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A multidimensional magnetic resonance histology atlas of the Wistar rat brain

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ABSTRACT

We have produced a multidimensional atlas of the adult Wistar rat brain based on magnetic resonance histology (MRH). This MR atlas has been carefully aligned with the widely used Paxinos–Watson atlas based on optical sections to allow comparisons between histochemical and immuno-marker data, and the use of the Paxinos–Watson abbreviation set. Our MR atlas attempts to make a seamless connection with the advantageous features of the Paxinos–Watson atlas, and to extend the utility of the data through the unique capabilities of MR histology: a) ability to view the brain in the skull with limited distortion from shrinkage or sectioning; b) isotropic spatial resolution, which permits sectioning along any arbitrary axis without loss of detail; c) three-dimensional (3D) images preserving spatial relationships; and d) widely varied contrast dependent on the unique properties of water protons. 3D diffusion tensor images (DTI) at what we believe to be the highest resolution ever attained in the rat provide unique insight into white matter structures and connectivity. The 3D isotropic data allow registration of multiple data sets into a common reference space to provide average atlases not possible with conventional histology. The resulting multidimensional atlas that combines Paxinos–Watson with multidimensional MRH images from multiple specimens provides a new, comprehensive view of the neuroanatomy of the rat and offers a collaborative platform for future rat brain studies.

Introduction

The evolution of histological brain atlases has been marked by the progressive introduction of new technologies, each of which has created new opportunities for more accurate mapping and greater understanding. Until 1982, the most widely used atlases of the rat brain were based on myelin-stained sections (König and Klippel, 1963) or Nissl-stained coronal sections (Pellegrino et al., 1979), and none offered a satisfactory stereotaxic system. The Paxinos and Watson (1982) rat brain atlas was the first to take advantage of histochemical staining, the first to offer a comprehensive and accurate stereotaxic system, and the first to picture labeled brain sections in all three cardinal planes. This atlas also marked the introduction of a new abbreviation set, which has since become the most widely used in the field of neuroscience, and has been adopted by almost all major mammalian and avian brain atlases (Ashwell and Paxinos, 2008; Franklin and Paxinos, 2007; Mai et al., 2008; Morin and Wood, 2001; Paxinos et al., 2007, 2009a, 2009b, 2010; Puelles et al., 2007; Watson and Paxinos, 2010).

Since its first release in 1982, the Paxinos and Watson (for simplicity "P–W") atlas has gone through rapid evolution, culminating in the 6th edition in 2007 (Paxinos and Watson, 1982, 1986, 1997, 1998, 2005, 2007). The new features include the use of a wide range of immunohistochemical markers (Paxinos et al., 2009a, 2009b) and gene expression patterns (www.alleninstitute.org), the delineation of about 1000 structures (versus 400 in the 1st edition), and the development of a web-based version featuring 3D reconstructions (*BrainNavigator* www.brainnav.com). In addition, the comprehensive nomenclature set has been structured in the form of an ontology based on developmental gene expression patterns (available on *BrainNavigator*).

Conventional histological atlases of the rat brain will continue to evolve. This article describes the addition of magnetic resonance histology (MRH) data, accompanied by a range of new benefits to the user. The evolution of atlases does not mean the replacement of the old by the new, but the continuing emergence of new types that take advantage of new technologies. The new types adopt the important features that developed in earlier atlases. A modern MRH atlas should incorporate all of the best features of the conventional histological atlases, while offering new technological advantages to users.

Johnson et al. introduced the first magnetic resonance imaging (MRI) atlas of the live rat in 1987 (Johnson et al., 1987). The

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realization in 1993 that MRI could be used to study the structure of fixed tissue introduced the concept of magnetic resonance histology (MRH) (Johnson et al., 1993). Over the last 25 years, MR atlases have evolved with higher spatial resolution, 3D acquisitions with isotropic voxels (Suddarth and Johnson, 1991), and an increasingly rich set of contrast mechanisms. Table 1 summarizes some of this work for the rat. The table covers a literature search using the terms "MRI," "atlas," and "rat" over the last 25 years. Magnetic field strength ranges from 1.5 T to 9.4 T. A number of sequences have been used, which emphasize different contrasts in the tissue (column 4): T_1—emphasizing difference in spin lattice relaxation time; T_2—emphasizing difference in spin relaxation time; PD—based on differences in proton density; and diffusion tensor images (DTI)—providing a suite of contrast parameters based on tissue-specific diffusion of water. Both two-dimensional (2D) and three-dimensional (3D) imaging sequences have been used. To allow comparisons of resolution between two sequences, we include the slice thickness and in-plane resolution. The product of slice and in-plane resolution yields the voxel volume (column 7), which is the most appropriate comparison of resolution. By normalizing this voxel volume to the voxel volume in this work (15.6×10^{-6} mm^3), one is able to readily compare the differences in resolutions. For example, the first rat MRI atlas in 1987 was based on contiguous slices that were 1.2-mm thick within in-plane resolution of 0.115 mm. The atlas shown here with 25-micron slices represents an increase in resolution along the *z* axis of 50 times. But the voxels of this first atlas ($0.115\times0.115\times1.2$ mm = 15.9 nl) are 1025-times larger than the voxels of this new atlas ($0.025\times0.025\times0.025$ mm = 15.6 pl).

