1. Title: Fornix fractional anisotropy mediates the association between Mediterranean diet adherence and memory four years later in older adults without dementia

2. Running head: MeDiAd, Fornix, and memory

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7. Abbreviations: AD: Alzheimer's disease; AIC: Akaike information criterion; ApoE: apolipoprotein E; BIC: Bayesian information criterion; BMI: body-mass-index; CFI: comparative fit index; DELCODE: German Center for Neurodegenerative Diseases Longitudinal Cognitive Impairment and Dementia Study; DWI: diffusion-weighted imaging; FA: fractional anisotropy; LGCM: latent growth curve modeling; MCI: mild cognitive impairment; MeDi: Mediterranean diet; MeDiAd: MeDi adherence; RMSEA: root mean square error of approximation; SCD: subjective cognitive decline; SRMR: standardized root mean square residual; TLI: Tucker–Lewis index

ABSTRACT

Background. The mechanisms in the brain that explain the benefits of adherence to the Mediterranean diet (MeDiAd) for cognition are incompletely understood. Here, we investigated whether fractional anisotropy (FA) in hippocampus-relevant white-matter tracts mediates the association between baseline MeDiAd and verbal episodic memory over four years.

Methods. Participants with baseline diffusion-weighted imaging data from the DELCODE cohort study were selected, including healthy older adults with and without subjective cognitive decline and patients with mild cognitive impairment (*n* = 376; age: 71.47 ± 6.09 years; 48.7% female). Demographic, MeDiAd, and diffusion data were obtained at baseline. Verbal episodic memory was assessed at baseline and four yearly follow-ups. The association between baseline MeDiAd and verbal episodic memory's *mean* and *rate of change* over four years and the mediation of that association by baseline white-matter tracts were tested with latent growth curve modeling. Potential mediators were selected based on the association with hippocampal volume.

Results. Baseline MeDiAd was associated with verbal episodic memory four years later (latent intercept; 95% confidence interval, CI [0.01, 0.32]) but not with its rate of change (latent slope) over this period. Only baseline Fornix FA, among four potential mediators (the cingulum ventral, corticospinal tract, and superior longitudinal fasciculus II), mediated this association (latent intercept; 95% CI [0.002, 0.09]).

Conclusions. Higher Fornix FA explains the association between higher baseline MeDiAd and better memory four years later in pre-dementia stages. Fornix FA may be a useful response biomarker of Mediterranean diet interventions on memory.

Keywords: *diet; latent growth curve modeling; longitudinal analysis; mild cognitive impairment; white matter*

INTRODUCTION

Lifestyle factors such as physical activity, diet, education, or social interaction can modify the risk of cognitive impairment in older age [1–3]. Understanding how lifestyle factors influence cognition is therefore a crucial element in the path toward dementia prevention. Diet offers a promising approach to implementing effective large-scale programs for dementia prevention [4]. The Mediterranean diet (MeDi), an example of a healthy diet in modern Western society, is characterized by abundant plant foods; low to moderate consumption of dairy; low to moderate fish and poultry; olive oil as the main source of fat; and eggs and red meat in low amounts [5]. In older adults [6,7], moderate to higher adherence to MeDi has been associated with reduced risk for cognitive impairment in epidemiological cohorts [8], conversion to dementia in mild cognitive impairment (MCI) [9], and better subjective [10] and objective [11] cognitive functions. So far, it is only partially understood what brain mechanisms explain the benefits induced by MeDi adherence (MeDiAd) on objective cognitive functions [3]. Importantly, ascertaining the potential role of specific brain measures in those benefits will help us better understand MeDiAd's association with cognition and identify biomarkers of MeDiAd's effects on the individual.

The brain's white matter is vulnerable to risk factors modulated by diet, such as hypertension and obesity [12]. Hence, white matter can help explain the relationship between MeDiAd and cognition. White matter properties can be measured with diffusion-weighted imaging (DWI) [12–14], which is based on the diffusion of water molecules. In broad terms, fractional anisotropy (FA) is a parameter that represents the degree of diffusion directionality [12]. In non-demented community dwellers, higher MeDiAd has been associated with higher FA measured nine years later [15]. Higher FA in hippocampus-relevant tracts has been shown to correlate with greater memory

recognition in patients with prodromal Alzheimer's disease (AD) [16]. Therefore, FA might mediate the association between MeDiAd and memory. A *cross-sectional* FA mediation of the association between dietary patterns, including Omega-3 and 6 fatty acids and vitamin E, and memory has been shown previously in healthy older adults [17,18]. However, *longitudinal* evidence on the FA mediation between MeDiAd and memory is missing. The present study set out to address this gap by (a) focusing on white-matter tracts relevant for AD, (b) capitalizing on latent variable analysis to properly control for baseline performance and measurement error across occasions, and (c) including participants at different risk levels for cognitive decline.

Here we investigated whether baseline MeDiAd is associated with the mean and rate of change in episodic memory over four years at the latent level and whether hippocampus-relevant white-matter tracts mediate this association. Given the previously-demonstrated association between MeDiAd, memory, and hippocampal volume [19], we expected hippocampus-relevant tracts (e.g., fornix, hippocampal cingulum, or uncinate fasciculus) [16] at baseline to mediate the association between baseline MeDiAd and latent mean and rate of change in verbal episodic memory over four years.

RESULTS

Descriptive statistics

Table 1 shows the descriptive statistics along with the number of missing values. None of the demographic or clinical variables (i.e., diagnosis group, age, sex, education, physical activity score, depressive symptoms, BMI, MMSE) or sites were associated with MeDiAd scores ($F_{19,197}$ = 1.01, p = 0.454).

Hippocampus-relevant white-matter tracts

The right hippocampal volume was positively associated with average FA in the left superior longitudinal fasciculus (SLF) II (b = 1879, standard error, SE = 826.5, p = 0.023) and right fornix (b = 443.4, SE = 156.2, p = 0.005) and negatively associated with average FA in the right corticospinal tract (CST; b = -1615, SE = 676, p = 0.017) while keeping all other variables constant. The left hippocampal volume, in turn, was positively associated with average FA in the right CST (b = 1307, SE = 652, p = 0.046) and in the left cingulum bundle, ventral (b = 778, SE = 347, p = 0.025) while keeping all other variables constant. Therefore, we tested the latent growth curve modeling (LGCM) using these four white-matter tracts simultaneously as potential mediators, thereby controlling for one another.

Baseline associations between MeDiAd, white matter, and memory

Average FA of all four hippocampus-relevant white-matter tracts (Fig 2) positively correlated with baseline verbal episodic memory (Table 2). Baseline MeDiAd also positively correlated with verbal episodic memory and, among the tracts, only with the fornix (Table 2).

