Journal of Psychiatric Research 111 (2019) 186–192

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Hippocampal subfield-specific connectivity findings in major depressive disorder: A 7 Tesla diffusion MRI study

John W. Rutland[a,*], Stephanie Brown[a], Gaurav Verma[a], Rebecca E. Feldman[a], Himanshu Sharma[a], Matthew Markowitz[a], Molly Schneider[b], Bradley N. Delman[c], James Murrough[b,d,1], Priti Balchandani[a,1]

[a] Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States
[b] Mood and Anxiety Disorders Program, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, United States
[c] Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, United States
[d] Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, United States

ARTICLE INFO

Keywords:
Depression
Diffusion-weighted imaging
Hippocampal subfields
Tractography
Biomarkers

ABSTRACT

Objective: Diffusion magnetic resonance imaging (dMRI) enables non-invasive characterization of white matter (WM) structures *in vivo*. Prior studies suggest that certain WM tracts may be affected in major depressive disorder (MDD), however, hippocampal subfield-specific dMRI measures have not yet been explored in MDD. We use 7 Tesla dMRI to investigate differences in hippocampal subfield connectivity of MDD patients.

Methods: Eighteen MDD patients and eighteen matched healthy volunteers underwent 7 Tesla MRI. The hippocampal formations were segmented by subfields and tractography was performed to determine streamline count (SC), fractional anisotropy (FA), and mean diffusivity (MD) in patients and controls. Significant subfield connectivity measures were also correlated with age at depression onset.

Results: Compared with controls, MDD patients exhibited reduced SC in the molecular layer of the left dentate gyrus ($p < 0.001$). SC count in the left dentate gyrus was shown to positively correlate with age at disease onset ($p < 0.05$). Increased MD was observed in streamlines emanating from both the left ($p = 0.0001$) and right ($p < 0.001$) fimbriae in MDD patients.

Conclusions: Increased MD of tracts in the fimbriae suggests compromised neuronal membranes in the major hippocampal output gate. Reduced SC of the dentate gyri indexes a disruption of normal cellular processes such as neurogenesis. These findings may have significant implications for identifying biomarkers of MDD and elucidating the neurobiological underpinnings of depression.

1. Introduction

Major depressive disorder (MDD) is a highly disabling and costly psychiatric disease, affecting approximately 6.6% of the US population (Kessler et al., 2003, Greenberg et al., 2003). Correct diagnosis of MDD remains challenging, and current diagnostic tools are limited in both sensitivity and specificity (Paris, 2014). Despite significant efforts to develop new pharmacological therapies, fewer than 40% of MDD patients achieve remission after initial treatment (McGrath et al., 2013). While clinical features of MDD such as anhedonia and melancholia are relatively easy to classify and may be indicative of prognosis, they are not reliable predictors of the efficacy of specific drug treatments within individual patients (Leuchter et al., 2009).

Neuroimaging is increasingly being applied to further the understanding of the neurobiology of psychiatric illness, and may help to overcome current limitations in diagnosis and monitoring of patients with psychiatric disease. Much of this research has focused on MDD, and various imaging findings, such as altered cerebral metabolism and white matter atrophy, have been associated with a depressed phenotype and even related to treatment outcome (Leuchter et al., 2009). However, further biomarker research is necessary to augment our understanding of MDD pathophysiology, provide more tailored methods of diagnosing MDD subtypes, and better monitor treatment response in depressed patients. Novel imaging techniques acquired at ultra-high field with high spatial resolution offer promise for detecting subtle

* Corresponding author. Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, 1470 Madison Avenue, Floor 1, New York, NY, 10029, United States.
E-mail address: jack.rutland@icahn.mssm.edu (J.W. Rutland).
[1] Drs. Balchandani and Murrough contributed equally to this work.

https://doi.org/10.1016/j.jpsychires.2019.02.008
Received 19 September 2018; Received in revised form 7 February 2019; Accepted 14 February 2019

differences in the brain that underlie MDD symptomatology, especially in areas that are implicated in mood regulation.

The hippocampus is well-studied in the context of depression due to its role in cognition and emotion (Thomas et al., 2007; Sapolsky, 2000; Bremner et al., 2000; McKinnon et al., 2009; MacQueen et al., 2003; Pham et al., 2003). The hippocampus is also a highly stress-sensitive structure (Thomas et al., 2007) that is adversely affected by elevated levels of glucorticoids in MDD patients (Sapolsky, 2000). Therefore, there is reason to believe that subtle structural differences may exist in the hippocampus in MDD patients. While reduced hippocampal volume has been reported in a number of studies (Bremner et al., 2000; McKinnon et al., 2009; MacQueen et al., 2003), recent research has focused on measuring differences that occur in the anatomically and functionally distinct hippocampal subfields (Huang et al., 2013; Teicher et al., 2012; Ballmaier et al., 2008). The unique molecular profiles of these subfields confer regional vulnerability to neurotoxic damage (Sapolsky, 2000). The dentate gyrus and Cornu Ammonis (CA) 3 are among the most implicated subfields in MDD (Sapolsky, 2000), however, evidence suggests that other subfields may also be affected by exposure to prolonged stress (Teicher et al., 2012). A number of studies have exploited volumetric analysis on the hippocampal subfields to confirm that volumes of dentate gyrus and CA3 are reduced in MDD patients (Huang et al., 2013; Teicher et al., 2012; Ballmaier et al., 2008). While these studies add to our understanding of the morphometric differences that exist in MDD, they do not provide information about the structural connectivity of the hippocampal subfields, and to our knowledge no such studies have been performed. In addition to augmenting our understanding of the etiology of MDD, detailed examination of hippocampal sub-regions may be valuable in monitoring response to antidepressant therapy in individuals. Recent work suggests that structural changes within certain hippocampal sub-regions are apparent as a result of antidepressant therapy (Cao et al., 2018), and we believed a high-resolution characterization of the hippocampal-subfields could also be useful in monitoring pharmacologic treatments in future studies.

