The role of the fornix in human navigational learning

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Abstract

Studies in rodents have demonstrated that transecting the white matter pathway linking the hippocampus and anterior thalamic nuclei - the fornix - impairs flexible navigational learning in the Morris Water Maze (MWM), as well as similar spatial learning tasks. While diffusion MRI studies in humans have linked fornix microstructure to scene discrimination and memory, its role in human navigation is currently unknown. We used high-angular resolution diffusion MRI to ask whether inter-individual differences in fornix microstructure would be associated with spatial learning in a virtual MWM task. To increase sensitivity to individual learning across trials, we adopted a novel curve fitting approach to estimate a single index of learning rate. We found a significant correlation between learning rate and the microstructure (mean diffusivity) of the fornix, but not that of a control tract linking occipital and anterior temporal cortices (the inferior longitudinal fasciculus, ILF). Further, this correlation remained significant when controlling for hippocampal volume. These findings extend previous animal studies by demonstrating the functional relevance of the fornix for human navigational learning, and highlight the importance of a distributed neuroanatomical network, underpinned by key white matter pathways, such as the fornix, in complex spatial behaviour.

Key words: hippocampus; navigation; spatial learning; cognitive map; diffusion MRI; connectivity
Introduction

The ability to navigate, and learn the location of rewards and goals in the environment, is a fundamental and highly adaptive cognitive function across species (Landau and Lakusta, 2009; Wolbers and Hegarty, 2010; Murray et al., 2016).

Lesion studies in animals suggest that this ability depends, in part, on several key brain regions, including the hippocampus, mammillary bodies, and the anterior thalamic nuclei (Sutherland and Rodriguez, 1989; Warburton and Aggleton, 1998; Jankowski et al., 2013), which in turn connect with a broader network including entorhinal, parahippocampal, retrosplenial, and posterior parietal cortex, all thought to be important for navigation (Ekstrom et al., 2017). In particular, the hippocampus, mammillary bodies, and anterior thalamic nuclei are connected anatomically by an arch-shaped white matter pathway called the fornix (Saunders and Aggleton, 2007).

Given the role of these interconnected structures in spatial learning and navigation (Jankowski et al., 2013), the ability for these distributed regions to communicate via the fornix may also be critical for successful spatial learning and navigation.

Indeed, transecting the fornix in rodents and monkeys impairs learning for objects-in-place, but not the objects themselves (Gaffan, 1992, 1994; Simpson et al., 1998).

These findings also extend to performance on spatial navigation tasks, most notably the Morris Water Maze (MWM). The MWM is one of the most widely used laboratory tasks in studies of navigational behaviour across non-human species and has been recognized as an excellent candidate for a universal test of spatial navigation ability (Morris, 1984; Possin et al., 2016). In this task, animals are placed in a circular pool and required to swim to a hidden platform beneath the surface using allocentric cues.
outside the pool. Several studies have shown that fornix-transected rodents are impaired on the MWM, particularly when required to navigate flexibly from multiple positions within the maze (Eichenbaum et al., 1990; Packard and McGaugh, 1992; Warburton et al., 1998; Warburton and Aggleton, 1998; De Bruin et al., 2001; Cain et al., 2006). Fornix transection also impairs allocentric place learning in other maze tasks (O’Keefe et al., 1975; Olton et al., 1978; Packard et al., 1989; Dumont et al., 2015).

Critically, while these animal studies highlight a key role for the fornix in spatial learning - across both visuo-spatial discrimination and navigation tasks - the role of this white matter pathway in human wayfinding is currently unknown. Studies using diffusion magnetic resonance imaging (dMRI), which allows white matter microstructure to be quantified in vivo, have reported associations in healthy human subjects between fornix microstructure and inter-individual differences in scene and spatial context processing across both memory (Rudebeck et al., 2009; Hodgetts et al., 2017) and perceptual tasks (Postans et al., 2014; Hodgetts et al., 2015). Given differences in the visuospatial representations underpinning navigation across rodents and humans (Ekstrom, 2015), it begs the question whether this same extended functional system, structurally linked by the fornix, is similarly important for navigational learning in humans.

To test this, we acquired dMRI data in healthy human subjects who performed a human analogue of the MWM (Figure 1). In this task, individuals were required to learn, over trials, the location of a hidden sensor within a virtual art gallery. Similar to
the rodent paradigm, subjects were required to navigate from multiple starting
positions, thus placing greater demand on flexible allocentric processing (Figure 1).

