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A diffusion tensor MRI atlas of the postmortem rhesus macaque brain

Evan Calabrese [a], Alexandra Badea [a], Christopher L. Coe [b], Gabriele R. Lubach [b], Yundi Shi [c], Martin A. Styner [c], G. Allan Johnson [a,*]

[a] Center for In Vivo Microscopy, Department of Radiology, Duke University Medical Center, Durham, NC 27710, USA
[b] Harlow Center for Biological Psychology, University of Wisconsin, Madison, WI 53715, USA
[c] Department of Computer Science, Department of Psychiatry, University of North Carolina, Chapel Hill, NC 27599, USA

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ABSTRACT

The rhesus macaque (*Macaca mulatta*) is the most widely used nonhuman primate for modeling the structure and function of the brain. Brain atlases, and particularly those based on magnetic resonance imaging (MRI), have become important tools for understanding normal brain structure, and for identifying structural abnormalities resulting from disease states, exposures, and/or aging. Diffusion tensor imaging (DTI)-based MRI brain atlases are widely used in both human and macaque brain imaging studies because of the unique contrasts, quantitative diffusion metrics, and diffusion tractography that they can provide. Previous MRI and DTI atlases of the rhesus brain have been limited by low contrast and/or low spatial resolution imaging. Here we present a microscopic resolution MRI/DTI atlas of the rhesus brain based on 10 postmortem brain specimens. The atlas includes both structural MRI and DTI image data, a detailed three-dimensional segmentation of 241 anatomic structures, diffusion tractography, cortical thickness estimates, and maps of anatomic variability among atlas specimens. This atlas incorporates many useful features from previous work, including anatomic label nomenclature and ontology, data orientation, and stereotaxic reference frame, and further extends prior analyses with the inclusion of high-resolution multi-contrast image data.

1. Introduction

The rhesus macaque (*Macaca mulatta*) is the primary nonhuman primate used to test theories about human brain structure and function. Brain atlases have become important tools for studying normal neuroanatomy and for localizing neuropathology that is the basis of disease states. Many different brain atlases have been generated for the rhesus macaque including those based on conventional histology (Paxinos et al., 2009; Saleem and Logothetis, 2012), anterograde/retrograde neuronal tracer studies (Schmahmann and Pandya, 2009), and magnetic resonance imaging (MRI) (Adluru et al., 2012; Dubach and Bowden, 2009; Frey et al., 2011; McLaren et al., 2009; Rohlfing et al., 2012; Saleem and Logothetis, 2012; Zakszewski et al., 2014). Although it is generally of lower resolution than other brain atlasing methods, MRI has several important advantages: 1) it is three-dimensional (3D) and volumetrically accurate; 2) it can provide multiple different image contrasts from the same tissue; and, 3) it allows probabilistic atlases to be generated from multiple subjects through non-linear image registration techniques. Previous MRI-based atlases of the rhesus brain have been limited by low resolution, a lack of multiple image contrasts, and/or absent or insufficient anatomic delineations.

In humans, diffusion tensor imaging (DTI) has emerged as an important imaging strategy for MRI-based brain atlases (Oishi et al., 2008, 2010). DTI provides a variety of unique MR image contrasts including fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) (Mukherjee et al., 2008). These contrasts provide highly sensitive, quantitative metrics of tissue microstructure (Basser and Pierpaoli, 1996) and can improve the ability to segment many brain structures from MR images (Calabrese et al., 2012). DTI also allows 3D tractography of diffusion pathways, which has become a popular method for structural connectivity mapping in the brain (Mori et al., 1999). An increasing number of studies have reported on DTI changes resulting from neurologic and psychiatric diseases, both in humans and animal models (Konrad and Eickhoff, 2010; Kubicki et al., 2007; Song et al., 2002; Sundaram et al., 2008). These studies highlight the need for high-quality DTI-based brain atlases, particularly for important model systems like the rhesus macaque.

