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In vivo mapping of macroscopic neuronal projections in the mouse hippocampus using high-resolution diffusion MRI

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ABSTRACT

Recent developments in diffusion magnetic resonance imaging (MRI) make it a promising tool for non-invasive mapping of the spatial organization of axonal and dendritic networks in gray matter regions of the brain. Given the complex cellular environments, in which these networks reside, evidence on the capability of diffusion MRI-based tractography to study these networks is still lacking. In this study, we used a localized diffusion MRI approach to acquire high spatial and angular resolution images of the live mouse hippocampus. The diffusion MRI and tractography results were compared with histology and the Allen mouse brain connectivity atlas using a multi-step image registration pipeline. The results demonstrated that *in vivo* diffusion MRI data at 0.1 mm isotropic resolution revealed the organization of axonal and dendritic networks in the hippocampus and the tractography results shared remarkable similarity with the viral tracer data in term of their spatial projection patterns. Quantitative analysis showed significant correlations between tractography- and tracer-based projection density measurements in the mouse hippocampus. These findings suggest that high-resolution diffusion MRI and tractography can reveal macroscopic neuronal projections in the mouse hippocampus and are important for future development of advanced tractography methods.

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Introduction

Mapping structural connectivity of the brain is critical for understanding its structural and functional organization. An array of imaging techniques has been used to dissect structural connectivity from the synaptic level using electron micrograph (Helmstaedter et al., 2013; Takemura et al., 2013) up to the system level using magnetic resonance imaging (MRI) (Lazar, 2010; Van Essen et al., 2013). The last 15 years have witnessed rapid development in diffusion MRI-based tract reconstruction, or tractography (Mori and van Zijl, 2002; Tournier et al., 2007; Tuch et al., 2002; Wedeen et al., 2008). To date, it is the primary tool for non-invasive mapping of large white matter tracts in the brain and an important component of the Human Connectome Project (Toga et al., 2012; Van Essen et al., 2013).

With rapid advances in MRI techniques, high-resolution diffusion MRI data from the human brain are increasingly available (Gaggl et al., 2014; Setsompop et al., 2013; Uğurbil et al., 2013), and increasing efforts have been made towards tracing small neuronal connections deep in gray matter regions, e.g., small white matter tracts and axonal projections in the hippocampus and cortex (Dell'Acqua et al., 2013; Gomez et al., 2015; Kurniawan et al., 2014; Yassa et al., 2010; Zeineh et al., 2012), in order to map the organization of various anatomical units in these regions. Along these fine connections, tractography faces a vastly different landscape from those in large white matter tracts, as the proximity of small axonal bundles to neurons, astrocytes, and adjacent axonal bundles makes it challenging to determine their trajectories and microstructural properties. Even though the strengths and limitations of diffusion MRI tractography have been investigated in large white matter tracts with tracer-based histological data (Choe et al., 2012; Dauguet et al., 2007; Dyrby et al., 2007; Leergaard et al., 2010; Seehaus et al., 2013; Thomas et al., 2014), evidence on its capability to resolve small neuronal connections in gray matter regions remains scarce.

In this study, we examined the mouse hippocampus and the spatial organization of its axonal and dendritic networks using *in vivo* high-resolution diffusion MRI. We chose the hippocampus because it plays an important role in memory, spatial navigation, and emotion (Eichenbaum et al., 1996; Strange et al., 2014), and contains an intrinsic network between its subfields with distinct functions (van Strien et al., 2009). Modem viral tracing techniques, such as those used by the Allen Mouse Brain Connectivity Atlas (AMBCA) (Oh et al., 2014), have generated extensive tracer-based neuronal connectivity data in the mouse brain, including the hippocampus. With our recently developed localized high-resolution diffusion MRI technique (Wu et al., 2014) that can "zoom-in" a selected region in the live mouse brain to reach a high spatial resolution, and advanced image processing techniques to

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co-register histological and MRI data, we examined the capability of high-resolution diffusion MRI by direct comparison of diffusion MRI tractography data and co-registered anterograde viral tracing data from AMBCA.