To create a single index of navigational learning rate, we used a curve fitting
approach to model the time taken to reach the sensor across trials (for similar
approaches, see Stepanov and Abramson, 2008; Pereira and Burwell, 2015; Kahn et
al., 2017). We predicted, based on previous work (Packard and McGaugh, 1992;
Warburton and Aggleton, 1998; Cain et al., 2006; Hodgetts et al., 2015), that
microstructure of the fornix, but not a control tract connecting occipital and anterior
temporal cortices (the “inferior longitudinal fasciculus”, ILF) (Latini, 2015), would be
significantly related to spatial learning rate in a virtual MWM task.

Methods

Participants

Thirty-three healthy volunteers (15 males, 18 females; mean age = 24 years; SD =
3.5 years) were scanned at the Cardiff University Brain Research Imaging Centre
(CUBRIC). These same participants completed a virtual Morris Water Maze task in a
separate behavioural session. All subjects were fluent English speakers with normal
or corrected-to-normal vision. Participation in both sessions was undertaken with the
understanding and written consent of each subject. The research was completed in
accordance with, and approved by, the Cardiff University School of Psychology
Research Ethics Committee.
Figure 1. The virtual reality Morris Water Maze. (A) Birds-eye schematic of the virtual art gallery that the participants explore during the task. The artwork on the outer walls of the gallery are the “landmarks” in the virtual arena. An example first person perspective from within the maze is shown. (B) Movement trajectories and (C) location heatmap across all 20 trials for an example participant.

Virtual Morris Water Maze Task

We used the virtual MWM task developed by Kolarik et al. (2016). This task was created using Unity 3D (Unity Technologies, San Francisco) and required participants to explore, from a first-person perspective, a virtual art gallery using the arrow keys on the computer keyboard (Figure 1A). The room was 8 x 8 virtual m² in
size, and contained four distinct paintings, one on each wall of the environment. On a given trial, the participants’ task was to locate a hidden sensor on the floor as quickly as possible. This sensor occupied 0.25% of the total floor space (i.e., an 0.8 x 0.8 m² square). When the participant walked over the hidden platform it became visible and the caption ‘You found the hidden sensor’ was displayed in the centre of the screen. At this point, the exploration time was recorded automatically and a 10 second countdown appeared in the centre of the display during which the participants could freely navigate the room. After this countdown, an inter-trial window appeared and the participants could click on a button to start the next learning trial. The maximum duration of each learning trial was 60 seconds. If the participant did not find the target location within this period, the sensor became visible. The task involved 20 learning trials, which comprised five blocks of four trials. Within each block, participants started from each of the four starting positions (arbitrary North, South, East, West). The movement trajectories and location heatmap for an example participant is shown in Figure 1B-C.

**MRI acquisition**

Whole brain dMRI data were acquired at the Cardiff University Brain Research Imaging Centre (CUBRIC) using a 3T GE HDx Signa scanner with an eight-channel head coil. Single-shell high-angular resolution dMRI (HARDI) (Tuch et al., 2002) data were collected with a single-shot spin-echo echo-planar imaging pulse sequence with the following parameters: 30 directions; TE= 87 ms; 60 continuous slices acquired along an oblique-axial plane with 2.4 mm thickness and no gap. The scans were cardiac-gated using a peripheral pulse oximeter placed on the participants’ fingertips. A T1-weighted 3D FSPGR sequence was also acquired with the following...
parameters: TR= 7.8 ms; TE= 3 ms, TI= 450 ms, flip angle= 20°; FOV= 256 mm*192 mm*172 mm; 1 mm isotropic resolution.

Diffusion MRI preprocessing

Diffusion MRI data were corrected for subject head motion and eddy currents using ExploreDTI (Version 4.8.3; Leemans and Jones, 2009). The bi-tensor 'Free Water Elimination' (FWE) procedure was applied post hoc to correct for voxel-wise partial volume artifacts arising from free water contamination (Pasternak et al., 2009). Free water contamination (from cerebrospinal fluid) is a particular issue for white matter pathways located near the ventricles (such as the fornix), and has been shown to significantly affect tract delineation (Concha et al., 2005). Following FWE, corrected diffusion-tensor indices FA and MD were computed. FA reflects the extent to which diffusion within biological tissue is anisotropic, or constrained along a single axis, and can range from 0 (fully isotropic) to 1 (fully anisotropic). MD (10^{-3}mm^2s^{-1}) reflects a combined average of axial diffusion (diffusion along the principal axis) and radial diffusion (diffusion along the orthogonal direction).