Several MRI and even DTI-based atlases of the rhesus macaque brain exist, but they are often limited by low contrast and/or low spatial resolution (Adluru et al., 2012; Frey et al., 2011; McLaren et al., 2009; Saleem and Logothetis, 2012; Wisco et al., 2008b; Zakszewski et al., 2014). Image resolution is particularly important because the macaque brain is 10–15 fold smaller volumetrically than the human brain (Herculano-Houzel, 2009), so image voxels must be 10–15 fold smaller to have comparable structural resolution. Postmortem MRI with exogenous contrast agents allows considerably higher resolution

* Corresponding author at: Center for In Vivo Microscopy, Box 3302 Duke University Medical Center, Durham, NC 27710, USA. Fax: +1 919 684 7158.
E-mail address: gjohnson@duke.edu (G.A. Johnson).

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and higher quality images than would be possible with in vivo studies (Johnson et al., 2002; Lerch et al., 2012). Postmortem MRI brain atlasing has been used to great effect in small animal models (Calabrese et al., 2013; Chuang et al., 2011; Jiang and Johnson, 2011; Johnson et al., 2012; Veraart et al., 2011), but has seen only limited use in nonhuman primates (Ghosh et al., 1994; Hikishima et al., 2012), partially due to the difficulty with high resolution imaging of large specimens (D'Arceuil et al., 2007). In this article we present a high-resolution DTI atlas of the postmortem rhesus macaque brain based on 10 specimens. The atlas consists of eight different MRI image contrasts at microscopic image resolution as well as a volumetric segmentation of 241 anatomic structures. The anatomic variability of atlas specimens has been characterized, and correlated with demographic information. We further demonstrate the use of this atlas for cortical thickness measurements, and white matter modeling with diffusion tractography.

2. Methods

2.1. Brain specimens

The atlas was constructed based on a population of 10 young adult rhesus macaque brains. The sample population included both males ($n = 7$) and females ($n = 3$) with ages ranging from 1.8 to 11 years (average $= 5.3 \pm 2.8$ years) and perimortem body weights ranging from 2.2 to 10 kg (average $= 5.7$ kg $\pm$ 2.8). Detailed information on age, weight, and gender is provided in Table 1. The brains were acquired following approved protocols from the Pathology Services and Tissue Distribution unit of the Wisconsin National Primate Research Center. All specimens were obtained at necropsy immediately following euthanasia for non-study related reasons. Brain specimens were extracted from the skull and submerged in a 500 mL solution of 10% formalin with 1% (5 mM) gadoteridol (ProHance, Bracco Imaging, Princeton, NJ) within minutes of euthanasia. After a fixation period of at least 4 weeks, brain specimens were transferred to a 500 mL solution of phosphate buffered saline with 0.5% (2.5 mM) gadoteridol for one week. Immediately prior to imaging, specimens were transferred to MRI compatible tubes and immersed in liquid fluorocarbon (Galden PFPE, Solvay, Brussels, Belgium) for susceptibility matching.

2.2. MRI acquisition

Imaging was performed on a 7 Tesla small animal MRI system (Magnex Scientific, Yarnton, Oxford, UK) controlled with an Agilent console running VnmrJ 4.0 (Agilent Technologies, Santa Clara, CA). Radiofrequency (RF) transmission and reception were achieved with a 65 mm inner-diameter quadrature RF coil (m2m Imaging, Cleveland, OH). Each brain specimen was imaged using two protocols, a high-resolution gradient echo anatomic scan and a DTI series.

Gradient echo anatomic images were acquired with a standard 3D gradient echo pulse sequence (TR/TE $= 50/6.2$ ms, $\alpha = 60°$, bandwidth $= 62.5$ kHz). The acquisition matrix was $1060 \times 800 \times 680$ over a 79.5 mm $\times$ 60 mm $\times$ 51 mm field of view (FOV) for a voxel size of $75 \times 75 \times 75$ μm^3.

