictor was associated with lower AIC score than its counterpart model that excluded the predictor (for fornix, AIC = 424.18 vs. 433.83, for cingulum, AIC = 419.61 vs. 432.99), indicating that modeling the relationship between FA and memory change improved fit of the data.

3.5 Specificity of the relationships between fornix and cingulum FA and memory change

We went on to examine the specificity of the relationships between FA metrics and memory change. Table 3 lists the main results from linear mixed effects regression analyses in which we controlled for mean performance averaged across the non-memory cognitive domains ($M_{oth\text{-}cog}$) as well as mean FA of other white matter tracts (allbut-FA) in a stepwise manner (see Supplemental Tables 4 and 5 for the full results for each model). As is evident from the table, both fornix FA and cingulum FA remained significant predictors of memory change in the presence of the additional covariates. For both fornix and cingulum, the allbut-FA metric was unrelated to either memory performance or memory change.

3.6 Fornix and cingulum FA independently predict memory change

We constructed a final linear mixed effects regression model that included fornix FA and cingulum FA as joint predictors of memory performance. As is shown in Table 4, the FA of each tract significantly interacted with test session, indicating that the two metrics accounted for unique variance in memory change. The AIC score for this model (408.35) was lower than that for the analogous model in which the interaction terms were excluded (425.59).

4. Discussion

In the present study, we examined associations in cognitively healthy older adults between free water-corrected FA metrics derived from the fornix and cingulum and memory performance and 3-year memory change. Neither fornix nor cingulum FA correlated with memory performance at baseline. By contrast, FA of each tract was predictive of memory change, such that greater FA was associated with less longitudinal decline. These associations remained significant after controlling for FA in other white matter tracts and for performance in other cognitive domains. Furthermore, fornix and cingulum FA explained unique variance in memory change.

The present study employed a burst measurement design to mitigate re-test and regression to the mean effects (see Methods). Our rationale for the employment of this approach is described and discussed in detail in Hou et al. (2021). We partially reprise that discussion here to note that when baseline memory performance was operationalized as the mean of the first two neuropsychological test sessions, memory performance at session 3 did not demonstrate a reliable decline at the group level. However, this finding does not imply that the memory performance of our sample remained stable over this period, given the likely positive bias in the session 3 scores resulting from re-test effects (e.g. Salthouse and Tucker-Drob, 2008; Salthouse, 2009). If it is assumed that session 2 performance provides the best correction for session 3 re-test effects, then memory scores declined significantly and robustly at the group level [$t(54) = 5.31$, $p < 0.001$]. Moreover, when session 2 scores were employed as the baseline measure, our main findings were essentially identical.

4.1 Comparisons between conventional and free water-corrected FA in fornix and cingulum

As was noted in the introduction, most prior studies examining relationships between the integrity of fornix and cingulum and memory performance and change have employed conventional DTI metrics. These metrics can be influenced by extracellular free water and may not accurately reflect white matter integrity, especially in older adults (Alexander et al., 2001; Giorgio et al., 2010; Vos et al., 2011). The impact of free water is especially likely to be evident for the fornix because of its proximity to the third ventricle, as is evidenced by the findings from the analyses comparing the conventional and free water-corrected FAs (see Supplementary material: section 7). Briefly, conventional FA metrics were lower than the corrected metrics in both the fornix and cingulum, with the difference being substantially larger in the fornix. In addition, uncorrected, but not corrected fornix FA was significantly correlated with chronological age (respectively, $r = -0.46$, $p < 0.001$ vs. $r = -0.16$, $p = 0.203$). Age was uncorrelated with either of these FA metrics in the cingulum (absolute rs < 0.08, ps > 0.558). Similar findings were reported in Chad et al. (2018), where the relationship between age and fornix FA also was nonsignificant after free water correction. Together, the present and prior results suggest that the magnitude of age effects on FA can be inflated by partial volume effects, especially for tracts that are adjacent to the ventricles. Contrary to our findings and those of Chad et al. (2018), however, Metzler-Baddeley et al. (2012) reported that the correlation between age and fornix FA became slightly stronger after free-water correction ($r = -0.56$ vs. $r = -0.65$ before and after correction). These diverse findings might be attributable to sample differences between the studies, or to differences in DTI analysis approaches. Clearly, additional research is required to establish the circumstances in which corrected and uncorrected FA estimates differ in their sensitivity to chronological age.

Whereas the corrected and uncorrected FA metrics were significantly correlated across participants for both tracts (for fornix, $r = 0.61$, $p < 0.001$; for cingulum, $r = 0.88$, $p < 0.001$), the relationship was significantly weaker for the fornix (Steiger's Z test: $Z = 3.82$, $p < 0.001$).