Tractography

Deterministic whole brain white matter tractography was performed using the ExploreDTI graphical toolbox. Tractography was based on constrained spherical deconvolution (CSD) (Jeurissen et al., 2011), which can extract multiple peaks in the fiber orientation density function (fODF) at each voxel. This approach permits the representation of crossing/kissing fibers in individual voxels. Each streamline was reconstructed using an fODF amplitude threshold of 0.1 and a step size of 1 mm, and followed the peak in the fODF that subtended the smallest step-wise change in
orientation. An angle threshold of 30° was used and any streamlines exceeding this threshold were terminated.

Three-dimensional reconstructions of each tract were obtained from individual subjects by using a waypoint region of interest (ROI) approach, based on an anatomical prescription. Here, “AND” and “NOT” gates were applied, and combined, to extract tracts from each subject’s whole brain tractography data. These ROIs were drawn manually on the direction-encoded FA maps in native space by one experimenter (MS) and quality assessed by other experimenters (CJH, ANW).

**Fornix**

A multiple region-of-interest (ROI) approach was adopted to reconstruct the fornix (Metzler-Baddeley et al., 2011). This approach involved placing a seed point ROI on the coronal plane at the point where the anterior pillars enter the fornix body. Using a mid-sagittal plane as a guide, a single AND ROI was positioned on the axial plane, encompassing both crus fornici at the lower part of the splenium of the corpus callosum. Three NOT ROIs were then placed: (1) anterior to the fornix pillars; (2) posterior to the crus fornici; and (3) on the axial plane, intersecting the corpus callosum. Once these ROIs were placed, and the tracts reconstructed, anatomically implausible fibers were removed using additional NOT ROIs (see Hodgetts et al., 2017).

**Inferior longitudinal fasciculus (ILF)**

Fiber-tracking of the ILF (control tract) was performed using a two-ROI approach in each hemisphere (Wakana et al., 2007). First, the posterior edge of the cingulum...
bundle was identified on the sagittal plane. Reverting to a coronal plane at this position, a SEED ROI was placed that encompassed the whole hemisphere. To isolate streamlines extending towards the anterior temporal lobe (ATL), a second ROI was drawn at the most posterior coronal slice in which the temporal lobe was not connected to the frontal lobe. Here, an additional AND ROI was drawn around the entire temporal lobe. Similar to the fornix protocol above, any anatomically implausible streamlines were removed using additional NOT ROIs. This approach was carried out in both hemispheres; diffusion properties of the left and right ILF (for both FA and MD) were averaged across hemispheres to provide a bilateral measure of ILF FA and MD in each participant.

**Grey matter volumetry**

Bilateral hippocampal volume was derived using FMRIB's Integrated Registration & Segmentation Tool (FIRST; Patenaude et al., 2012). As temporal lobe substructures have been shown to correlate with intracranial volume (Moran et al., 2001), individual-level hippocampal volumes were divided by total intracranial volume (eTIV) to create proportional scores (Westman et al., 2013).

**Statistical analysis of maze learning**

To increase sensitivity to individual-level performance across learning trials, and to derive a single index of learning rate, we analysed the relationship between spatial learning and fornix tissue microstructure using a curve fitting approach (see e.g., Pereira and Burwell, 2015; Kahn et al., 2017). Performance on each learning trial was defined by the time (in seconds) to reach the hidden sensor. As can be seen in Figure 2A, there was high inter-individual variability in spatial learning, with subjects
varying in both learning speed and the shape of their learning pattern. Here, individual learning data was fit using a power function: \( \text{Time to sensor} = a \times x^b \), where \( b \) specifies the slope of the fitted power model.