Longitudinal measurement invariance

The configural model had an adequate model fit (Table 3). However, this fit (e.g., ΔCFI, see *Materials and Methods*) decreased as more constraints were incrementally imposed on the model. This result indicated that especially scalar invariance (i.e., the equivalence of item intercepts) was not supported. On further inspection, we could determine that releasing the equality constraints for the first and second time-points improved model fit and ensured partial scalar invariance (*cf.*

"Scalar," Table 3; CFI = 0.981; χ^2 = 26.8, df = 10; AIC = 2508.6; BIC = 2547.9; ΔCFI = 0.01; $\Delta\chi^2$ = 12.16, Δdf = 3). Residual invariance was further ensured after those changes by releasing the variances of the corresponding time points (*cf.* "Residual," Table 3; CFI = 0.972; χ^2 = 35.2, df = 11; AIC = 2515.0; BIC = 2550.3; ΔCFI = 0.009; $\Delta\chi^2$ = 8.42, Δdf = 1). However, to be able to freely estimate the *latent* variable intercepts and because this assumption is more tenable in future studies, we kept all equality constraints on intercepts and residuals. We ran a sensitivity analysis with the above-mentioned modifications and confirmed that our results still held.

Longitudinal associations between MeDiAd, white matter, and (latent) memory

The individual trajectories of memory scores are shown in Fig 3, color-coded by each diagnosis subgroup. With LGCM, we analyzed whether baseline MeDiAd is associated with the variability in individual trajectories (slope) and/or individual scores four years later (intercept) and whether white-matter tracts mediate those associations. A LGCM including baseline MeDiAd and all four tracts as mediators (CFI = 0.962, TLI = 0.948, χ^2 (33) = 99.76, p < 0.0001, RMSEA = 0.073, SRMR = 0.043) showed that both MeDiAd (β = 0.15, b = 0.16, SE = 0.08, p = 0.032, 95% confidence interval, CI [0.01, 0.32]) and Fornix FA (β = 0.27, b = 0.29, SE = 0.07, p < 0.0001, 95% CI [0.15, 0.42]), but none of the other white-matter tracts, were associated with memory at time point 5 (latent intercept; Table 4). Neither MeDiAd nor any white-matter tract was associated with the *rate* of change in memory over four years (latent slope; all p-values > 0.164), although there was an overall (mean) decline in performance (β = -0.51, b = -0.08, SE = 0.01, p < 0.0001, 95% CI [-0.11, -0.05]). Adding sex and age as covariates resulted in poor model fit (CFI = 0.915, TLI

= 0.886, χ^2 (47) = 207.96, p < 0.0001, RMSEA = 0.095, SRMR = 0.083). Thus, we continued without these covariates.

The right fornix FA significantly mediated the association between baseline MeDiAd and the latent intercept of memory, i.e., the score four years later (β = 0.04, b = 0.04, SE = 0.02, p = 0.039, 95% CI [0.002, 0.09]), but not the latent slope. No mediation was found for the other white-matter tracts (Table 4). The total indirect effects were not significant, indicating that the association between MeDiAd and memory is not explained by the *combined* effect of all four tracts' FA on the latent intercept or slope of memory. Therefore, we continued with a (simpler) single-mediator model (Fig 4 and Table 5; CFI = 0.935, TLI = 0.935, χ^2 (21) = 81.77, p < 0.0001, RMSEA = 0.088, SRMR = 0.046) for sensitivity analyses.

Sensitivity analyses

Including the MMSE as a covariate in the model slightly decreased model fit (CFI = 0.926, TLI = 0.920, χ^2 (25) = 100.08, p < 0.0001, RMSEA = 0.089, SRMR = 0.058), but results remained the same, including the association between MeDiAd and the latent intercept (β = 0.17, b = 0.17, SE = 0.07, p = 0.017, 95% CI [0.03, 0.32]) and Fornix FA mediation (β = 0.03, b = 0.04, SE = 0.02, p = 0.040, 95% CI [0.002, 0.07]). MMSE was significantly associated with both the latent intercept (β = 0.40, b = 0.41, SE = 0.06, p < 0.0001, 95% CI [0.29, 0.53]) and slope (β = 0.20, b = 0.03, SE = 0.01, p = 0.022, 95% CI [0.005, 0.06]).

An alternative model in which MeDiAd mediates the association between Fornix FA and memory (FA → MeDiAd → memory) was plausible (CFI = 0.935, TLI = 0.935, χ^2 (21) = 81.88, p < 0.0001, RMSEA = 0.088, SRMR = 0.046). However, the

indirect effects through MeDiAd on neither the latent intercept nor the latent slope were significant (both *p*-values > 0.128).

In a model including the right hippocampus volume as an additional potential mediator (CFI = 0.929, TLI = 0.936, χ^2 (25) = 86.47, $p < 0.0001$, RMSEA = 0.081, SRMR = 0.043), both indirect effects were significant for the latent intercept only, both individually and combined (hippocampus: β = 0.03, b = 0.04, SE = 0.02, p = 0.031, 95% CI [0.003, 0.07]; Fornix: β = 0.04, b = 0.04, SE = 0.02, p = 0.033, 95% CI [0.003, 0.08]; both: β = 0.08, b = 0.08, SE = 0.03, p = 0.004, 95% CI [0.03, 0.13]). A sequential mediation (Hippocampus → FA: 95% CI [-0.0004, 0.01]; FA → Hippocampus: 95% CI [-0.001, 0.01]) was not supported.

Post-hoc, we explored whether apolipoprotein E (ApoE) risk status (a) is associated with the latent intercept and/or the latent slope of memory and (b) modulates the association between MeDiAd and the latent intercept and/or the latent slope of memory. This model (CFI = 0.935, TLI = 0.926, χ^2 (29) = 91.17, $p < 0.0001$, RMSEA = 0.076, SRMR = 0.039) showed a significant association for ApoE status with the latent intercept (β = -0.17, b = -0.17, SE = 0.06, p = 0.006, 95% CI [-0.29, -0.05]) but not the latent slope of memory (95% CI [-0.02, 0.03]). ApoE status did not modulate the association between MeDiAd and the latent intercept (95% CI [-0.1, 0.20]) or latent slope (95% CI [-0.03, 0.04]) of memory. The Fornix mediation remained unchanged (95% CI [0.004, 0.09]).

Finally, *post hoc*, we used FA weighted by the tracts' posterior probabilities instead of FA averaged over the entire tract for the single-mediator model to address potential partial volume effects, especially in the fornix (e.g., closeness to cerebrospinal fluid). With this model (CFI = 0.936, TLI = 0.936, χ^2 (21) = 80.54, $p <$ 0.0001, RMSEA = 0.087, SRMR = 0.045), results remained the same (*Cf.* Fig 4 and

Table 5), including the association between Fornix FA and the latent intercept (95% CI [0.19, 0.44]) of memory and the Fornix mediation (95% CI [0.002, 0.09]).