Unsurprisingly, given the strength of the correlation, findings for uncorrected cingulum FA were consistent with those obtained with the corrected metric. By contrast, uncorrected fornix FA was unrelated to longitudinal memory change (Supplementary material: section 7). We conjecture that one reason for the inconsistency of prior findings in respect of white matter integrity and memory performance and change might be variability in the effects of free water on the DTI metrics.

4.2 Fornix and cingulum FA and baseline memory performance

Neither fornix nor cingulum FA demonstrated a reliable association with baseline memory performance. Thus, in agreement with some prior reports (Ly et al., 2016; Sasson et al., 2013), these findings imply that at least one putative measure of the integrity of fornix and cingulum is not predictive of concurrent memory performance in cognitively healthy older adults. Of potential importance, although fornix and cingulum FA are inconsistently associated with memory performance in cognitively healthy participants, reliable correlations between cingulum integrity and memory performance have been reported with more consistency in participants with MCI or AD (for reviews, see Bubb et al., 2018; Douet and Chang, 2015). Thus, it is possible that fornix and cingulum integrity are predictive of memory performance when their integrity is compromised by neuropathology.

4.3 Fornix and cingulum FA and longitudinal memory change

Although uncorrelated with baseline performance, both fornix and cingulum FA were predictive of 3-year memory change. Thus, consistent with the reports of Rabin et al (2019b) and Lancaster et al. (2016), these findings suggest that DTI metrics of fornix and cingulum integrity are predictors of future memory change in cognitively healthy older adults.

Reliable associations have been reported previously between whole brain FA and performance in multiple cognitive domains (Rabin et al., 2019a). In light of this report, we examined whether the relationships we observed between fornix and cingulum FA and memory change remained after controlling for whole brain FA and performance in domains other than memory. As described in the results section, controlling for these variables did not attenuate the relationships between fornix and cingulum FA and memory change. The findings are consistent with those of Rabin et al. (2019b), who also reported that fornix FA predicted memory change after controlling for whole brain FA. In contrast to our findings, however, in a companion paper Rabin et al. (2019a) reported no significant relationship between cingulum FA and memory change, even before controlling for whole brain FA. The discrepancy between our findings and those of Rabin et al. (2019a) might be attributable to sampling and methodological differences between the studies. Notably, the age range of the participants in Rabin et al. (2019a) (63-90 years old) was broader than the range in the present study (63-76 years old), raising the possibility that age-related variability in memory performance attributable to factors other than white matter integrity overshadowed any relationship with FA in that study.

In the current study, both fornix and cingulum FA were predictive exclusively of memory change, demonstrating no relationship with change in fluency or crystallized IQ. In line with these findings, Rabin et al (2019b) also reported a relationship between fornix FA and change in memory but not in executive function or processing speed. Since Lancaster et al. (2016) restricted their analyses to memory change, it is unclear whether the association between cingulum FA and memory change reported in that study was unique to memory.

When included in the same model, fornix and cingulum FA independently predicted memory change, suggesting that the two tracts have at least partially non-overlapping mnemonic functions. A small number of prior studies have examined associations between fornix or cingulum FA and different forms of memory. For example, Rudebeck et al. (2009) reported that, in young adults, fornix FA was sensitive to behavioral estimates of recollection but not familiarity. There is also some evidence that fornix, but not cingulum, FA is associated with performance on the 'mnemonic similarity test' across the lifespan (Bennett et al., 2015, but see Introduction and Bennett and Stark, 2016). Further understanding of the differential roles of the fornix and cingulum in supporting memory performance would benefit from the administration of a broad range of different memory tests to the same participants.

It should be noted that fornix and cingulum FA are not the only brain measures reported to predict longitudinal memory change in older adults. Notably, we previously identified a significant relationship between functional activity in the hippocampus (functional MRI 'recollection effects') and memory change in the same sample we report on here (Hou et al., 2020). Of interest, the functional effects and the FA metrics turn out to be unique predictors of memory change, collectively accounting for more than 23% of the total variance (see Supplementary material: section 6). These findings indicate that longitudinal memory change likely reflects the influence of multiple mechanisms. Delineating their relative importance will be an important challenge for future research.