One aspect of this data is that some subjects learned quickly (and plateaued) before displaying variable, or slow, performance in the later trials (e.g., subjects 9, 13, and 20; Figure 2B). This presents a challenge for a curve fitting approach across all trials (and potentially produces counterintuitive results), as some of the fastest learners will show the poorest model fits. For instance, both subjects 9 and 16 display an initial steep learning curve and an early plateau (Figure 2B), but a power model fit to all trials provides a poor fit of the subject who does not sustain performance until the end of the task. In order to account for this complexity in learning patterns, we adopted a data-driven approach to determine a cut-off in individual subjects. Specifically, a second-order polynomial model was fit to all trials in each subject using the curve fitting toolbox in Matlab (Mathworks, Inc.). The cut-off was defined as the trough of this curve, which is where the first derivative of the second-degree polynomial crosses zero (Figure 2C). Trials up to and including this cut-off were then modelled using a power function (mean trials included = 14.3; range = 7 – 20).
Figure 2. Modelling navigational learning in individual participants. MWM task learning at the (A) group-level and (B) individual-level. Y-axes represent the time to reach the hidden sensor in seconds. The number of trials (total = 20) is shown on the x-axis. (C) Method for determining the number of learning trials to-be-modelled. Some participants appeared to learn rapidly and plateau before displaying variable performance in later trials. For instance, a power model fits the example participant’s latency data poorly when all trials are considered. In order to capture initial learning, therefore, we fitted the latency data (across all trials) with a second-order polynomial in each subject. The point at which the first derivative of this polynomial crossed zero was used to define the number of trials to-be-modelled. The trials up to this point were then fit with a power function and the b parameter derived to index learning rate. Power fits are shown by linearly fitting the log-transformed data. (D) Learning rate measures were correlated with diffusion metrics (FA, MD) from the fornix (blue) and the ILF (yellow). Tract reconstructions are shown against an inflated brain for visualisation purposes.
Using this approach, we derived a single measure of learning rate, denoted by the \( b \) parameter (or slope) of the fitted power model (\( b; \text{mean} = -0.32, \text{SD} = 0.08, \text{range} = -0.49 \text{ to } -0.19 \)). The \( b \) parameter reflects slope curvilinearity in each subject, where lower, negative values reflect more convex downward curves and thus faster learning rates. As such, we predict a positive association between fornix MD and learning rate, and negative associations between fornix FA and learning rate.

Directional Pearson correlations were conducted between the learning rate and free water corrected MD and FA values for the fornix and ILF (Figure 2D). The resulting coefficients were compared statistically using directional Steiger Z-tests (Steiger, 1980) within the ‘cocor’ package in R (Diedenhofen and Musch, 2015). Pearson correlations were Bonferroni-corrected by dividing \( \alpha = 0.05 \) by the number of statistical comparisons for each DTI metric (i.e., \( 0.05/2 = 0.025 \)) (Lakens, 2016). Prior to correlational analyses, outliers for each tract and metric were identified and removed using the Tukey method in R. This excluded an extreme value for fornix MD, fornix FA, and ILF FA. To exclude poor performers who were not engaging with the task, we used a resampling approach where individual-level data was shuffled over 500 permutations and confidence intervals (CIs) derived. Participants with a model \( R^2 \) that fell outside the CI of their individually-defined random distribution were excluded (Subjects 10, 15, 17, 18 and 21).

We also conducted Bayesian correlation analyses using JASP (https://jasp-stats.org). From this, we report default Bayes factors and 95% Bayesian credibility intervals (BCI). The Bayes factor, expressed as \( BF_{10} \) grades the intensity of the evidence that the data provide for the alternative hypothesis (H1) versus the null
(H0) on a continuous scale. A $BF_{10}$ of 1 indicates that the observed finding is equally likely under the null and the alternative hypothesis. A $BF_{10}$ much greater than 1 allows us to conclude that there is substantial evidence for the alternative over the null. Conversely $BF_{10}$ values substantially less than 1 provide strong evidence in favour of the null over the alternative hypothesis (Wetzels and Wagenmakers, 2012).

Complementary Spearman’s rho tests were also conducted for our key correlations. The strength of Spearman’s correlations were compared directly using a robust bootstrapping approach (Wilcox, 2016), as implemented using ‘comp2dcorr’ in Matlab (https://github.com/GRousselet/blog/tree/master/comp2dcorr).

**Results**

**Correlating navigational learning with tract microstructure**

There was a significant positive correlation between the derived learning rate and fornix MD, as shown in Figure 3. This suggests that those subjects with lower fornix MD had faster learning rates ($r = 0.44, p = 0.01, 95\% \text{ BCI } [0.09, 0.68], B_{+0} = 5.5$; Figure 3). There was no significant relationship between individual learning rate and MD in a control tract - the inferior longitudinal fasciculus (ILF; $r = -0.06; p = 0.62$, 95\% BCI [0.37, 0.01], $B_{0+} = 5.38$). A directional Steiger Z-test (Steiger, 1980) revealed that the correlation between derived learning rate and fornix MD was significantly greater than with ILF MD ($z = 2.26, p = 0.01$).