DTI data were acquired using a 3D diffusion weighted spin echo pulse sequence (TR/TE $= 100/21.5$ ms, bandwidth $= 62.5$ kHz). Diffusion preparation was accomplished using a pair of unipolar, half-sine diffusion gradient waveforms ($\Delta = 14$ ms, $\delta = 4$ ms, amplitude $=$ 50 G/cm, $b = 1500$ s/mm^2) on either side of a 2 ms hyperbolic-secant adiabatic inversion (180°) RF pulse. Twelve diffusion weighted images and a single non-diffusion weighted ($b = 0$) were collected for each specimen. Diffusion sensitization vectors were generated using an electrostatic repulsion model and are provided as Supplementary Table 1. The acquisition matrix was $530 \times 400 \times 340$ over a 79.5 mm $\times$ 60 mm $\times$ 51 mm field of view (FOV) for a voxel size of $150 \times 150 \times 150$ μm^3. Total acquisition time was approximately 46 h per specimen.

Table 1
Descriptive information, including age and gender, for the 10 rhesus macaque brain specimens included in the atlas.

Specimen ID	Sex	Scan date	Age (years)	Weight (kg)	Brain volume (mL)
Rhesus 01	M	16-Dec-2013	2.8	3.4	80.80
Rhesus 02	M	20-Dec-2013	4.9	10.0	79.55
Rhesus 03	M	22-Jan-2014	5.2	8.0	77.50
Rhesus 04	F	27-Jan-2014	9.4	6.5	86.63
Rhesus 05	M	5-Feb-2014	5.2	10.0	79.00
Rhesus 06	M	9-Feb-2014	3.8	3.6	84.91
Rhesus 07	M	20-Feb-2014	4.1	3.4	69.47
Rhesus 08	F	2-May-2014	11.0	4.5	79.51
Rhesus 09	M	4-May-2014	1.8	2.2	69.85
Rhesus 10	F	15-Jun-2014	4.8	5.0	78.30

2.3. Image processing and registration

All data processing was done on a high-performance computing cluster with 96 physical cores and 1.5 TB of RAM. After initial image reconstruction, the gradient echo image and all 12 diffusion-weighted images were registered to the $b = 0$ image using the Advanced Normalization Tools (ANTs, http://picsl.upenn.edu/software/ants/) 12-parameter affine transform model to correct for the linear component of eddy current distortions. The rotational component of the affine transform for each diffusion-weighted image was applied to the corresponding gradient vector prior to tensor estimation. Diffusion tensors were estimated with Diffusion Toolkit (http://trackvis.org). Isotropic diffusion weighted images (DWI) were generated by taking the average of all 12 diffusion weighted images.

A laterally symmetric average image template was generated using an unbiased minimum deformation template strategy (Kochunov et al., 2001). In short, images were divided along the midline and the left side mirrored across the midline to match the right, resulting in 20 hemisphere images. Each unique pair of hemisphere images (190 pairs in total) was registered using a rigid-body transform followed by a non-linear (diffeomorphic) transform. All transforms from a given image to the rest of the group were averaged to generate a final transform between the individual and the final template. The DWI images were used to drive registration. Non-linear registration employed the ANTs greedy symmetric normalization model with cross-correlation as the image similarity metric (Avants et al., 2008). An iterative, multi-resolution scale-space approach was used with six down-sampling levels ranging from 8× to 1× and Gaussian smoothing ranging from $\sigma = 1.2$ mm to 0 mm. Individual image volumes were transformed into the template space, averaged, and mirrored across the midline to generate the final atlas images. Diffusion tensors were transformed and averaged using Log-Euclidean math operations (Arsigny et al., 2006). Atlas images were rigidly aligned to an orientation consistent with previous work, and image origins were set to the center of the midline crossing of the anterior commissure (Frey et al., 2011; Paxinos et al., 2009). Average atlas templates for all eight image contrasts are available for download at http://www.civm.duhs.duke.edu/rhesusatlas/.

2.4. Anatomic segmentation

Anatomic segmentation was initialized through automated label registration using labels from the Paxinos et al. histology atlas of the rhesus macaque brain (Paxinos et al., 2009). This Nissl-stained histology atlas primarily focuses on forebrain gray matter structures, and features detailed delineations of brain nuclei and neocortical regions. First, digitized label diagrams were interpolated to 3D label volumes with each label represented by a unique integer value. Next, a binary version of the label volume was generated by setting all gray matter labels to one and all non-gray mater labels to zero. A corresponding binary mask of the average brain template was generated by simple