4.4 Free water correction for single-shell data

In the present study, we applied the procedure developed by Pasternak et al. (2009) to DTI data acquired with one *b*-value (single shell acquisition). Unlike in the case of multi-shell acquisition, applying the procedure to single-shell data requires spatial regularization, which might limit the accuracy of the free water estimates (Pasternak et al., 2009, 2012; Hoy et al., 2014). Nonetheless, recent studies have reported that, even when free-water is estimated from single shell DTI data, corrected FA metrics demonstrate higher test-retest reliability (Albi et al., 2017), and greater accuracy in detecting age- and disease-related white matter differences than do uncorrected metrics (Bergamino et al., 2017, 2021; Chad et al., 2023; Edde et al., 2019). These advantages underscore the utility of the free water correction procedure over the conventional DTI methods for single-shell data.

5. Limitations

There are a number of limitations to the present study. First, the sample size was modest, limiting the power to detect small effects. Second, as already noted, the diffusion weighted data were acquired with a single *b*-value diffusion shell, possibly limiting the accuracy of the free water model in estimating subtle spatial features of the data. Third, because we acquired diffusion weighted data only at baseline, we were unable to examine change-change relationships between FA and memory. Fourth, the age range of our sample is relatively narrow. Thus, future research would benefit from the employment of larger samples with wider age ranges, multi-shell diffusion acquisition, and longitudinal measurement of white matter integrity.

6. Conclusions

The present study provides novel evidence of an association between free water-corrected measures of the integrity of the fornix and parahippocampal cingulum and longitudinal memory change in cognitively healthy older adults. The findings for the fornix in particular highlight the utility of correcting for free water when estimating DTI metrics of white matter integrity.

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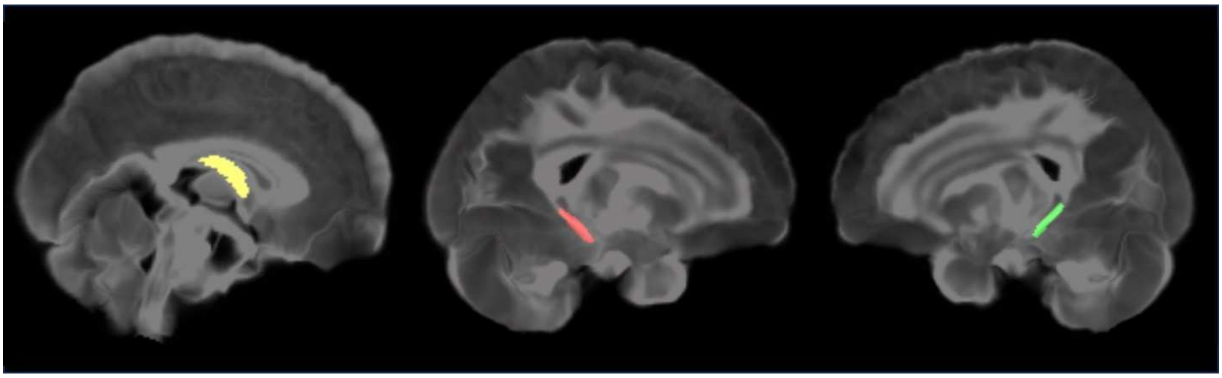

Fig 1. Regions of interest depicted on a mean FA map derived from all participants (fornix in yellow, left cingulum in red and right cingulum in green).