A moderate trend was observed between fornix FA and learning rate but this did not reach our experiment-wise significance level ($r = -0.34, p = 0.04, 95\% \text{ BCI } [-0.62, -0.04], B_{-0} = 1.99$; Figure 3). There was no significant correlation between ILF FA and
learning rate \((r = -0.17; p = 0.2, \text{95\% BCI }[-0.51, -0.01], B_{0} = 1.68)\). These two correlations did not differ significantly \((z = 0.22, p = 0.21)\).

\hspace{1cm}

Figure 3. The correlation between tract microstructure and learning rate (b parameter) for the fornix (top row) and the inferior longitudinal fasciculus (ILF).

Controlling for hippocampal volume

To examine whether hippocampal volume contributes to the microstructural-behavioural correlations reported above, partial correlations (both frequentist and Bayesian) were conducted. The significant positive correlation between the learning rate parameter and fornix MD remained when controlling for bilateral hippocampal volume \((r = 0.4, p = 0.02, BF_{0} = 3.59)\), as seen in prior studies (Hodgetts et al.,...
2017). For fornix FA, a slightly stronger negative trend was observed ($r = -0.35$, $p = 0.04$, $BF_{-0} = 0.09$) when hippocampal volume was controlled for, though this did not reach our experiment-wise significance level (i.e., $p = 0.025$). When examining hippocampal volume, independent of fornix microstructural measures, there was no significant association found between hippocampal volume and learning rate ($r = 0.03$, $p = 0.94$, 95% BCI [-0.25, -0.002], $B_{-0} = 10.2$).

Non-parametric correlations between tract microstructure and learning

Finally, we also conducted complementary directional Spearman’s rho tests for our key correlations, with such tests robust to univariate outliers (Croux and Dehon, 2010). As above, Spearman’s correlations were Bonferroni-corrected by dividing $\alpha = 0.05$ by the number of statistical comparisons for each DTI metric (i.e., $0.05/2 = 0.025$). A significant positive association was observed between learning rate and fornix MD ($\rho = 0.4$, $p = 0.02$). No significant association was found with ILF MD ($\rho = -0.18$, $p = 0.82$). A strong trend was found between the $b$ parameter and fornix FA ($\rho = -0.32$, $p = 0.05$) but not ILF FA ($\rho = -0.21$, $p = 0.14$).

A direct comparison between these correlations revealed a significant difference between fornix MD and ILD MD and their association with navigation learning rate, as indicated by the bootstrap distribution not overlapping with zero (95% CI = 0.2 – 0.88, $p = 0$). There was no significant difference between the FA correlations (95% CI = -0.7191 - 0.2962, $p = 0.4$).
General discussion

Using a virtual-reality analogue of a classic navigational paradigm, the Morris Water Maze (Morris, 1984), we asked whether inter-individual variation in the microstructure of the fornix (linking hippocampus with medial diencephalon and prefrontal cortex) is related to individual differences in navigational learning. To increase sensitivity to individual learning across trials we adopted a curve fitting approach (Kahn et al., 2017), which generated a single index of learning rate (‘b’) in each individual. We found that fornix microstructure (particularly MD) was significantly associated with navigational learning rate in a virtual MWM task, as defined by the slope of the fitted power model, and this association remained when controlling for bilateral hippocampal volume. Furthermore, this effect was significantly stronger than that seen for the ILF, a control tract linking occipital and anterior temporal cortices, which has previously been implicated in semantic learning (Qi et al., 2015; Ripollés et al., 2017).

These results build upon previous animal studies that highlight a potential key role for the fornix in mediating place learning and navigational behaviour. Critically, we provide novel evidence, using a MWM task analogous to that used in animals (Kolarik et al., 2016; Possin et al., 2016), that the fornix supports navigational learning in humans. In rodents, fornix transection has been shown to impair MWM learning, as characterised by more gradual learning slopes and slower latencies in finding the hidden platform (Eichenbaum et al., 1990; Packard and McGaugh, 1992; Warburton and Aggleton, 1998; Cain et al., 2006). By applying a curve fitting approach, we were able to characterise the steepness of learning slopes at the
individual participant level, and relate this directly with fornix microstructure.