DISCUSSION

We aimed to shed light on the brain mechanisms of the positive effects of MeDiAd on memory in older individuals without dementia. Using LGCM, we investigated the association between baseline MeDiAd and verbal episodic memory assessed longitudinally as well as the mediation of this association via hippocampus-relevant white-matter tracts. MeDiAd was associated with verbal episodic memory four years later but not with its rate of change over this period. FA of the cingulum bundle (ventral), fornix, corticospinal tract, and superior longitudinal fasciculus II correlated with hippocampal volume and thus were used as candidate mediators in the analyses. However, only Fornix FA mediated the association between baseline MeDiAd and memory four years later. These results indicate that higher MeDiAd is associated with higher Fornix FA and that this association predicts better verbal episodic memory four years later in healthy older adults with and without subjective cognitive decline (SCD) and patients with MCI. This result suggests that MeDiAd contributes to verbal episodic memory in old age by supporting Fornix FA.

Effective prevention of cognitive impairment and dementia possibly necessitates multi-domain interventions (i.e., targeting more than one lifestyle factor) [3]. Establishing dose-response relationships requires first understanding how individual interventions work [12]. In this context, the Fornix FA mediation found here suggests that MeDiAd could help maintain the white matter in tracts that support hippocampal function [20] and connectivity with regions involved in food intake

regulation (e.g., hypothalamus) [21]. Such maintenance of fornix white matter would be critical for the preservation of verbal episodic memory [22] over at least four years. This implies that Fornix FA can be used to evaluate the potential effectiveness of MeDi interventions on verbal and possibly non-verbal memory in older adults. The influence of MeDi on white-matter tracts, which is widespread throughout the brain [15], is thought to occur through the enhancement of a healthy vasculature and metabolic state [23,24]. Accordingly, future work has to identify the neurobiological substrate of that influence as well as that of potentially modifiable dementia risk factors.

A cross-sectional association of MeDiAd with episodic memory and hippocampal volume in an overlapping DELCODE sample was recently described [19]. Here we extended those insights by focusing on longitudinal modeling at the latent level. The Fornix FA mediation was non-sequential and independent from that of the hippocampal volume. Beyond previous *cross-sectional* approaches focused on white matter [17,18], our study demonstrated (i) a temporal sequence from baseline MeDiAd and FA to memory four years later and (ii) a mediation at the latent level specifically for memory four years later, independently of global cognitive status or ApoE risk. The lack of a significant mediation for the rate of change in memory can be explained in two ways. First, testing the *combined* effect of modifiable (e.g., lifestyle or vascular risk) and unmodifiable (e.g., genetic or pathological) factors [7], rather than either of them alone, might capture better the variability in trajectories. Second, the rate of *change* in MeDiAd or FA, rather than their level on a *single* occasion – as we had here –, could be associated with the rate of change in memory.

Four white-matter tracts were identified as potential mediators between MeDiAd and verbal episodic memory, based on the association with hippocampal volume, a well-validated AD biomarker [16,25]. Although FA of all four tracts correlated

with verbal episodic memory at baseline, only Fornix FA was associated with the latent verbal episodic memory four years later. This is in concert with the fornix's particular role in memory recall [22], regulation of learned aspects of food intake [26], and greater vulnerability (than other limbic tracts, e.g., parahippocampal cingulum) to early neurodegenerative processes in the course of AD, indicating that it could be a clinically useful biomarker for interventions in pre-dementia stages [27]. Acetylcholine, a neurotransmitter crucial for memory encoding, is sent from the medial septum/diagonal band of Broca to the hippocampus through the fornix [26].

Some limitations of our study ought to be considered. First, MeDiAd was based on participants' report. Dietary assessments relying on reports are generally limited by memory requirements [28]. We mitigated this impact by excluding the data of participants with marked cognitive impairment. Second, the identified mediation might reflect the influence of additional factors related to cognitive or brain reserve and brain maintenance, such as physical or cognitive activity levels [28]. Although those factors can hardly be dissociated from MeDiAd, we evaluated their relationships in this sample. There might be reverse causation due to preclinical disease affecting behavior in unknown ways, given that it can be present even within 15 years of follow-up [29]. Nevertheless, in our study, no baseline demographic or clinical variable was associated with MeDiAd, LGCM included baseline memory, and the sequence of events was handled by centering the latent intercept in the last time point. The fornix is surrounded by cerebrospinal fluid, which makes it vulnerable to partial volume effects (i.e., cerebrospinal fluid being included in the fornix's voxels) [26], a problem inherent to diffusion tensor imaging (DTI). Using FA weighted by the tracts' posterior probabilities yielded comparable results, but fully overcoming this limitation might require models beyond DTI. Finally, our results may not directly

generalize to patients with dementia, under/overweight older adults, or other ethnicities.

To conclude, our study demonstrated that higher MeDiAd contributes to better verbal episodic memory four years later through baseline Fornix FA. Our results imply that Fornix FA is a potential response biomarker of MeDi interventions on memory.

MATERIALS AND METHODS

Participants

Data from the DELCODE (German Center for Neurodegenerative Diseases Longitudinal Cognitive Impairment and Dementia Study) [30] cohort were used for the present study. The general procedure, study design, and selection criteria for DELCODE have been described earlier [30]. In short, participants with MCI and AD dementia fulfilled the respective clinical criteria according to the National Institute on Aging-Alzheimer's Association workgroup guidelines (MCI: [31]; probable AD dementia: [32]). Participants with or without SCD had no objective cognitive impairment in standard neuropsychological tests and no history of neurological or psychiatric disease. Contrary to those without, participants with SCD reported self-perceived cognitive decline unrelated to an acute event, which lasted for at least 6 months. All participants with available baseline DWI data were selected (*n* = 503; healthy controls: *n* = 123; SCD: *n* = 200; MCI: 82; AD dementia: *n* = 62; healthy relatives of patients with dementia: *n* = 36). Participant data were used for: (i) white-matter tract selection based on the association with hippocampal volume and (ii) testing the association between white-matter tracts, MeDiAd, and episodic memory. For (i) and (ii), participants with low head-motion (i.e., < 2 interquartile ranges, IQR, from the

sample median in the total motion index) in DWI were included (*n* = 486; 71.38 ± 6.37 years; age range: 59 – 90; 49.8% female). For (ii), only participants *without* dementia or family history of dementia were included (*n* = 405), as those with dementia lacked dietary data (because cognitive impairment makes retrospective data collection infeasible) and those with a family history of dementia constituted a different at-risk group exploratorily included in DELCODE [30]. From these 405, 10 data sets were excluded due to high head motion during DWI (i.e., > 2 IQR of the sample median); 7 were excluded due to high (i.e., > 2 IQR of the sample median) body mass index (BMI; as in [15]), and 5 had no episodic memory data. Finally, to reduce the influence of cognitive impairment on the food report, data from participants with baseline Mini-Mental State Examination (MMSE) ≤ 25 (*n* = 7) were excluded [1]. The final sample for (ii) was then *n* = 376 (71.47 ± 6.09 years; age range: 59 – 87; 48.7% females; Table 1), including healthy participants without a family history of dementia (*n* = 122), participants with SCD (*n* = 192), and patients with MCI (*n* = 62). Genetic risk for AD was identified for each participant based on the presence of at least one risk allele (i.e., ε4) in the ApoE gene [30] (1: present; 0: not present). All participants gave written informed consent prior to enrollment in DELCODE, which was approved by the local ethics committees of all participating centers and conducted in accordance with the Declaration of Helsinki [30].