Diffusion magnetic resonance imaging (dMRI) is a noninvasive technique that can be used to characterize white matter architecture *in vivo* (Rodrigues et al., 2018; Le Bihan et al., 2001). In an unrestricted medium, such as bulk water, the diffusion behavior of water molecules lacks directional bias and is termed “isotropic; ” this pattern can be modeled with a Gaussian distribution (Rodrigues et al., 2018; Le Bihan et al., 2001). With increasing structure, diffusion of water may be restricted in one or more directions. This is particularly apparent relative to axonal myelin sheaths of white matter, since water diffuses more rapidly along the longitudinal axis of axons than it does perpendicular to these axons (Le Bihan et al., 2001). This “anisotropic” water movement is used to model the fiber orientation distribution within white matter, and to derive measures of microstructural integrity (Le Bihan et al., 2001). Streamline count (SC) is a metric that quantifies the number of streamlines between brain regions, and reflects the number of axons coupling these regions (Jones et al., 2015). Recent evidence suggests that SC may be a useful metric of characterizing connectivity differences in psychiatric illness (Callahan et al., 2018). Fractional anisotropy (FA) is a measure of microstructure that reflects the degree to which water movement is directionally constrained, and diminished FA is usually associated with loss of white matter organization or demyelination (Bijanki et al., 2015). Mean diffusivity (MD) is a non-directional measure of total magnitude of diffusion within a voxel. While pathology, such as acute ischemia, can be associated with reduced MD (Mukherjee et al., 2008), increased MD is more frequently associated with pathology (Bijanki et al., 2015; Mukherjee et al., 2008; Rovaris et al., 2005). Common disease processes that modify biological membranes and increase permeability of confining barriers in white matter include multiple sclerosis (Rovaris et al., 2005), temporal lobe epilepsy (McDonald et al., 2008), and autism spectrum disorders (Nagae et al., 2012). Lastly, axial diffusivity (AD) and radial diffusivity (RD) are two specific markers of white matter integrity that can detect subtle changes in fiber integrity (Kumar et al., 2014) and are important characteristics to measure in MDD pathology. The resolution of dMRI is benefitted by ultra-high field strengths, such as those employed in 7 Tesla (7T) scanners, as they provide increased signal to noise ratio (SNR) and superior spatial resolution (Polders et al., 2011).

The primary objective of this study was to measure hippocampal subfield-specific connectivity using high-resolution dMRI-based tractography and evaluate group differences in SC, FA, and MD between MDD patients and healthy controls. We hypothesized that tracts emanating from a number of hippocampal subfields would be reduced in MDD patients. We also expected that damage to neural connectivity would be associated with age at disease onset, due to increased length of disease duration and the brain's vulnerability to damage earlier in life. Identification and characterization of imaging markers of depression could facilitate diagnosis of biological subtypes of depression and help differentiate similar diagnostic categories. Additionally, since some preclinical studies have indicated that successful antidepressant treatment may diminish or even reverse stress-induced alterations in the hippocampus (Pham et al., 2003, Malberg and Duman, 2003), we may identify imaging biomarkers capable of detecting subtle structural differences that might serve as valuable tools for monitoring treatment efficacy.

2. Material and methods

2.1. Participants

Eighteen MDD patients (6 females with mean age of 34.2 years, standard deviation (SD) 10.4 years; 12 males, mean 42.2 years, SD 11.4 years) were recruited through the Mood and Anxiety Disorders Program at the Icahn School of Medicine at Mount Sinai. Eligible patients had a primary diagnosis of major depressive disorder, without psychotic features, assessed by the Structured Clinical Interview for DSM-IV Disorders (SCID-IV) (First et al., 1995) or the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV) (First et al., 2015). MDD symptom severity was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS), which was administered by trained clinical raters at screening, within 4 weeks of the MRI scan. Participants were excluded if they had a current diagnosis of obsessive-compulsive disorder, alcohol or substance abuse in the previous year, or lifetime history of a psychotic illness, bipolar disorder, or neurological disease. Patients were also required to have not taken medication for their depression for at least four weeks leading up to their scan. Each patient was age and gender-matched with neurologically and psychiatrically healthy volunteer participants (6 females: mean 36.5 years, SD 12.1 years; 13 males, mean 42.2 years, SD 11.4 years). Healthy controls had no current or lifetime neurological or psychiatric disorder as determined by the SCID-IV or SCID-5-RV. The protocol was approved by the local Institutional Review Board, and after the study was fully explained to participants, written informed consent was obtained by a member of the study team.

2.2. Imaging acquisition

All imaging was acquired on a 7 Tesla whole body scanner (Magnetom, Siemens Healthcare, Erlangen, Germany). A SC72CD gradient coil was used (max slew rate = 200 T/m/s, G_{max} = 70 mT/m), with a single channel transmit and 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). The MRI protocol included a T_1-weighted MP2RAGE sequence: TR = 6000 ms, TE = 3.62 ms, field of view (FOV) = 240 mm × 320 mm, resolution = 0.7 mm isotropic. A coronal-oblique T_2-weighted turbo spin echo (T_2TSE) sequence was also acquired: TR = 6900 ms, TE = 69 ms, FOV = 202 mm × 202 mm, resolution = 0.4 mm × 0.4 mm x 2 mm. Lastly, a high-angular-resolved diffusion-weighted imaging (HARDI) dMRI sequence was acquired: b = 1200 s/mm^2, TR = 7200 ms, TE = 67.6 ms, FOV = 2400 mm × 2400 mm, resolution = 1.05 mm isotropic. The dMRI images were skull-stripped and corrected for eddy currents using FMRIB Software

Library (FSL) (www.fmrib.ox.ac.uk/fsl), and corrected for gradient non-linearities.

2.3. Hippocampal segmentation

Freesurfer (http://surfer.nmr.mgh.harvard.edu) version 6.0 was used to perform an automated cortical reconstruction and volumetric segmentation of the T_1-weighted images. The hippocampi were then segmented using Freesurfer by leveraging the high in-plane resolution of the T_2-TSE images (Fig. 2). Freesurfer segmented the hippocampus into the following subregions: presubiculum, subiculum, parasubiculum, hippocampal fissure, CA1, CA2/3, CA4, granule cell layer of dentate gyrus (GC-DG), hippocampal-amygdala transition area (HATA), fimbria, and molecular layer of the dentate gyrus.