Strikingly consistent with the animal studies described above, reduced structural connectivity in the fornix (indexed by higher MD) was related to more gradual learning rates. Further, by identifying individual learning plateaus in a data-driven way, this approach also accounts for potential fatigue, mind-wandering or other factors that may affect performance later in the learning session.

Similar to lesioning hippocampus and anterior thalamic nuclei, learning deficits following fornix transection in rodents are also more severe when the animal is required to navigate from multiple start positions (Eichenbaum et al., 1990). Such findings suggest, therefore, that this broader neuroanatomical system, structurally underpinned by the fornix (Aggleton et al., 2010), supports spatial learning in a flexible manner (i.e., from novel start positions, or from different perspectives), rather than response-based learning, that appears to recruit regions outside this extended hippocampal system, specifically the caudate nucleus (Packard and McGaugh, 1992; Devan et al., 1996; Chersi and Burgess, 2015). Consistent with this, we observed an association between navigational learning and fornix properties in a task which required participants to navigate to the goal from multiple starting positions.

Overall, this study provides support for the idea that an individual’s spatial navigation ability (Wolbers and Hegarty, 2010) is underpinned, at least in part, by the integrated functioning of a distributed neuroanatomical network, comprising not only individual regions (such as the hippocampus and anterior thalamic nuclei), but also the white
matter connections linking these brain areas (Jankowski et al., 2013; Murray et al., 2016). While MWM performance is considered to depend, at least partly, on the ability to form and utilise detailed allocentric mental representations, or “cognitive maps” (Tolman, 1948; O'Keefe and Nadel, 1976), human and animal studies suggest that the role of the fornix in spatial processing may be linked to mechanisms beyond spatial mapping per se.

For instance, while fornix transection impairs, or at least slows, navigational learning in the MWM (Warburton and Aggleton, 1998), as discussed above, these impairments are not as severe as that seen following lesions to the anterior thalamic nuclei or the hippocampus proper (Eichenbaum et al., 1990; Warburton and Aggleton, 1998; Cain et al., 2006). This is not to suggest that fornix connectivity is not important for place representations (Miller and Best, 1980; Shapiro et al., 1989), but rather that the fornix may support processes which help build and support detailed cognitive maps (e.g., scene-based processing, path integration) in conjunction with other brain areas involved in a broader navigation network (Whishaw and Maaswinkel, 1998; Gaffan et al., 2001). For instance, evidence from non-human primates suggests a potential key role in forming conjunctive scene representations (Gaffan, 1991; Hodgetts et al., 2015; Murray et al., 2017). The ability to learn and remember object-in-scene associations, as well as naturalistic scenes, is impaired significantly following fornixectomy (Gaffan, 1992; Gaffan et al., 2001; Buckley et al., 2008).
Convergent with scene learning deficits reported in monkeys, diffusion MRI studies in humans have reported associations between fornix microstructure and scene recollection (Rudebeck et al., 2009), complex scene discrimination (Postans et al., 2014; Hodgetts et al., 2015) and the ability to retrieve spatiotemporal detail in real-world memories (Hodgetts et al., 2017). Rather than suggesting a selective role in allocentric spatial navigation per se, these studies support the view that the connections established by the fornix may be critical for integrating scenes into coherent spatial representations, which then may contribute to the generation of detailed map-like representations useful for navigation (Ryan et al., 2010; Fidalgo and Martin, 2016). An alternative account (Relational Memory Theory), by contrast, posits that while the extended hippocampal system is essential to spatial navigation via a cognitive map, its role derives from the relational organization and flexibility of cognitive maps and not from a selective role in the spatial domain (Eichenbaum, 2017; see also Ekstrom and Ranganath, 2017). The initial formation of such flexible spatial relations has been argued to critically rely on cholinergic system modulation of the hippocampus (Ikonen et al., 2002), which is dependent on the fornix (Alonso et al., 1996), consistent with our findings.

Note, it is possible that some individual differences in navigational performance may actually reflect differences in types of spatial strategies employed. For instance, while some individuals may use a strategy akin to cognitive mapping, i.e., based on allocentric vectors from the “landmarks” to the hidden sensor, some individuals may use a strategy based on matching and integrating disparate viewpoints from the sensor location; a strategy more akin to building a model of the broader scene and
While participants were not asked about their use of spatial strategies in the current study, this would be an interesting avenue for disentangling scene-based and cognitive mapping approaches in future studies.