Materials and methods

In vivo high-resolution diffusion magnetic resonance imaging

All experimental procedures were approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine. Twelve adult mice (C57BL/6, three-month old, female) from the Jackson Laboratory (Bar Harbor, ME) were used in this study. *In vivo* MRI was performed on a horizontal 11.7 Tesla MR scanner (Bruker Biospin, Billerica, MA, USA) with a triple-axis gradient system. Images were acquired using a quadrature volume excitation coil (72 mm diameter, for excitation) and a receive-only planar surface coil (15 mm diameter). The planar surface coil was placed on top of the head, above the region containing the right hippocampus. During imaging, the mice were anesthetized with isoflurane (1–1.5%) together with air and oxygen mixed at a 3:1 ratio via a vaporizer. The mice were restrained in an animal holder with ear pins and a bite bar. Respiration was monitored via a pressure sensor (SAII, Stony Brook, NY, USA) and maintained at 40–60 breaths per minute. After imaging, animals recovered within 5 minutes.

To locate the hippocampus in each mouse brain, multi-slice T_2-weighted images were first acquired as an anatomical reference. In this study, the region for localized imaging, or field of excitation (FOE), was set to cover the right hippocampus and surrounding regions, which had a spatial dimension of 8 mm (dorsoventral, the x axis) × 5 mm (mediolateral, the y axis) × 5 mm (rostrocaudal from bregma 0 to −5 mm, the z axis). To achieve localized imaging, spatially selective excitation radio frequency (RF) pulses were designed based on a linear class of large tip-angle (LCLTA) pulses (Pauly et al., 1989), and calculated in Matlab (MathWorks, Natick, MA, USA), with a pulse duration of 2.5 ms and a maximum pulse amplitude of 15 μT, to excite a rectangular FOE in the x–y plane. The performance of the spatially selective excitation RF pulses had been evaluated in our previous report (Wu et al., 2014).

Localized high-resolution diffusion MRI data were acquired using a modified 3D diffusion-weighted gradient- and spin-echo (DW-GRASE) sequence (Aggarwal et al., 2010; Wu et al., 2014) with a spatially selective excitation pulse and a slice-selective refocusing pulse (Mao et al., 1988) that restricted the imaging slab in the z-direction. The sequence acquired 20 echoes after each excitation using a double-sampled EPI readout (Yang et al., 1996). A twin-navigator scheme (Mori and van Zijl, 1998) was implemented to correct motion-induced phase errors, and no respiration trigger was used. The diffusion MRI parameters were: echo time (TE)/repetition time (TR) = 21/500 ms; two signal averages; field of view (FOV) = 9.6 mm × 5.6 mm × 5 mm, resolution = 0.1 mm × 0.1 mm × 0.1 mm; four non-diffusion weighted image (b_0); 30 ($n = 5$) or 60 ($n = 7$) diffusion directions (Jones et al., 1999); gradient pulse duration = 4 ms; diffusion separation = 12 ms; and $b = 2500$ s/mm^2 (Alexander and Barker, 2005). It took less than 2 minutes to acquire a single diffusion-weighted image. The total scan time was 63 and 118 minutes for the 30- and 60-direction high angular resolution diffusion imaging (HARDI) (Frank, 2001; Tuch et al., 2003) data, respectively. Raw data from the scanner were Fourier transformed after zero-padding (to 50 μm isotropic resolution) with navigator-based phase corrections in Matlab. Images at lower spatial resolution (0.15 mm, 0.2 mm, and 0.3 mm isotropic) than the actual resolution were obtained by cropping the raw data before zero-padding (also padded to 50 μm isotropic resolution). Using the log-linear fitting method implemented in DTIStudio (http://www.mristudio.org), diffusion tensor was calculated at each pixel along with the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (Basser et al., 1994).