The hippocampal fissure and the HATA were excluded from the analysis due to highly variable segmentation results in these regions. Additionally, because of the significant challenges with accurately delineating the CA4 *in vivo* (Yushkevich et al., 2015), the authors chose to exclude this region from the analysis as well. The authors also decided to combine the subiculum, presubiculum and parasubiculum into one region (subicular complex) and the molecular and granule cell layers of the dentate gyrus into one region (dentate gyrus) due to similar concerns about Freesurfer's ability to segment such small structures (Yushkevich et al., 2015).

Statistical Parametric Mapping 12 was used to co-register these datasets in MATLAB (r2017a, The MathWorks, Natick, MA). Because the regions examined in this study were very small and the imaging contrasts of the dMRI and structural images are quite different, rigorous quality assurance steps were taken to ensure proper alignment between the diffusion images and the structural images that were used to segment the hippocampus.

2.4. Tractography

The T_1-weighted images were segmented into 5 tissue types: gray matter, white matter, ventricles, non-ventricular cerebrospinal fluid and lesion(s) using Freesurfer. A gray-white matter boundary was created from this segmentation, and points along this interface were used to seed anatomically constrained tractography. Constrained spherical deconvolution was used to calculate the fiber orientation distributions, and tensor metrics were obtained throughout the whole brain using MRtrix3 (Brain Research Institute, Melbourne, VIC, Australia). Anatomically constrained whole-brain probabilistic tractography was performed in MRtrix3 (10 million streamlines, FA cutoff = 0.1). The SIFT2 (spherical-deconvolution informed filtering of tractograms) algorithm was applied to reduce streamline biases. The resulting streamlines were used to generate structural connectomes.

To demarcate streamlines emanating from individual subfields, the hippocampal subfield segmentation image was inserted into Freesurfer's default parcellation image (Desikan-Killiany atlas), replacing the left and right hippocampi. Whole-brain connectomes were generated using the 'tck2connectome' command in MRtrix3. The underlying FA, MD, AD, and RD images were sampled to generate connectomes containing diffusion indices for streamlines emanating from each subfield. Fig. 1 shows the workflow used to perform hippocampal subfield-specific tractography.

2.5. Statistical analysis

The SC of each subfield was calculated by adding all of the streamlines between the subfield and the rest of the brain regions. The average FA and MD of all of the streamlines emanating from each subfield were also calculated. Statistical analyses were carried out in *R* Version 3.3.3 (https://www.r-project.org/). Differences between groups were investigated using independent two-tailed Mann-Whitney U t-tests. Because 6 regions of the hippocampus were analyzed for three separate analyses, a Bonferroni-corrected significance threshold of 0.002 was set. All p-values that fell below this threshold were considered significant. Multiple linear regression was used to assess the correlations between the significant group differences and age at disease onset in depressed patients.

3. Results

3.1. Streamline counts of the hippocampal subfields

Subfield-specific probabilistic tractography was acquired in the 18 MDD patients and 18 matched controls (Fig. 3). MDD patients exhibited a significantly reduced SC of left dentate gyrus (Mean: 5574.4 streamlines, SD: 1690.0 streamlines) compared with the controls (Mean: 8784.9 streamlines, SD: 1287.7 streamlines), $p < 0.001$ (Table 1). The volumes of the hippocampal sub-regions were also compared between patients and controls to determine whether SC differences were being driven by differences in volume. There were no sub-regions that significantly differed between patients and controls. Multiple linear regression revealed a significant positive correlation between SC in the left dentate gyrus and age at disease onset ($r = 0.51$, $p = 0.035$). This correlation indicates that there is reduced SC with younger age of disease onset and greater duration of disease. There were no other subfields that showed abnormal SC. Total MADRS scores were not significantly correlated with SC in any of the subfields.

3.2. Diffusion characteristics of the hippocampal subfields

The MD of the hippocampal subfield streamlines in MDD patients and controls are shown in Table 2. The average MD of the streamlines emanating from the left fimbria in MDD patients (M: $7.66 \times 10^{-4}\,mm^2/s$, SD: $4.9 \times 10^{-5}\,mm^2/s$) was significantly increased compared with controls (M: $7.14 \times 10^{-4}\,mm^2/s$, SD: $5.3 \times 10^{-5}\,mm^2/s$), $p < 0.001$. The streamlines from the right fimbria also displayed significantly increased MD in MDD patients (Mean: $7.87 \times 10^{-4}\,mm^2/s$, SD: $5.5 \times 10^{-5}\,mm^2/s$) compared with controls (Mean: $7.25 \times 10^{-4}\,mm^2/s$, SD: $4.8 \times 10^{-5}\,mm^2/s$), $p < 0.001$. Neither the left ($r = -0.18$, $p = 0.50$) nor the right fimbria ($r = -0.05$, $p = 0.86$) were significantly correlated with age at disease onset.

The average RD of the fibers in the left fimbria was significantly greater in MDD patients (M: $8.91 \times 10^{-4}\,mm^2/s$, SD: $1.94 \times 10^{-4}\,mm^2/s$) compared with controls (M: $5.05 \times 10^{-4}\,mm^2/s$, SD: $4.83 \times 10^{-5}\,mm^2/s$), $p = 0.02$. Patients also exhibited increased RD in the right fimbria (M: $6.85 \times 10^{-4}\,mm^2/s$, SD: $2.51 \times 10^{-4}\,mm^2/s$), compared with controls (M: $4.50 \times 10^{-4}\,mm^2/s$, SD: $2.82 \times 10^{-4}\,mm^2/s$), however this finding was jot significant after correction for multiple comparisons, $p = 0.09$. No other subfields displayed statistically significant differences in RD. These results are shown in Table 3.

The FA and AD of the hippocampal subfield streamlines were not significantly different in MDD patients compared with controls. There were no diffusion characteristics that were significantly correlated with total MADRS scores in any of the subfields.

4. Discussion

This study is the first to obtain hippocampal subfield-specific connectivity measures in MDD patients by employing high-resolution dMRI at 7T. We believe that this method can uniquely probe the hippocampal sub-regions and provide new information about the effect of depression on hippocampus. We measured SC, FA, and MD to evaluate potential abnormalities in both the number of streamlines going into and coming out of each subfield (SC) as well as the microstructural integrities of those streamlines (FA and MD).