Data missingness

Due to the selection procedure, all participants had DWI data. Almost two-thirds (61.7%, *n* = 232) had MeDi data, and one-third (35.4%, *n* = 133) had complete longitudinal memory data (10.1% had baseline data only). Little's MCAR test (X^2 (75, N = 376) = 164, $p < 0.0001$, missing patterns = 24) indicated that data were not

missing *completely* at random, i.e., missingness might depend on other observed variables. A multiple linear regression analysis revealed that missing data on the MeDiAd score was positively associated with SCD diagnosis (b = 0.87, SE = 0.29, *p* = 0.002) – while holding age, sex, education, MMSE, and site constant. Having more missing episodic memory scores (i.e., the sum of missing observations: “1” for each missing observation; “0” otherwise) was associated with both MCI (b = 0.67, SE = 0.22, *p* = 0.003) and SCD (b = 0.61, SE = 0.16, *p* = 0.0002) diagnosis, lower MMSE (b = -0.15, SE = 0.07, *p* = 0.038), and with one of the participating sites (b = 1.22, SE = 0.43, *p* = 0.005), while holding all the other variables constant. Therefore, to avoid potential bias in our longitudinal analyses, no cases with missing information were excluded, no comparisons between groups were performed, and MMSE scores were added as a control covariate in our longitudinal model (sensitivity analysis) to safely assume missingness at random [33] in implementing full information maximum likelihood.

Study setting

DELCODE is a multicenter cohort study across memory clinics in Germany. Baseline data were collected between April 2014 and August 2018, with the fourth yearly follow-up between June 2018 and February 2021. Two further follow-ups (fifth and sixth) were not considered for the present study because, at the time of the analyses (between August 2021 and March 2022), 83.78% and 97.07% of the sample had no episodic memory data in the fifth and sixth follow-ups, respectively. The mean follow-up duration for the current sample was 12.81 months (range: 8.2 – 28.0 months) and the mean total duration of follow-up was 4.19 years (range: 3.9 – 5.3 years).

Mediterranean diet adherence (MeDiAd)

Participants without dementia [30] filled out the European Investigation into Cancer and Nutrition (EPIC) Food Frequency Questionnaire (FFQ) [34] at baseline only. A value of 1 was assigned to the consumption of 'beneficial' components (i.e., fruit and nuts, legumes, vegetables, cereal, and fish) at or above the median or the consumption of 'detrimental' components (i.e., meat, high-fat dairy, and poultry) below the median, and 0 otherwise [35]. A value of 1 was assigned to moderate alcohol consumption (i.e., males/females: 10 – 50 / 5 – 25 g/day, respectively), and 0 otherwise [35]. Component values were added up (0 – 9), with a greater score indicating higher MeDiAd. MeDiAd data of a sample partly overlapping with that of the current study have been reported previously [19].

Verbal episodic memory

The 16-item Free and Cued Selective Reminding Test with Immediate Recall [36] was used. In the learning phase, four cards were presented individually. Each card depicts four objects of four different categories. Participants pointed to and named the object belonging to the category given by the examiner. Then the card was removed and participants were asked to name the object after a verbal cue and reminded of objects not recalled. After the learning phase, memory was tested by a free and cued recall. Free recall was used in the present study because it has no ceiling or floor effects and is a reliable and sensitive measure of episodic memory [37]. Verbal episodic memory was assessed at baseline and four follow-ups, one year apart.

Other measures

BMI was calculated as weight (kg)/height (m)^2. Physical activity was quantified with the Physical Activity Scale for the Elderly [38]. The MMSE [39] was used to assess global cognitive status; the Clinical Dementia Rating (CDR) scale – sum of boxes [40–42] was used to quantify global clinical state; and the geriatric depression scale [43] was used to assess depressive symptoms.

Diffusion MRI data

Acquisition

Diffusion data were acquired using DWI, with single-shot echo-planar imaging (EPI) in 3-Tesla MRI scanners (i.e., Siemens MAGNETOM TrioTim, Verio, Skyra, and Prisma; Siemens Healthcare, Erlangen, Germany). Acquisition parameters were the same across all scanners: 72 axial slices; repetition time = 12,100 ms; echo time = 88.0 ms; GRAPPA acceleration factor = 2; phase encoding = anterior-to-posterior; voxel size = 2.0 mm isotropic; field-of-view = 240 mm; matrix size = 120 × 120; flip angle = 90°; diffusion directions = 70 (10 without diffusion weighting); diffusion weightings: b-values = 700 and 1000 s/mm^2 (30 directions each); total acquisition time = 14 min 45 s.

Analysis

To identify white-matter tracts, we used TRACULA (TRActs Constrained by UnderLying Anatomy; https://surfer.nmr.mgh.harvard.edu/fswiki/Tracula) [44]. TRACULA automatically reconstructs tracts by using prior knowledge of the relative positions of white-matter pathways with respect to their surrounding anatomical structures. We selected the FA averaged over the entire path distribution for further

analysis. FA reflects the difference between a modeled ellipsoid and a perfect sphere in each white-matter voxel, thus representing the normalized variance, which ranges from 0 (no anisotropy) to 1 (high anisotropy) [14].

Major white-matter tract selection

We selected white-matter tracts (from the TRACULA set of 42) based on the association with right and left hippocampal volumes by multiple linear regressions in the entire sample adjusting for diagnosis group, DWI head motion, age, sex, education, MMSE, site, white matter hypointensities in T1, total gray matter volume, and the contralateral hippocampal volume. We followed this data-driven approach to reduce selection bias and select tracts relevant to the AD context, thereby controlling for Type-II and Type-I errors, respectively.