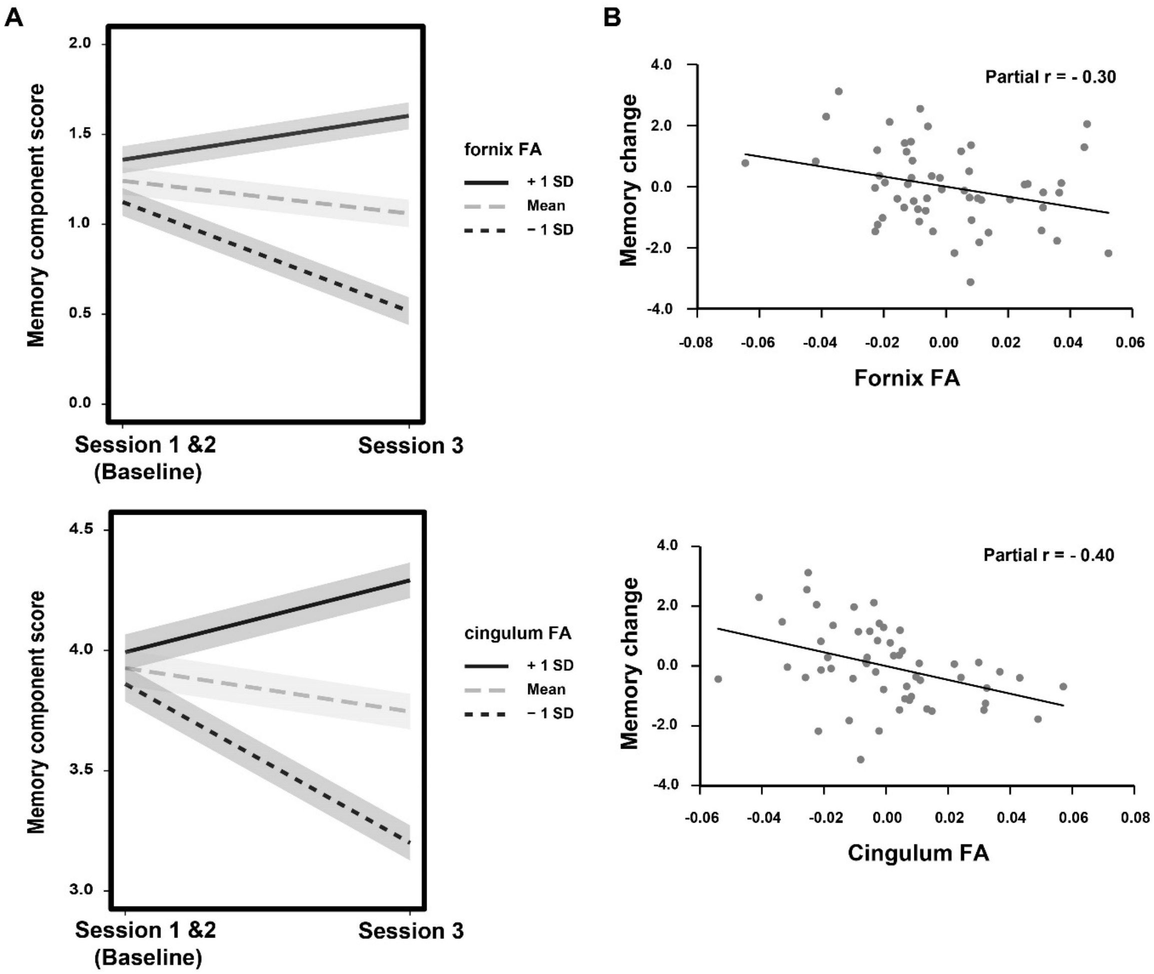

Fig 2. A: fornix (upper) and cingulum (lower) FA × session interactions visualized with simple slopes (mean +/- 1 SD). B: scatter plots depicting the relationships between memory change scores (baseline minus session 3) and fornix and cingulum FA at baseline, controlling for age, sex, years of education and baseline scores.

Table 1. Demographic information, summary measures of FA and neuropsychological data for the study participants (standard deviations in parentheses).

Task	Session				
	1	2	1	2	3
	Full group		Longitudinal subgroup		
N	67 (37 F)		55 (28 F)		
Age	68.2 (3.6)		68.3 (3.7)		
Education	17.2 (2.3)		17.3 (2.3)		
Conventional fornix FA	0.25 (0.03)		0.25 (0.03)		
Corrected fornix FA	0.48 (0.03)		0.48 (0.03)		
Conventional cingulum FA	0.21 (0.02)		0.21 (0.02)		
Corrected cingulum FA	0.26 (0.02)		0.26 (0.02)		
FAS[a, b]	45.21 (12.53)	49.09 (12.74)	45.04 (12.63)	48.40 (11.58)	47.56 (13.26)
Logical Memory Composite[a, b, c]	27.59 (5.39)	31.73 (5.45)	27.51 (5.57)	31.93 (5.39)	28.39 (5.57)
SDMT[a, b, c]	49.46 (8.50)	51.91 (8.20)	49.45 (9.18)	51.80 (8.73)	49.09 (8.46)
Trail A (ms)	32.69 (11.24)	30.25 (11.10)	31.96 (9.40)	30.75 (11.71)	31.89 (10.18)
Trail B (ms)[a, b, c]	75.01 (45.70)	59.82 (18.51)	71.44 (30.60)	59.44 (18.32)	69.69 (30.54)
Digit Span	18.27 (4.36)	17.87 (4.24)	18.25 (4.30)	17.71 (4.09)	18.15 (4.26)
Category Fluency (Animals)[a, b]	22.45 (5.56)	23.96 (5.39)	22.35 (5.50)	23.69 (5.44)	22.93 (5.80)
WTAR (Full-Scale IQ)[c]	112.64 (5.43)	113.00 (5.16)	112.95 (5.21)	113.04 (5.15)	112.11 (4.63)
Raven's	9.57 (2.13)	9.91 (1.87)	9.49 (2.25)	9.85 (1.87)	9.56 (2.63)
CVLT Hits[a, b]	14.82 (1.31)	15.42 (.96)	14.84 (1.33)	15.36 (1.01)	15.25 (1.13)
CVLT False Alarms	1.97 (2.18)	1.84 (2.53)	1.93 (2.13)	1.93 (2.71)	2.09 (2.52)
CVLT recall Composite[a, b, c]	12.00 (2.47)	13.63 (2.02)	11.95 (2.57)	13.54 (2.17)	12.80 (2.55)