One of the main findings in this study was reduced SC of the left dentate gyrus in patients with MDD. Yushkevich et al. reviewed

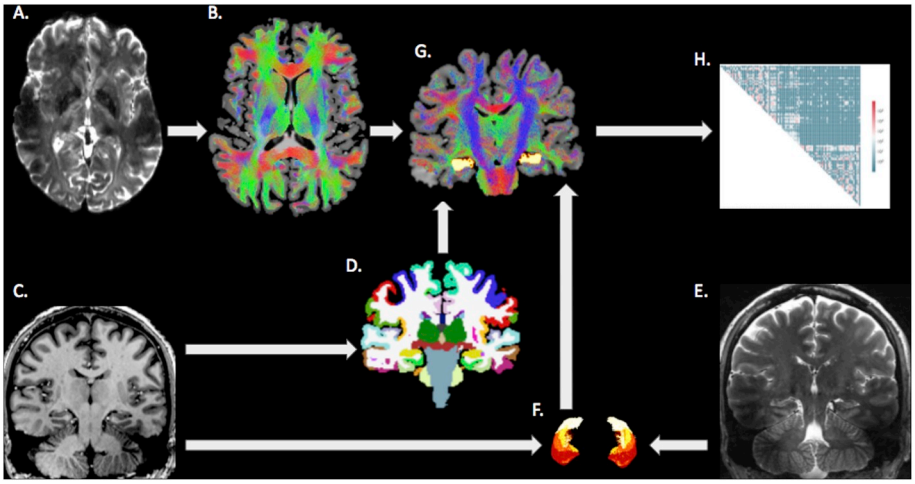

Fig. 1. A schematic of the work-flow. The dMRI images (A) were used to perform whole-brain probabilistic tractography (B). The T_1-weighted images (C) were used for the whole-brain segmentation (D). Both the T_1-weighted and T_2-TSE images (E) were used to segment the hippocampus (F). Tractography was performed in the hippocampal subfields (G) and structural connectomes (H) were generated.

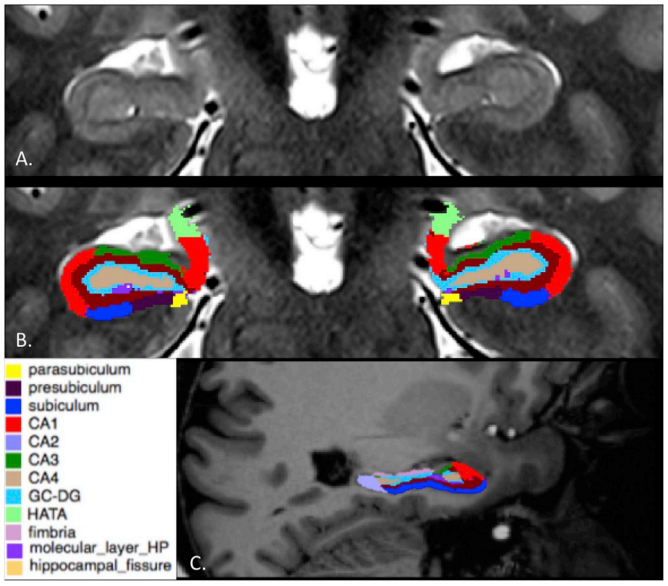

Fig. 2. Coronal T_2-TSE slice through the hippocampi (A), Coronal T_2-TSE slice through the hippocampi with Freesurfer subfield overlays (B), Sagittal T1-wighted image through the left hippocampus with Freesurfer segmentation overlays (C).

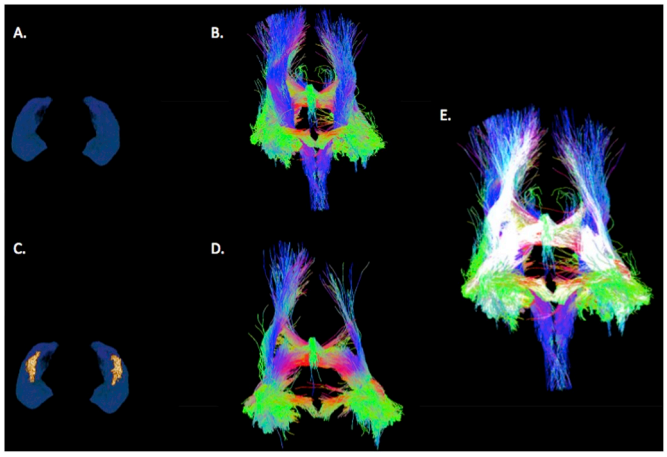

Fig. 3. 3-dimensional rendering of the hippocampi (A), probabilistic tractography of the hippocampi (B), the fimbriae (orange) within the hippocampi (C), streamlines to/from only the fimbriae (D), a subtraction image with tractography from the whole hippocampi and the streamlines from only the fimbriae in white (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

numerous methodologies for hippocampal segmentation, including Freesurfer, and found that segmentation of the dentate gyrus shows very consistent agreement between softwares (Yushkevich et al., 2015). The ability to accurately delineate the boundaries of the dentate gyrus is likely because the stratum radiatum and lacunosomoleculare layer separating CA from dentate gyrus is very hypointense on T2-weighted imaging and provides an objective boundary to identify automatically (Yushkevich et al., 2015). Precise segmentation, combined with the fact that we combined the two constituent layers of the dentate gyrus into the larger structure, allowed us to perform accurate subfield-specific tractography in this hippocampal sub-region.

The dentate gyrus is well studied in the context of anxiety and depression, in part because it is one of only two areas in the adult brain that exhibits neurogenesis (Samuels and Hen, 2011). The neurogenesis hypothesis of depression postulates that reduced production of new neurons in the dentate gyrus is a neurobiological substrate underlying depression (Samuels and Hen, 2011). The mechanism behind this hypothesis is believed to involve elevated concentrations of glucocorticoids in MDD that impair neurogenesis (Samuels and Hen, 2011). A number of neuroimaging studies have found reduced dentate gyrus volume in MDD patients (Treadway et al., 2015; Frodl et al., 2002). However, the findings presented in this study are the first to report reduced connectivity of the dentate gyrus *in vivo*. While this finding aligns with the neurogenesis hypothesis and many prior studies (Thomas et al., 2007, Sapolsky, 2000, Pham et al., 2003; Malberg and Duman, 2003; Samuels and Hen, 2011), there are conflicting reports of the effects of stress of the hippocampus (Conrad, 2008), and more work is required to validate this finding. Additionally, connectivity differences of the dentate gyrus may serve as useful markers for monitoring antidepressant efficacy, and is an area of future investigation in our group.