Anatomical imaging measures

A high resolution, T1-weighted, anatomical volume was acquired with a 3D magnetization prepared-rapid gradient echo (MPRAGE) sequence, with the following parameters: 192 sagittal slices; TR = 2500 ms; TE = 4.37 ms; GRAPPA acceleration factor = 2; phase encoding = anterior to posterior; voxel size = 1.0 mm isotropic; field-of-view, FOV = 256 mm; matrix size = 256 × 256; flip angle = 7°; inversion time, TI = 1100 ms; and TA = 5 min 8 s. This image was used for the tract reconstruction in the DWI analysis. Based on the T1-weighted, high-resolution MRI, total gray-matter volume, total white-matter hypointensities (i.e., a surrogate of white matter hyperintensities), and hippocampal volumes were computed using FreeSurfer [45,46].

Longitudinal analysis

LGCM [47] within a structural equation modeling framework was used. LGCM allows estimating between-person differences in within-person patterns of change over time [48] and explicitly handles measurement errors across time, thereby increasing power [49]. Individual longitudinal trajectories are captured by a latent intercept (mean) and a latent slope (rate of change). Using LGCM, we simultaneously tested (a) the association between baseline MeDiAd and verbal episodic memory mean *and* rate of change over four years (five time points) and (b) the mediation of this association via baseline FA of hippocampus-relevant white-matter tracts (Fig 1–2). The latent intercept was centered on the last time point, i.e., where the slope factor corresponded to zero ('Mem4', Fig 1). This factor-loading sequence permitted us to interpret the latent intercept as the 'level' of memory at the fifth time point (instead of at baseline). Model fit was evaluated with comparative fit index (CFI) or Tucker–Lewis index (TLI) ≥ .95; root mean square error of approximation (RMSEA) < .08; and standardized root mean square residual (SRMR) ≤ .08. The longitudinal measurement invariance (i.e., psychometric equivalence) of memory scores for the LGCM shown in Fig 1 was also tested. LGCM was conducted using *'lavaan'* (v. 0.6-11) [50] (https://lavaan.ugent.be/) in R (v. 4.2.0) [51].

Sensitivity analyses

We adjusted for MMSE, which represents the diagnosis groups, following the data missingness analysis. To confirm the hypothesized path sequence (MeDiAd → FA → memory), we swapped MeDiAd and FA in an alternative model (FA → MeDiAd → memory). Finally, we tested a model including baseline hippocampal volume as a mediator, additional to FA [19].

Other statistical analyses

Pearson's correlations and multiple regression were used to describe baseline associations between relevant variables. A two-tailed $\alpha = 0.05$ determined significance. Analyses were run on R.

Data availability

Data are available from DELCODE upon request. The analysis scripts used to generate these results are openly available and can be downloaded from [https://osf.io/8x7mc/?view_only=d9c58fbe68704a8e8503446065c3f779].

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CREDIT AUTHOR CONTRIBUTIONS

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D. Melo van Lent; R. Yakupov: Resources, Writing – review & editing.

N. Roy; A. Spottke: Investigation, Supervision.

F. Jessen; M. Wagner: Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

E. Düzel: Investigation, Resources, Writing – review & editing.

R. Perneczky; B. Rauchmann: Conceptualization, Investigation, Methodology, Writing – review & editing.

DELCODE Study Group: Data acquisition (see members in the Supplement)

POTENTIAL CONFLICTS OF INTEREST

Nothing to report

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FIGURE LEGENDS

Fig. 1. Latent growth curve model (LGCM). This LGCM tested the association of baseline Mediterranean diet adherence (MeDiAd) with the latent rate of change (S, slope) in the verbal episodic memory score (Mem) and its latent mean after four years (I, intercept), as indicated by the arrows labeled as “c1” and “c2,” respectively. The mediation of those associations through a hippocampus-relevant white-matter tract (Fornix) was also tested, as indicated by the arrows labeled as “b1” and “b2”. One-headed arrows indicate causal effects, whereas double-headed arrows indicate correlations or residuals. Dotted gray lines show fixed coefficients, whereas coefficients of the continuous black lines were estimated. The “e” in the Mem residuals indicates an equality constraint imposed for the analysis across time points (0, 1, 2, 3, 4).

Fig. 2. Hippocampus-relevant white-matter tracts. Major white matter tracts of which average fractional anisotropy was significantly associated with hippocampal volume across healthy participants and patients with MCI and AD dementia. Both the 2D images and the 3D render show the first healthy participant’s brain. The red dotted line on the coronal section (lower right) indicates the position of the sagittal silhouette (center). A: anterior, P: posterior, R: right.

Fig. 3. Individual longitudinal trajectories of verbal episodic memory scores across five time points. Scores in the Free and Cued Selective Reminding Test (FCSRT) - free recall, are shown for baseline (BL) and each one of four yearly follow-ups (FU). For visualization purposes, individual (thin) lines are shown

separately for each subgroup (healthy controls: HC; subjective cognitive decline: SCD; and mild cognitive impairment: MCI).

Fig. 4. Model estimates of the model with the Fornix FA as a single mediator of the association between Mediterranean adherence (MeDi) adherence and verbal episodic memory. The model structure is the same as in Fig. 1. I: latent intercept; S: latent slope. The unstandardized estimates of significant effects are underlined in red (all p-values < 0.038). The indirect effect (mediation) estimate of "Fornix → I" is depicted in gray font. See the summary results in Table 5.

TABLES

Table 1. Demographic, clinical, and MRI variables of the sample at baseline

Variable	**Overall sample**, *n* = 376 [a]
Demographic	
Age [years]	71.5 ± 6.1
Sex	
Male	193 (51.3%)
Education [years]	14.7 ± 3.0
Missing values	2
Diagnosis group	
Healthy older adults without a family history of dementia	122 (32.4%)
Older adults with subjective cognitive decline	192 (51.1%)
Patients with mild cognitive impairment	62 (16.5%)
Handedness	
Left-handed	15 (4.8%)
Right-handed	287 (91.1%)
Ambidextrous	13 (4.1%)
Missing values	61
Lifestyle	
MeDiAd / 9	4.6 ± 1.7
Missing values	144
Clinical and cognitive	
Clinical state [CDR (SumBoxes / 18)]	0.19 ± 0.68
Missing values	97
Depression [GDS / 15]	1.52 ± 1.89
Missing values	9
MMSE score / 30	29.2 ± 1.0
Genetic risk	

ApoE ε4 allele present	124 (33.3%)
Missing values	4
Neuroimaging	
Total motion index DWI	-0.1 ± 1.2

[a] Mean ± SD; *n* (%). The vast majority of the sample was of German/European origin and/or ancestry. *Abbreviations*: ApoE: apolipoprotein E; DWI: diffusion-weighted imaging; MeDiAd: Mediterranean diet adherence; MMSE: MiniMental state examination

Table 2. Bivariate correlations between the variables of interest at baseline

	Memory	**Right CST**	**Right Fornix**	**Left cingulum**	**Left SLF II**
MeDi	0.15 (***p* = 0.026**) *n* = 231	0.03 (*p* = 0.651) *n* = 232	0.15 (***p* = 0.025**) *n* = 232	-0.02 (*p* = 0.713) *n* = 232	-0.01 (*p* = 0.854) *n* = 232
Memory	-	0.13 (***p* = 0.013**) *n* = 373	0.30 (***p* < 0.001**) *n* = 373	0.21 (***p* < 0.001**) *n* = 373	0.10 (***p* = 0.042**) *n* = 373
Right CST	-	-	0.35 (***p* < 0.001**) *n* = 376	0.75 (***p* < 0.001**) *n* = 376	0.79 (***p* < 0.001**) *n* = 376
Right Fornix	-	-	-	0.41 (***p* < 0.001**) *n* = 376	0.22 (***p* < 0.001**) *n* = 376
Left cingulum	-	-	-	-	0.74 (***p* < 0.001**) *n* = 376

Note. CST = Corticospinal tract; SLF: superior longitudinal fasciculus II. Significant correlations are in bold.