Registration of MRI data to the Allen Reference Atlas (ARA)

Projection mapping images from 18 tracer experiments (with injection sites in the hippocampus, listed in Supplementary Table S1) were downloaded from the AMBCA (http://connectivity.brain-map.org/). These images were labeled using injected recombinant adeno-associated virus (AAV) tracers expressing enhanced green fluorescent protein (EGFP) and acquired using serial two-photon microscopy (Ragan et al., 2012). A series of image registration procedures (Supplementary Fig. S1(A)) was taken to co-register the MRI and tracer data, which were both registered to the Allen Reference Atlas (ARA) (Goldowitz, 2010). Since the localized high-resolution HARDI data only covered partial brain volume, an *in vivo* whole mouse brain MRI atlas from our previous study (Wu et al., 2013) was used to bridge the localized HARDI datasets and the ARA. First, the whole brain MRI atlas was aligned to the ARA (both re-sampled to 50 μm isotropic resolution) using landmark-based rigid transformation (Diffeomap, www.mristudio.org), followed by intensity-based 12-degree affine transformation and large deformation diffeomorphic metric mapping (LDDMM) (Ceritoglu et al., 2009), utilizing the similar but inverted tissue contrasts between the ARA and fractional anisotropy (FA) image from the MRI atlas. Second, the HARDI datasets were aligned to the transformed whole brain MRI atlas (obtained from the previous step and cropped to match the localized volume in the HARDI datasets) through landmark-based rigid transformation, affine transformation, and LDDMM. The registration accuracy between the ARA and the HARDI datasets was evaluated by measuring the distances between 15 landmarks manually placed throughout the hippocampus in the two datasets (Fig. S1(B)). The average registration error was 0.28 ± 0.14 mm as evaluated by two independent raters. After the registration steps, structural segmentations in the ARA (including 17 hippocampal subfields) were transferred into the HARDI datasets (Fig. S1(C)). Because the hippocampus is a C-shaped structure, we used the septotemporal axis (van Strien et al., 2009) in addition to the rostrocaudal, dorsoventral, and mediolateral axes to locate sub-regions of the hippocampus (Fig. 1A).

Diffusion MRI-based tractography

Using MRtrix (http://www.brain.org.au/software/mrtrix/) (Tournier et al., 2012), fiber orientation distribution (FOD) at each voxel was estimated using the constrained spherical deconvolution (CSD) method (Tournier et al., 2007) with a maximum harmonic order of 6. Both deterministic and probabilistic fiber-tracking (Jones and Pierpaoli, 2005; Lazar and Alexander, 2005) was performed based on the FOD data with a step size of 0.005 mm, a minimal length of 0.5 mm, and a maximum angle of 45° between steps. Tracking terminated when the FOD amplitude became less than 0.005, or when fibers exited the manually segmented hippocampal region. The method has been demonstrated in postmortem mouse brain specimens to provide the most sensitive detection of white matter pathways (Moldrich et al., 2010). Whole hippocampus tract density images (TDIs) at a grid size of 10 μm isotropic were generated based on short streamlines (0.4–1 mm in length) from random seed points over the hippocampal region to visualize the microstructural organization (Calamante et al., 2012). Specific fiber streamlines were generated from specific regions of interest (ROIs) as seed regions, which were defined according to the viral tracer injection sites in the corresponding tracer experiments. The resulting seed-based probabilistic streamlines were used to generate TDIs at a grid size of 50 μm isotropic. The tract density values equal the fractions of fibers in a voxel compared to the total number of fibers in the entire volume. TDIs generated from individual mice were averaged to obtain group-averaged TDI, and then registered to the ARA using the same transformation that was used to align the HARDI data.

In each of the 17 hippocampal subfield structural delineations in the ARA, the tracer-based projection density was defined as the fraction of tracer-projected pixels to the total number of pixels in a subfield

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