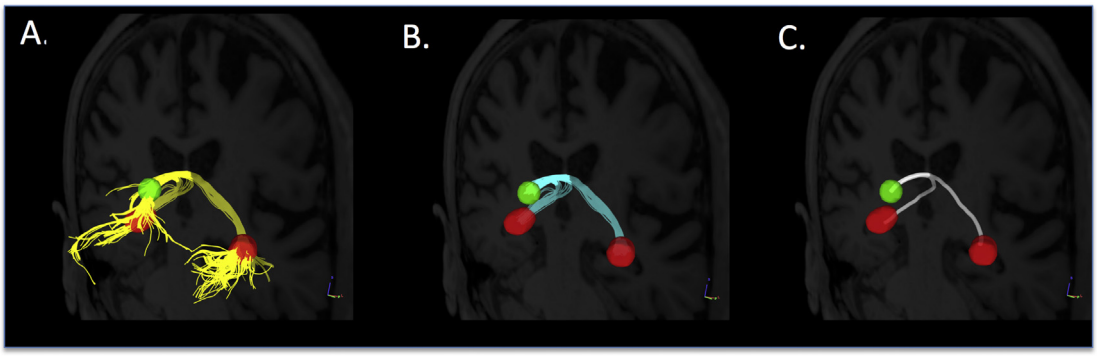

Fig. 3. Demonstration of streamline tractography and reconstruction of continuous fornix bundles. In every participant's native diffusion space, A) streamline tractography (yellow streamlines) was performed using the spherical (green) and fimbriae (red) regions of interest as separate seeds and limited by a subject-specific region of avoidance (not shown). B) To enable group comparisons guided by anatomically-based landmarks, the portion of each streamline that extended beyond the ROIs was trimmed, keeping only that portion between the ROIs (cyan). D) Finally, the single streamline with the highest average fractional anisotropy (white streamline) was selected for each participant and used to extract quantitative metrics for statistical analyses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

beginning at the midline and moving laterally to the left, then to the right. The coronal and axial views were used to verify tracings and to identify stopping points. Co-registered T2-weighted images were used as reference when needed to verify contrast differences (as between gray and white boundaries). The manual tracings included the body and the crus of the fornix, and the posterior aspect of the fimbria. Tracing of the body was stopped ventrally at the point corresponding to the first axial slice on which the columns of the fornix were clearly separated. Tracing of the fimbria stopped at the sagittal slice in which the hippocampal sulcus was no longer visible. The columns of the fornix, the alveus, and the anterior extent of the fimbria were not traced because they would not be included in the algorithmic method. Although manual tracing has an inherently subjective component, conservative criteria for labeling each voxel were applied to limit partial volume contamination. Volume of each manually traced mask was computed. Diffusion-weighted images were co-registered to the T1 image space for each participant. Diffusion metrics of fractional anisotropy, radial, axial, and mean diffusivity, GFA, and NQA0 were extracted from each manually traced mask and averaged across all voxels in the mask.

2.2.7. Streamline tractography for the genu of the corpus callosum

We applied a similar tractography methodology to the genu of the corpus callosum in each participant to determine whether diffusion characteristics were different between groups in another major bundle with curved anatomy that was not expected to be altered in Alzheimer's disease patients (Head et al., 2004; Di Paola et al., 2010). After manually identifying the most anterior coronal slice where the SDF image displayed a continuous connection of the corpus callosum (Supplementary Fig. S1-A), we moved 3 slices anteriorly and manually created two square ROIs (5 × 5 voxels) centered on the most anterior-posterior SDFs indicative of the anatomy of the genu on the left and right side (Supplementary Fig. S1-B). We performed streamline tractography using the two ROIs as filtered-in regions, applying 10,000 seeds in the left ROI, a 40-degree turning angle, a minimum and maximum length of 40 and 110, and using the automatically-calculated QA threshold using Otsu's method (Otsu, 1975). We trimmed these streamlines to contain only the portion between the ROIs (Supplementary Fig. S1-C, cyan) and isolated the streamline containing the highest average fractional anisotropy (Supplementary Fig. S1-D, white), from which we extracted fractional anisotropy and radial, axial, and mean diffusivity, as well as GFA and NQA0, as above.

2.3. Statistical analyses

Statistical analyses were performed using the statistical toolbox in Matlab R2016a (The Mathworks Inc., Natick, MA). Parametric analyses of variance (ANOVA) and chi-square tests were used to evaluate group differences in clinical and demographic characteristics. To investigate structural differences in the fornix or genu bundles, we used general linear models with volumetric and diffusion metrics (e.g. fornix bundle volume, fractional anisotropy, and radial, axial, and mean diffusivity, GFA, and NQA0) as dependent variables, diagnosis (normal control or Alzheimer's disease) as the independent variable, and co-varying for head motion. Estimated fornix volume was used as a covariate in analyses of the diffusion metrics. Fimbriae volumes were also entered as covariates when fimbriae ROIs were used as seeds. Absolute head motion during the scan acquisition was calculated using the b0 images spaced at every thirteenth diffusion image during acquisition. Because the diagnostic groups were age- and sex-matched, age and sex were not included as covariates. Due to our strong directional hypotheses that diffusion measures would be impaired in Alzheimer's disease relative to controls, alpha was set to 0.05 one-tailed for all analyses. Regional analyses of voxels along the fornix bundle were performed using 'randomise', a non-parametric permutation-based tool that corrects for multiple comparisons (Nichols and Holmes, 2002) and includes threshold-free cluster enhancement (Smith and Nichols, 2009). We set permutations to 5000 and described significant voxel locations at $p \leq 0.05$ corrected.

3. Results

3.1. Group characterization

Table 1 shows demographic characteristics, head motion during diffusion MRI acquisition, and MMSE scores of Alzheimer's and control participants. Due to matching, there were no significant differences in age or sex between the groups. There was no significant difference in head motion between groups ($p = 0.053$); nonetheless, head motion was used as a covariate in all subsequent analyses due to its potential impact on data quality (Mukherjee et al., 2008; Yendiki et al., 2014). As expected, Alzheimer's disease participants performed significantly worse on the MMSE.

3.2. Reconstruction and volumetric analyses

The volume estimates derived from the tractography reconstruction are displayed by group in Supplementary Table S1 and Supplementary Fig. S2. The estimated fornix volume did not differ significantly between the Alzheimer's disease and normal control groups in the left or right hemisphere either before ($p_{Left} = 0.36$, $p_{Right} = 0.11$) or after