Table 3. Longitudinal measurement invariance

Model	CFI	χ^2 (*df*)	AIC	BIC	ΔCFI	$\Delta\chi^2$ (Δdf)	*p*-value
Configural	0.991	14.6 (7)	2502.4	2553.5	-	-	-
Scalar	**0.921**	80.5 (11)	2560.3	2595.6	**0.07**	65.86 (4)	**< 0.001**
Residual	**0.906**	97.2 (15)	2569.0	2588.6	0.01	16.71 (4)	**0.002**

Note. Measurement models: configural (unconstrained, with free estimation of item intercepts), scalar (Configural + item intercepts constrained to be equal between the five time points), and residual (Scalar + item residuals constrained to be equal between all time points). Values in boldface indicate poor model fit or measurement non-invariance. AIC: Akaike information criterion; BIC: Bayesian information criterion; CFI: comparative fit index; df: degrees of freedom. Significant results are in bold face.

Table 4. Indirect effects (mediation) of each white-matter tract

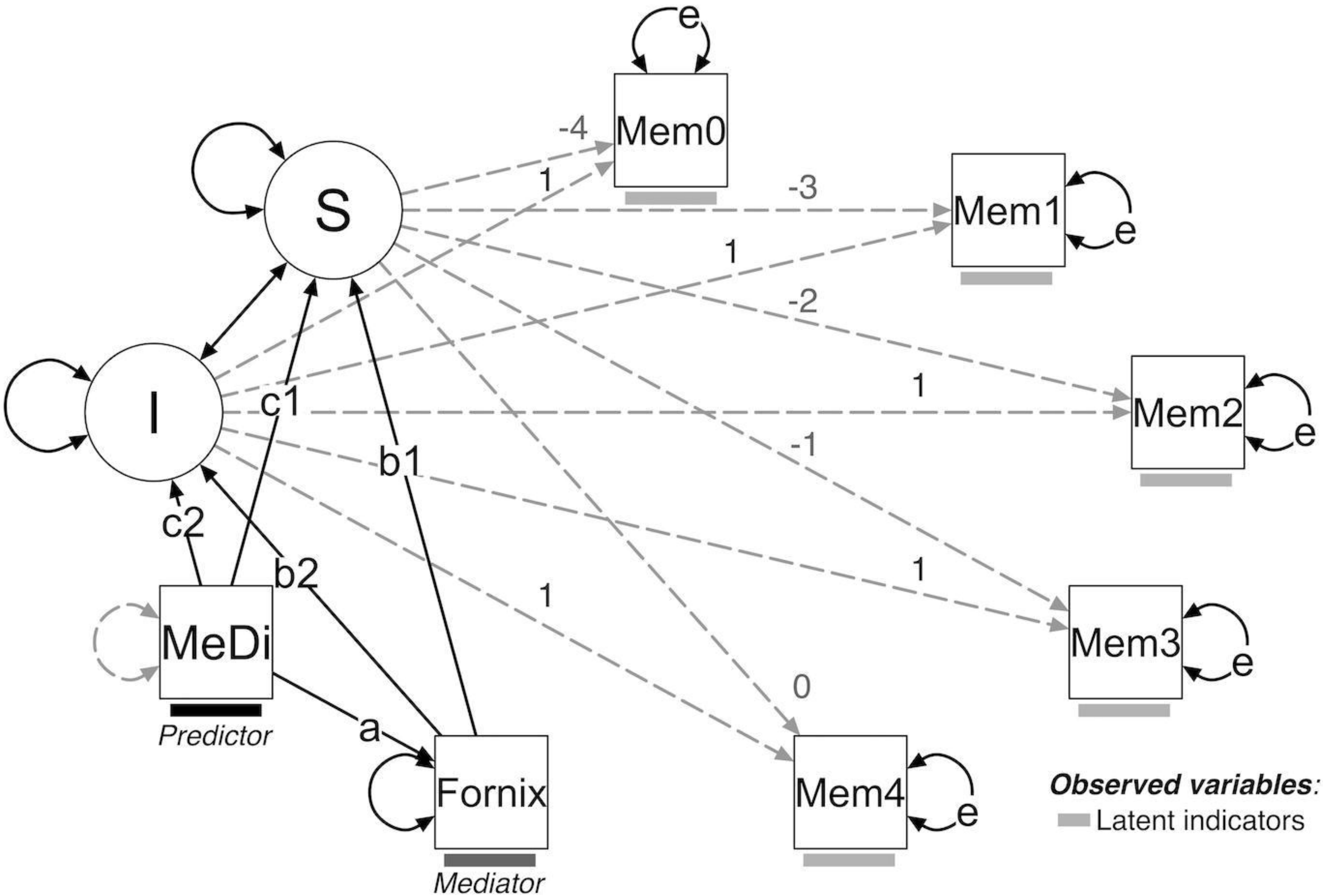
Mediator	β	b	SE	*p*-value	95% CI
MeDiAd → Verbal episodic memory's latent intercept (time point 5, after four years)					
Left cingulum ventral	-0.002	-0.002	0.009	0.845	-0.02, 0.02
Right fornix	0.04	0.04	0.02	**0.039**	**0.002, 0.09**
Right CST	-0.005	-0.005	0.01	0.598	-0.02, 0.01
Left SLF II	-0.0002	< -0.001	0.002	0.917	-0.004, 0.003
Total indirect effects	0.03	0.04	0.02	0.121	-0.01, 0.08
MeDiAd → Verbal episodic memory's latent slope (rate of change over four years)					
Left cingulum ventral	0.0002	< 0.001	< 0.001	0.918	-0.001, 0.001
Right fornix	0.02	0.003	0.003	0.229	-0.002, 0.01
Right CST	-0.002	< -0.001	0.001	0.764	-0.003, 0.002
Left SLF II	-0.001	< -0.001	0.001	0.901	-0.002, 0.002
Total indirect effects	0.02	0.003	0.003	0.337	-0.003, 0.01