Note. a: session 1≠session 2, $p < .05$ for full group; b: session 1 ≠ session2, for longitudinal subgroup, $p < .05$; c: session 2 ≠ session 3, $p < .05$. Unless noted, FA in the text refers to corrected FA.

Table 2. Linear mixed effects regression results for FA metrics predicting memory performance and memory change. Higher FA was associated with lower memory decline.

Parameter	B (SE)	df	t	p	Marginal R^2	Conditional R^2
Model 1						
Intercept	-1.01 (9.57)	53	0.11	0.916	0.291	0.882
Age	-0.10 (0.08)	50	1.31	0.195		
Sex	**2.59 (0.60)**	**50**	**4.30**	**< 0.001**		
Edu	0.27 (0.13)	50	2.03	0.048		
Fornix FA	4.70 (12.44)	58	0.38	0.707		
Session	**-8.34 (3.28)**	**53**	**2.54**	**0.014**		
Fornix FA × Session	**17.00 (6.82)**	**53**	**2.49**	**0.016**		
Model 2						
Intercept	3.17 (6.40)	52	0.50	0.622	0.292	0.885
Age	-0.14 (0.08)	50	1.79	0.080		
Sex	**2.71 (0.61)**	**50**	**4.49**	**< 0.001**		
Edu	0.25 (0.13)	50	1.90	0.063		
Cingulum FA	2.90 (13.20)	58	0.22	0.827		
Session	**-5.71 (1.94)**	**53**	**-2.94**	**0.005**		
Cingulum FA × Session	**21.11 (7.38)**	**53**	**2.86**	**0.006**		

Table 3. Beta estimates of linear mixed effects regression models employing fornix and cingulum FA as predictors of memory performance and change, before and after controlling for variance shared with other cognitive domains and other tracts (p values in the parentheses).

	Original Model	+ $M_{oth\text{-}cog}$	+ Allbut-FA	+ $M_{oth\text{-}cog}$ & Allbut-FA
Fornix FA predicting memory change				
FA	4.70 (0.707)	3.75 (0.718)	5.16 (0.682)	3.16 (0.764)
FA × Session	**17.00 (0.016)**	**14.19 (0.023)**	**19.05 (0.007)**	**15.76 (0.012)**
$M_{oth\text{-}cog}$		**0.74 (< 0.001)**		**0.71 (< 0.001)**
Allbut-FA			11.34 (0.683)	-8.02 (0.732)
Allbut-FA × Session			28.41 (0.053)	20.73 (0.115)
Cingulum FA predicting memory change				
FA	2.90 (0.827)	-1.09 (0.921)	1.04 (0.946)	2.36 (0.852)
FA × Session	**21.11 (0.006)**	**22.60 (< 0.001)**	**20.16 (0.019)**	**24.39 (0.001)**
$M_{oth\text{-}cog}$		**0.77 (< 0.001)**		**0.79 (< 0.001)**
Allbut-FA			8.19 (0.803)	-15.74 (0.564)
Allbut-FA × Session			4.29 (0.796)	-7.87 (0.578)

Table 4. Linear mixed effects regression results for fornix and cingulum FA as joint predictors of memory performance and change. Higher FA was associated with lower memory decline.

Parameter	B (SE)	df	t	p	Marginal R^2	Conditional R^2
Intercept	0.02 (9.65)	52	< 0.01	0.998	0.302	0.894
Age	-0.12 (0.08)	49	1.45	0.152		
Sex	**2.66 (0.61)**	**49**	**4.35**	**< 0.001**		
Edu	**0.27 (0.13)**	**49**	**2.01**	**0.050**		
Fornix FA	3.73 (12.75)	56	0.29	0.771		
Cingulum FA	1.54 (13.53)	56	0.11	0.910		
Session	**-11.93 (3.41)**	**52**	**3.50**	**< 0.001**		
Fornix FA × Session	**14.33 (6.57)**	**52**	**2.18**	**0.034**		
Cingulum FA × Session	**18.62 (7.22)**	**52**	**2.58**	**0.013**		