We also found a significant positive correlation with the SC of the left dentate gyrus and age at disease onset, suggesting that patients with early diagnosis of MDD exhibit greater reduction in SC in this region. Similarly, Schmaal et al. found that MDD patients diagnosed earlier in life exhibited significantly smaller hippocampal volumes than did patients with later disease onset (Schmaal et al., 2015). Greater connectivity abnormality in MDD patients diagnosed earlier in life may be attributable to longer duration of disease exposure or may be reflective of the adolescent brain's vulnerability to neural or hormonal changes in depression (Kerestes et al., 2014), and is an area of future investigation.

While we did not observe any significant group differences in the FA of fibers emanating from the hippocampal subfields, some regions did exhibit increased MD and RD in MDD patients. MD is a sensitive but

Table 1
Mean (standard deviation) of the SC for the hippocampal subfields. *p*-values below the Bonferroni-corrected significance threshold are shown in bold-face.

	Left hemisphere			Right hemisphere		
	MDD	Control	*p*-value	MDD	Control	*p*-value
Subicular complex	13590.8	14170.0	0.67	13908.8	17634.4	0.06
CA1	11699.8	11548.6	0.89	8962.1	8698.1	1.0
Dentate Gyrus	**5574.4**	**8784.9**	**0.0006**	7209.8	7836.0	0.44
CA2/3	5680.9	4019.3	0.09	4603.4	3887.8	0.42
Fimbria	2616.9	3235.0	0.31	2559.6	2950.2	0.46
Whole Hippocampus	39162.8	41757.7	0.56	37243.7	41006.6	0.42

Abbreviations: CA, Cornu Ammonis.

Table 2
Mean (*p*-value) of the mean diffusivities ($mm^2/s \times 10^{-4}$) of the tracts emanating from the hippocampal subfields. *p*-values below the Bonferroni-corrected significance threshold are shown in bold-face.

	Left hemisphere			Right hemisphere		
	MDD	Control	*p*-value	MDD	Control	*p*-value
Subicular complex	7.23	7.02	0.17	7.19	7.01	0.37
CA1	7.30	7.18	0.70	7.32	7.21	0.62
Dentate Gyrus	**7.35**	7.18	0.40	7.38	7.18	0.10
CA2/3	7.52	7.22	0.29	7.24	7.22	0.91
Fimbria	**7.66**	**7.14**	**0.0006**	**7.87**	**7.25**	**0.0001**
Whole Hippocampus	7.40	7.22	0.37	7.42	7.19	0.07

Abbreviations: CA, Cornu Ammonis.

Table 3
Mean (*p*-value) of the radial diffusivities ($mm^2/s \times 10^{-4}$) of the tracts emanating from the hippocampal subfields. *p*-values below the Bonferroni-corrected significance threshold are shown in bold-face.

	Left hemisphere			Right hemisphere		
	MDD	Control	*p*-value	MDD	Control	*p*-value
Subicular complex	6.82	7.87	0.70	8.21	6.56	0.27
CA1	7.64	8.67	0.82	9.09	7.25	0.09
Dentate Gyrus	7.06	8.78	0.11	5.68	4.75	0.80
CA2/3	5.37	3.83	0.27	4.25	4.78	0.67
Fimbria	**8.91**	**5.05**	**0.02**	6.85	4.50	0.09
Whole Hippocampus	8.55	6.50	0.09	5.03	5.02	0.80

Abbreviations: CA, Cornu Ammonis.

non-specific dMRI scalar known to increase as a result of tissue degeneration from neuronal membrane damage (Jones et al., 2015; Bijanki et al., 2015; Rovaris et al., 2005; Lutz et al., 2008). We observed significantly increased MD within the left and right fimbriae. Interestingly, this effect was shown bilaterally in the major output structure of the hippocampus (Lutz et al., 2008). Efferent fibers from the fimbriae project to other parts of the limbic system and the cortex, and damage to these connections may underlie or contribute to emotional disturbances in MDD patients (Lutz et al., 2008). While previous studies have found volumetric reductions in the fimbriae (MacQueen et al., 2003), ours is the first to report microstructural damage to fibers coming out of this sub-region in MDD patients.

A number of previous studies have reported increased MD in certain brain regions in depressed patients, including within the brainstem (Raison and Miller, 2012), cingulum, corpus callosum, and the prefrontal cortex (Nenonen et al., 2015). Because MD is non-specific and can be altered by numerous molecular processes (Mukherjee et al., 2008; Rovaris et al., 2005), it is difficult to pinpoint the exact mechanism responsible for elevated MD in the fimbriae. One possible explanation for our finding of increased MD in this region is inflammation. The relationship between inflammation and depression is still unclear, however, inflammatory processes are increasingly being recognized as important factors in depression pathophysiology (Song et al., 2014). MD is known to increase in the presence of inflammation, as a result of swelling and increased cellular water content, which allows increase water diffusion in biological tissue (Benedetti et al., 2011). Compromised neuronal fibers emanating from this output gate of the hippocampus may be an effect of neuroinflammation and contribute to fronto-limbic network dysfunction in MDD, accounting for some of the emotional and cognitive deficits observed in MDD patients.

We also report significantly increased RD in the left fimbriae, mirroring our MD findings. RD is a measure of microstructure that measures molecular water movement perpendicular to the axonal wall. As a subcomponent of MD, RD may detect microstructural alterations that may not be revealed by MD measures. Elevated RD is suggestive of reduced axonal wall integrity or increased axonal diameter, and has also been associated with inflammation and cellular swelling (Winklewski et al., 2018). While a significant effect was only observed in the left fimbria, the right fimbria also demonstrated increased RD in patients, however, this finding was not significant after correction for multiple comparisons ($p = 0.09$) and warrants further investigation with larger sample sizes.