Note. All tracts were included in one LGCM; 'Total indirect effects' is the sum of all individual indirect effects. CI: confidence interval; CST: corticospinal tract; SLF: superior longitudinal fasciculus

Table 5. Summary results of the single-mediator model (Fornix FA)

Path	β	b	SE	*p*-value	95% CI
MeDiAd → Slope	0.13	0.02	0.02	0.251	-0.01, 0.05
Fornix → Slope	0.13	0.02	0.01	0.151	-0.01, 0.05
MeDiAd → Intercept	0.15	0.16	0.08	**0.037**	**0.01, 0.31**
Fornix → Intercept	0.29	0.30	0.06	**< 0.001**	**0.18, 0.43**
MeDiAd → Fornix	0.15	0.15	0.07	**0.021**	**0.02, 0.28**
MeDiAd → Fornix → S	0.02	0.003	0.002	0.217	-0.002, 0.01
MeDiAd → Fornix → I	0.04	0.05	0.02	**0.033**	**0.004, 0.09**

Note. The two last rows show the indirect effects. CI: confidence interval; I, Intercept: latent intercept of verbal episodic memory; S, Slope: latent slope of verbal episodic memory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Mem1
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-1
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c2
b2
1
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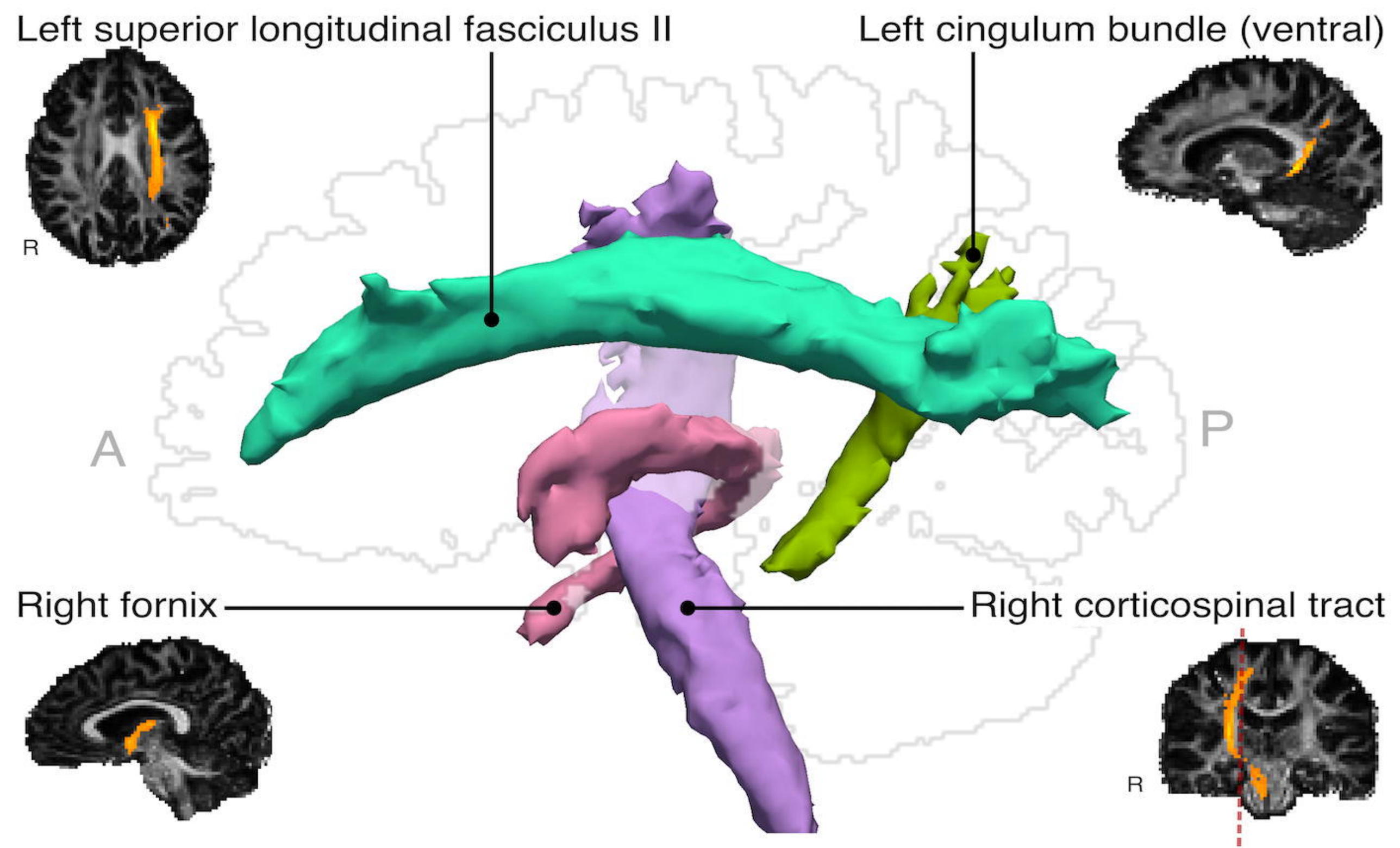
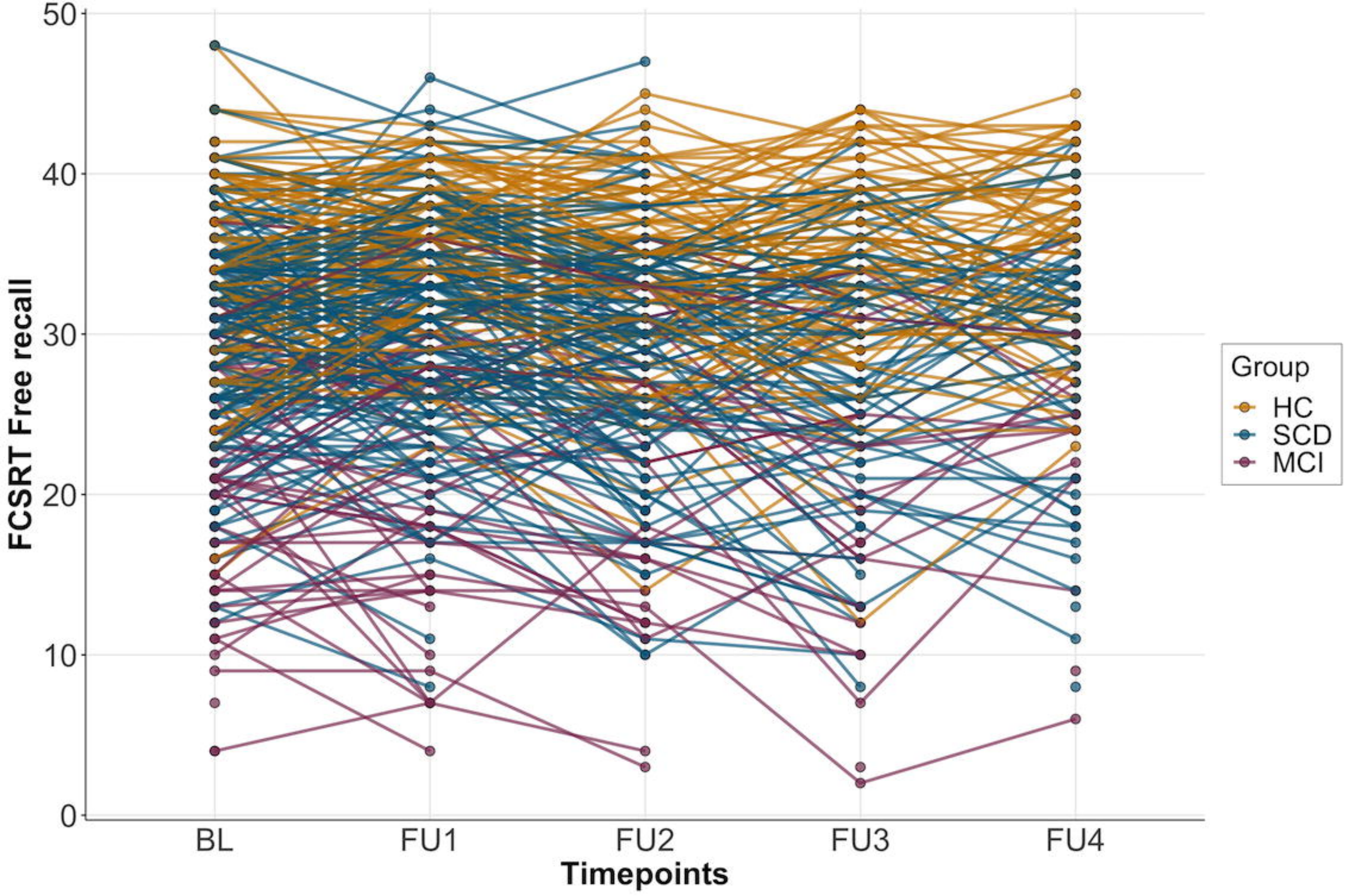
MeDi
Mem3
e
0
Predictor
a
Fornix
Mem4
e
Mediator
Observed variables:
Latent indicators