While our findings support the notion that an extended hippocampal-based system, mediated by the fornix, may be important for navigational learning in humans, it was notable that the fornix association was present when controlling for HC volume. Further, there was no independent association between place learning and HC volume in this task. Though some studies have found associations between hippocampal grey matter volume and navigational ability in humans (Maguire et al., 1997; Bohbot et al., 1998; Schinazi et al., 2013; Chrastil et al., 2017), others have shown that fornix microstructure (but not hippocampal volume) predicts individual differences in remembering spatiotemporal aspects of autobiographical memories (e.g., Hodgetts et al., 2017). In addition, studies of individuals with profound orientation deficits (termed development topographical disorientation, or DTD) similarly show impairments in connectivity patterns to the hippocampus (in this case, between hippocampus and prefrontal cortex). Interestingly, like in our study, hippocampal grey matter does not appear to explain these differences (Iaria et al., 2009; Iaria and Barton, 2010). This highlights that variation in broader neuroanatomical systems, rather than regional volumetric variation, may be particularly sensitive to individual differences in navigational learning.

Similar to our previous work, we observed stronger effects for fornix MD versus FA (Postans et al., 2014; Hodgetts et al., 2015). The biological interpretation of this
difference is not straightforward, as variation in either measure could arise from multiple aspect(s) of the underlying white matter, including axon density, axon diameter, myelination, and the manner in which fibres are arranged in a voxel (Beaulieu, 2002). A recent study reported strong correspondence between DTI microstructural indices and underlying myelin microstructure, where high FA was linked to high myelin density and a sharply tuned histological orientation profile, whereas high MD was related to diffuse histological orientation and low myelin density (Seehaus et al., 2015). Diffusion MRI studies applying more advanced biophysical models of white matter microstructure may be able to provide additional insight into the specific biological attributes underlying these brain-behaviour associations (Assaf et al., 2017; Huber et al., 2018).

The causes of inter-individual variation in white matter microstructure are not fully understood, but likely involve a complex interplay between genetic and environmental factors over the lifespan. Evidence from both adults and neonates, for instance, suggests that the microstructure of the fornix is highly heritable (Lee et al., 2015; Budisavljevic et al., 2016). The fornix is also one of the earliest white matter tracts to mature, reaching its peak FA and minimum MD before age 20 (Lebel et al., 2012), and potentially nearing maturation during infancy and childhood (Dubois et al., 2008). At the same time, evidence suggests that fornix microstructure displays learning-related plasticity, even over short time periods. For instance, short-term spatial learning, in both rodents and humans, has been shown to induce alterations in diffusion indices of fornix microstructure (Hofstetter et al., 2013). Similarly, navigational ability is influenced by both genetic factors and experience (Lee and Spelke, 2010; Wolbers and Hegarty, 2010). Thus, fornix microstructure is likely to
both shape, and be shaped by spatial navigation, in a bidirectional fashion (Bechler et al., 2018).

To conclude, by modelling learning performance on a virtual-reality water maze, we showed that the microstructure of the main white matter pathway linking the hippocampus and medial diencephalon – the fornix – predicted individual differences in human navigational learning. These results suggest that a full understanding of the biological underpinnings of individual differences in human navigational ability requires not only the analysis of individual processing regions, but of a distributed “navigation system”, underpinned by white matter. Critically, given the vulnerability of this brain system to the deleterious effects of aging (Lester et al., 2017), but also pathology in Alzheimer’s disease (Braak and Braak, 1991; Oishi et al., 2012), it is a key priority to develop behavioural markers of navigational ability that are sensitive to individual variation in this network, as seen here. One study in rodents, for instance, found that poorer learning on the MWM in early life predicted cognitive impairment in later life, but also that extensive training in poorer learners buffered against age-related learning impairments (Hullinger and Burger, 2015). Studies such as this highlight the potential of navigational learning, particularly as assessed using translation paradigms (Possin et al., 2016), for characterising, and potentially ameliorating, the effects of cognitive decline.
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Author contributions

CJH, MS, BK, ADE and KSG contributed to the conception and design of the experiment; MS collected imaging and behavioural data; CJH, MS, ANW, BK and JZ analysed the data; CJH wrote the manuscript with input from all other authors.

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