5. Limitations

The main limitation of this study was the relatively small number of participants. This may have limited detectability of subfield-specific findings. While 7T MRI provides excellent resolution and contrast, it also carries more contraindications than lower field strengths, making patient recruitment criteria more stringent than criteria for clinical imaging at lower field strengths (Feng et al., 2015; van der Kolk et al., 2013). Future work will expand the number of participants in this study, and focus on the subfields that were implicated in this preliminary investigation.

We also recognize the potential effects of partial voluming, imperfections in motion correction, and inherent spatial blur in this study. In order to minimize these effects, we excluded hippocampal subfields that were very small or could not by precisely delineating on T_2-TSE imaging (Yushkevich et al., 2015). We amalgamated a number of smaller constituent regions into their larger subfield (subicular complex and dentate gyrus) to mitigate the inherent effects of partial voluming, and believe that the sub-regions evaluated were i) large enough to be visualized and segmented on high-resolution T_2-TSE imaging, and ii) accurately probed with 1.05 mm isotropic dMRI resolution, and that our findings reflect true biological effects in the hippocampal sub-regions that are reflective of MDD pathology.

We are also aware that there is ongoing work focused on reaching a consensus on automated hippocampal subfield segmentation (Yushkevich et al., 2015). We decided to use Freesurfer version 6.0 for this initial exploration of subfield specific connectivity because to the algorithm's advanced version of Bayesian inference labeling and

promising initial results and validations (Mueller et al., 2018., NeuroImage Clinical). Lastly, we recognize the potential for false positives to affect the streamlines generated from the dMRI in this study (Zalesky et al., 2016). While this consideration is inherent to all dMRI-based tractography studies, we believe that our use of the SIFT2 algorithm adequately reduced such biases and minimize these potential effects in this study.

Lastly, we were unable to account for potential effects that prior medications may have contributed in this study. While we attempted to minimize these effects by requiring patients to be off of medications for at least four weeks leading up to the MRI scan, we recognize the possibility of confounding effects from prior drug therapies. Prospective drug trials should include the analyses developed in this study in order to determine whether subfield-specific connectivity can be used as a marker of drug response in depressed patients. Quantifying SC in the dentate gyrus and diffusivity measures in the fimbriae may prove valuable in determining efficacy of particular pharmacotherapies in certain individuals, especially since it has been shown that depression-related neuroplastic deficits can be reversed with effective antidepressant therapy (Nordanskog et al., 2010). For example, hippocampal volume has been shown to increase due to cell proliferation in MDD patients as a result of electroconvulsive therapy (Nordanskog et al., 2010). Recent indicates that subfield-specific volumetric changes in MDD patients that have underwent electroconvulsive therapy may useful in predicting clinical outcomes in depressed patients (Cao et al., 2018). We believe hippocampal subfield-specific connectivity differences may also have a role in predicting response to antidepressant therapy.

6. Conclusions

The significant public health cost as well as the increasing incidence of MDD has spurred research efforts to understand the neurobiological underpinnings of the disease (Klerman and Weissman, 1989). However, the paucity of neuroimaging biomarkers for MDD and other psychiatric disorders has delayed attempts aimed at finding new pharmacological therapies for depression (Savitz et al., 2013). Many promising experimental drugs have also failed at late stages of development, partly due to this lack of neuropathological targets (Savitz et al., 2013). Additionally, DSM-5 is currently used for clinical diagnosis of MDD and its empirical approach to diagnosis (trial and error) fails to accurately diagnose some patients (Savitz et al., 2013). Lastly, pharmacological therapy monitoring is a clinical challenge, as many MDD patients will take weeks to respond to initial drug treatment (Leuchter et al., 2009). Radiological biomarkers that can monitor progress and predict remission would be more desirable than the standard method of “watchful waiting”, and may allow clinicians to correct ineffective treatment courses more rapidly (Leuchter et al., 2009).

Ultra-high field MRI may be useful for identifying viable biomarkers for psychiatric disorders, by elevating previously unnoticed pathology above the threshold of detectability in MDD patients. In this study we used 7T dMRI to perform subfield-specific tractography in the hippocampus and measured SC, FA, and MD of tracts emanating from these subfields. We report for the first time, subfield-specific connectivity differences in MDD patients, and found abnormal connectivity in the hippocampi of MDD patients. We believe that these findings could have significant implications for the diagnosis, treatment, and monitoring of patients with MDD and help to further elucidate the neurobiological underpinnings of psychiatric illness.

Funding

This work was supported by the National Institutes of Health [NIH R01 MH109544, NIH R01 CA202911, NARSAD Young Investigator Grant]. And the Icahn School of Medicine Capital Campaign, Translational and Molecular Imaging, Institute and Department of Radiology, Icahn School of Medicine at Mount Sinai.

Conflict of interest

In the past 3 years, Dr. Murrough has provided consultation services to Allergan, Sage Therapeutics, Fortress Biotech, Novartis, Janssen Research and Development, Medavante-ProPhase, and Global Medical Education and has received research support from Avanir Pharmaceuticals. Dr. Murrough is named on a patent pending for neuropeptide Y as a treatment for mood and anxiety disorders. He is a full time employee of the Icahn School of Medicine at Mount Sinai, part of the Mount Sinai Health System. The Icahn School of Medicine is named on a patent and has entered into a licensing agreement and will receive payments related to the use of ketamine if it is approved for the treatment of depression. Dr. Murrough is not named on this patent and will not receive any payments.

The remaining authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.02.008.

References

Ballmaier, M., Narr, K.L., Toga, A.W., Elderkin-Thompson, V., Thompson, P.M., Hamilton, L., et al., 2008. Hippocampal morphology and distinguishing late-onset from early-onset elderly depression. Am. J. Psychiatry 165, 229–237. https://doi.org/10.1176/appi.ajp.2007.07030506.

Benedetti, F., Yeh, P.H., Bellani, M., Radaelli, D., Nicoletti, M.A., Poletti, S., et al., 2011. Disruption of white matter integrity in bipolar depression as a possible structural marker of illness. Biol. Psychiatry 69, 309–317. https://doi.org/10.1016/j.biopsych.2010.07.028.

Bijanki, K.R., Matsui, J.T., Mayberg, H.S., Magnotta, V.A., Arndt, S., Johnson, H.J., et al., 2015. Depressive symptoms related to low fractional anisotropy of white matter underlying the right ventral anterior cingulate in older adults with atherosclerotic vascular disease. Front. Hum. Neurosci. 9, 408. https://doi.org/0.3389/fnhum.2015.00408.

Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L., Charney, D.S., 2000. Hippocampal volume reduction in major depression. Am. J. Psychiatry 157, 115–118. https://doi.org/10.1176/ajp.157.1.115.

Callahan, F., Maller, J.J., Welton, T., Middione, M.J., Shankaranarayanan, A., Grieve, S.M., 2018. Toward personalised diffusion MRI in psychiatry: improved delineation of fibre bundles with the highest-ever angular resolution in vivo tractography. Transl. Psychiatry 8, 91. https://doi.org/10.1038/s41398-018-0140-8.

Cao, B., Luo, Q., Fu, Y., Du, L., Qui, Yang, X., et al., 2018. Predicting individual responses to the electroconvulsive therapy with hippocampal subfield volumes in major depression disorder. Sci. Rep. 8, 5434.

Conrad, C.D., 2008. Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis. Rev. Neurosci. 19, 395–411. https://doi.org/10.1515/REVNEURO.2008.19.6.395.

Feng, X.D., McCauley, J.P., Morgan-Curtis, F.K., Salam, R.A., Pennell, D.R., Loveless, M.E., et al., 2015. Evaluation of 39 medical implants at 7.0 T. Br. J. Radiol. 88, 20150633. https://doi.org/10.1259/bjr.20150633.

First, M.B., Spitzer, R.L., Williams, J.B.W., Gibbon, M., 1995. Structured Clinical Interview for DSM-IV Axis I Disorders–Patient Edition. New York Psychiatric Institute, New York.

First, M.B., Williams, J.B.W., Karg, R.S., Spitzer, R.L., 2015. Structured Clinical Interview for DSM-5—Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV). American Psychiatric Association, Virginia.

Frodl, T., Meisenzahl, E.M., Zetzsche, T., Born, C., Groll, C., Jager, M., et al., 2002. Hippocampal changes in patients with a first episode of major depression. Am. J. Psychiatry 159, 1112–1118. https://doi.org/10.1176/appi.ajp.159.7.1112.

Greenberg, P.E., Kessler, R.C., Birnbaum, H.G., Leong, S.A., Lowe, S.W., Berglund, P.A., Corey-Lisle, P.K., 2003. The economic burden of depression in the United States: how did it change between 1990 and 2000? J. Clin. Psychiatr. 64, 1465–1475.

Huang, Y., Coupland, N.J., Lebel, R.M., Carter, R., Seres, P., Wilman, A.H., Malykhin, N.V., 2013. Structural changes in hippocampal subfields in major depressive disorder: a high-field magnetic resonance imaging study. Biol. Psychiatry 74, 62–68. https://doi.org/10.1016/j.biopsych.2013.01.005.

Jones, J.T., DiFrancesco, M., Zaal, A.I., Klein-Gitelman, M.S., Gitelman, D., Ying, J., Brunner, H.I., 2015. Childhood-onset lupus with clinical neurocognitive dysfunction shows lower streamline density and pairwise connectivity on diffusion tensor imaging. Lupus 24, 1081–1086. https://doi.org/10.1177/0961203315572718.

Kerestes, R., Davey, C.G., Stephanou, K., Whittle, S., Harrison, B.J., 2014. Functional brain imaging studies of youth depression: a systematic review. Neuroimage: Clinical. 4, 209–231. https://doi.org/10.1016/j.nicl.2013.11.009.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., et al., 2003. The epidemiology of major depressive disorder results from the national comorbidity survey replication (NCS-R). J. Am. Med. Assoc. 289, 3095–3105. https://doi.org/:10.1001/jama.289.23.3095.

Klerman, G.L., Weissman, M.M., 1989. Increasing rates of depression. J. Am. Med. Assoc.