Left superior longitudinal fasciculus II
Left cingulum bundle (ventral)
R
A
P
Right fornix
Right corticospinal tract
R


50
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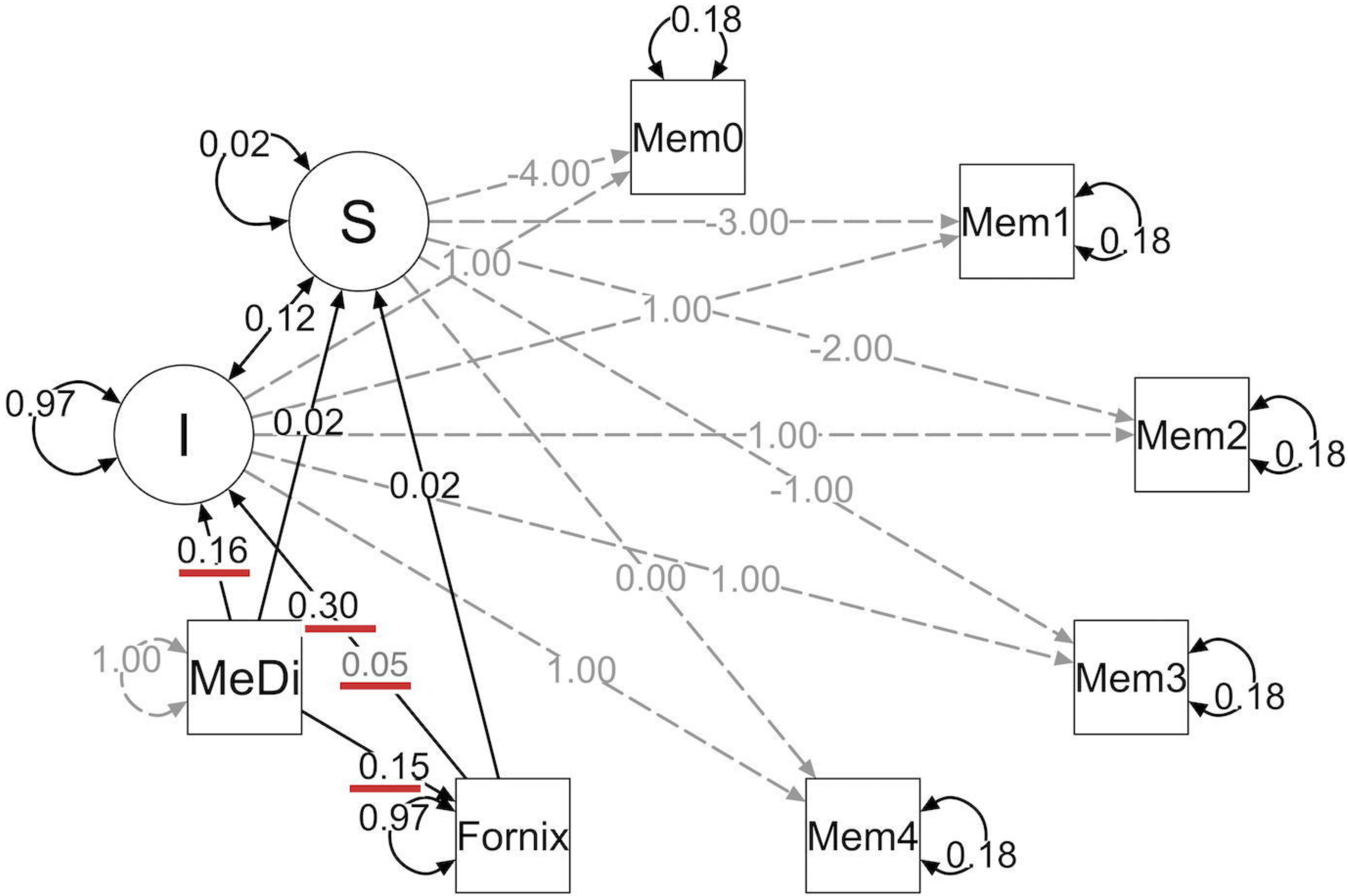
FCSRT Free recall
BL
FU1
FU2
FU3
FU4
Timepoints
Group
HC
SCD
MCI


0.18
Mem0
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S
-4.00
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Mem1
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MeDi
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Mem3
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Fornix
Mem4
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