261, 2229–2235. https://doi.org/10.1001/jama.1989.03420150079041.
Kumar, R., Chavez, A.S., Macey, P.M., Woo, M.A., Harper, R.M., 2014. Brain axial and radial diffusivity changes with age and gender in healthy adults. Brain Res. 1512, 22–36. https://doi.org/10.1016/j.brainres.2013.03.028.
Le Bihan, D., Mangin, J.F., Poupon, C., Clark, C.A., Pappata, S., Molko, N., Chabriat, H., 2001. Diffusion tensor imaging: concepts and applications. J. Magn. Reson. Imag. 13, 534–546.
Leuchter, A.F., Cook, I.A., Marangell, L.B., Gilmer, W.S., Burgoyne, K.S., Howland, R.H., et al., 2009. Comparative effectiveness of biomarkers and clinical indicators for predicting outcomes of SSRI treatment in Major Depressive Disorder: results of the BRITE-MD study. Psychiatr. Res. 169, 124–131. https://doi.org/10.1016/j.psychres.2009.06.004.
Lutz, J., Jager, L., de Quervain, D., Krauseneck, T., Padberg, F., Wichnalek, M., et al., 2008. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. Arthritis Rheum. 58, 3960–3969. https://doi.org/10.1002/art.24070.
MacQueen, G.M., Campbell, S., McEwen, B.S., Macdonald, K., Amano, S., Joffe, R.T., et al., 2003. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc. Natl. Acad. Sci. U.S.A. 100, 1387–1392. https://doi.org/10.1073/pnas.0337481100.
Malberg, J.E., Duman, R.S., 2003. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology 28, 1562–1571. https://doi.org/10.1038/sj.npp.1300234.
McDonald, C.R., Ahmadi, M.E., Hagler, D.J., Tecoma, E.S., Iragui, V.J., Gharapetian, L., et al., 2008. Diffusion tensor imaging correlates of memory and language impairments in temporal lobe epilepsy. Neurology 71. https://doi.org/10.1212/01.wnl.0000327824.05348.3b.
McGrath, C.L., Kelley, M.E., Holtzheimer, P.E., Dunlop, B.W., Craighead, W.E., Franco, A.R., et al., 2013. Toward a neuroimaging treatment selection biomarker for major depressive disorder. JAMA Psychiatry 70, 821–829. https://doi.org/10.1001/jamapsychiatry.2013.143.
McKinnon, M.C., Yucel, Y., Nazarov, A., MacQueen, G.M., 2009. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J. Psychiatr. Neurosci. 34, 41–54.
Mueller, S.G., Yushkevich, P.A., Das, S., Wang, L., Van Leemput, K., Iglesias, J.E., et al., 2018. Systematic comparison of different techniques to measure hippocampal subfield volumes in ADNI2. Neuroimage Clin. 17, 1006–1018. https://doi.org/10.1016/j.nicl.2017.12.036.
Mukherjee, P., Berman, J.I., Chung, S.W., Hess, C.P., Henry, R.G., 2008. Diffusion tensor imaging and fiber tractography: theoretic underpinnings. Am. J. Neuroradiol. 29, 632–641. https://doi.org/10.3174/ajnr.A1051.
Nagae, L.M., Zarnow, D.M., Blaskey, L., Dell, J., Khan, S.Y., Qasmieh, S., et al., 2012. Elevated mean diffusivity in the left hemisphere superior longitudinal fasciculus in autism spectrum disorders increases with more profound language impairment. Am. J. Neuroradiol. 33, 1720–1725. https://doi.org/10.3174/ajnr.A3037.
Nenonen, M., Hakulinin, U., Brander, A., Ohman, J., Dastidar, P., Luoto, T.M., 2015. Possible confounding factors on cerebral diffusion tensor imaging measurements. Acta Radiol. Open 4, 1–9. https://doi.org/10.1177/2047981614546795.
Nordanskog, P., Dahlstrand, U., Larsson, M.R., Larsson, E.M., Knutsson, L., Johanson, A., 2010. Increase in hippocampal volume after electroconvulsive therapy in patients with depression: a volumetric magnetic resonance imaging study. J. ECT 26, 62–67.
Paris, J., 2014. The mistreatment of major depressive disorder. Can. J. Psychiatr. 59, 148–151. https://doi.org/10.1177/070674371405900306.
Pham, K., Nacher, J., Hof, P.R., McEwen, G.S., 2003. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. Eur. J. Neurosci. 17, 879–886. https://doi.org/10.1046/j.1460-9568.2003.02513.x.
Polders, D.L., Leemans, A., Hendrikse, J., Donahue, M.J., Luijten, P.R., Hoogduin, J.M., 2011. Signal to noise ratio and uncertainty in diffusion tensor imaging at 1.5, 3.0, and 7.0 Tesla. J. Magn. Reson. Imag. 33, 1456–1463. https://doi.org/10.1002/jmri.22554.
Raison, C.L., Miller, A.H., 2012. Is depression an inflammatory disorder? Curr. Psychiatr. Rep. 13, 467–475. https://doi.org/10.1007/s11920-011-0232-0.
Rodrigues, N.B., Mithani, K., Meng, Y., Lipsman, N., Hamani, C., 2018. The emerging role of tractography in deep brain stimulation: basic principles and current applications. Brain Sci. 8, 23. https://doi.org/10.3390/brainsci8020023.
Rovaris, M., Gass, A., Bammer, R., Hickman, S.J., Ciccarelli, O., Miller, D.H., Filippi, M., 2005. Diffusion MRI in multiple sclerosis. Neurology 65. https://doi.org/10.3390/brainsci8020023.
Samuels, B.A., Hen, R., 2011. Neurogenesis and affective disorders. Eur. J. Neurosci. 33, 1152–1159. https://doi.org/10.1111/j.1460-9568.2011.07614.x.
Sapolsky, R.M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatr. 57, 925–935. https://doi.org/10.1001/archpsyc.57.10.925.
Savitz, J.B., Rauch, S.L., Drevets, W.C., 2013. Clinical application of brain imaging for the diagnosis of mood disorders: the current state of play. Mol. Psychiatr. 18, 528–539. https://doi.org/10.1038/mp.2013.25.
Schmaal, L., Veltman, D.J., van Erp, T.G.M., Samann, P.G., Frodl, T., Jahanshad, N., et al., 2015. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol. Psychiatr. 21, 806–812. https://doi.org/10.1038/mp.2015.69.
Song, Y.J.C., Korgaonkar, M.S., Armstrong, L.V., Eagles, S., Williams, L.M., Grieve, S.M., 2014. Tractography of the Brainstem in Major Depressive Disorder Using Diffusion Tensor Imaging. PLoS One 9, e84825. https://doi.org/10.1371/journal.pone.0084825.
Teicher, M.H., Anderson, C.M., Polcari, A., 2012. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. Proc. Natl. Acad. Sci. Unit. States Am. 109, 563–572. https://doi.org/10.1073/pnas.1115396109.
Thomas, R.M., Hotsenpiller, G., Peterson, D.A., 2007. Acute psychosocial stress reduces cell survival in adult hippocampal neurogenesis without altering proliferation. J. Neurosci. 27, 2734–2743. https://doi.org/10.1523/JNEUROSCI.3849-06.2007.
Treadway, M.T., Waskom, M.L., Dillon, D.G., Homes, A.J., Park, M.T.M., Chakravarty, M.M., et al., 2015. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. Biol. Psychiatry 77, 285–294. https://doi.org/10.1016/j.biopsych.2014.06.018.
van der Kolk, A.G., Hendrikse, J., Zwanenburg, J.J.M., Visser, F., Luijten, P.R., 2013. Clinical applications of 7 T MRI in the brain. Eur. J. Radiol. 82, 708–718. https://doi.org/10.1016/j.ejrad.2011.07.007.
Winklewski, P.J., Sabisz, A., Naumczyk, P., Jodzio, K., Szurowska, E., Szarmach, A., 2018. Understanding the physiopathology behind axial and radial diffusivity changes- what do we know? Front. Neurol. 9, 92. https://doi.org/10.3389/fneur.2018.00092.
Yushkevich, P.A., Amaral, R.S.C., Augustinack, J.C., Bender, A.R., Bernstien, J.D., Boccardi, M., et al., 2015. Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: towards a harmonized segmentation protocol. Neuroimage 111, 526–541. https://doi.org/10.1016/j.neuroimage.2015.01.004.
Zalesky, A., Fornito, A., Cocchi, L., Gollo, L.L., van den Heuvel, M.P., Breakspear, M., 2016. Connectome sensitivity or specificity: which is more important? Neuroimage 142, 407–420.