The majority of these rat atlases have been based on 2D multislice imaging strategies (Cross et al., 2004; Johnson et al., 1987; Nie et al., 2010; Ramu et al., 2006; Schwarz et al., 2006; Schweinhardt et al., 2003; Ting and Bendel, 1992). The resolution is limited in 2D sequences because of hardware capabilities and reduced signal relative to 3D sequences. The introduction of 3D sequences with large arrays (Suddarth and Johnson, 1991), the use of active staining to reduce T_1 (and allow shorter acquisitions) (Johnson et al., 2002), and the development of extended dynamic range (Johnson et al., 2007) have been crucial technical milestones required for isotropic imaging. While a number of mouse atlases have exploited these methods for isotropic imaging (Benveniste et al., 2000; Chen et al., 2006; Jiang and Johnson, 2011; Ma et al., 2005) the arrays have been much smaller. The $800\times800\times1600$ arrays used here represent an increase in data volume of more than 30-times that of the mouse atlases. 3D data allows the development of a whole new class of atlases based on population averages (Aggarwal et al., 2011; Bai et al., 2012; Chuang et al., 2011; Valdés-Hernández et al., 2011). Isotropic resolution allows one to align images sets from multiple specimens regardless of misalignments between specimens during scanning. The MR data are acquired in the skull, so there is no damage to the brain from extraction. The specimen preparation has been undertaken to minimize shrinkage and there is no distortion from physical sectioning.

As conventional histology atlases have evolved with varied chemical stains to highlight the chemoarchitecture, so too have the MRH atlases evolved with different MRI contrasts. Sophisticated image-processing methods developed for comparison of clinical images have been adapted to register multiple data sets into average atlases with enhanced signal-to-noise and contrast-to-noise.

We present here what we believe to be the highest-resolution multidimensional MR atlas of the rat brain, with the widest range of contrast mechanisms, including average atlases with a total of 8 different types of contrast. The data have been registered to the orientation defined by the Paxinos and Watson atlas, and provides a comprehensive new view of the adult Wistar rat.

Methods

Experimental animals

All experiments and procedures were done with the approval of the Duke University Institutional Animal Care and Use Committee. Five postnatal-day 80, male Wistar rats (Charles River Laboratories, Wilmington, MA, USA) weighing approximately 250 g were selected for imaging studies. Animals were perfusion fixed using the *active staining* technique (Johnson et al., 2002) to introduce the gadolinium-based MRI contrast agent Gadoteridol (ProHance, Bracco Diagnostics Inc., Princeton, NJ, USA) into the brain parenchyma. After flushing the vasculature with normal saline, perfusion fixation was achieved using a 10% solution of neutral buffered formalin (NBF) containing 10% (50 mM) gadoteridol. After perfusion fixation, rat heads were removed from the torso and immersed in 10% NBF for 24 h. Finally, fixed rat heads (i.e., with brains still in the cranium) were transferred to a 0.1 M solution of phosphate buffered saline containing 1% (5 mM) gadoteridol at 4°C for 5–7 days to ensure equilibration of contrast agent, and tissue rehydration. This final step of rehydration in normal buffered saline minimizes any shrinkage or swelling. The active staining technique reduces the T_1 relaxation time of the brain parenchyma to less than 100 ms and allows faster, higher-resolution imaging with higher signal-to-noise ratio (SNR) (Johnson et al., 2002). Prior to imaging, specimens were placed in custom-made, MRI-compatible tubes and immersed in a liquid fluorocarbon (Fomblin perfluoropolyether, Ausimont, Thorofare, NJ, USA) to reduce susceptibility artifacts at tissue interfaces and to prevent specimen dehydration. All imaging experiments were performed with the brain in the cranium to preserve its natural shape.

Table 1
Comparison of MR atlases.

Year	Reference	Field (T)	Contrast	Slice (mm)	In plane (Mm)	Voxel (mm^3)	Relative[a]
1987	(Johnson et al., 1987)	1.5	T_1	1.2	0.115×0.115	0.016	1025
1992	(Ting and Bendel, 1992)	4.7	T_1, T_2, PD	0.6	0.175×175	0.018	1177
2003	(Leergaard et al., 2003)	3.0	T_1	0.39	0.390×0.390	0.059	3782
2003	(Schweinhardt et al., 2003)	4.7	T_1, T_2	0.5	0.117×0.117	0.007	448
2004	(Cross et al., 2004)	1.5	T_1	0.5	0.273×250	0.034	2179
2006	(Ramu et al., 2006)	7.0	T_2	1.0	0.137×0.137	0.019	1203
2006	(Schwarz et al., 2006)	4.7	T_2	1.0	0.156×0.156	0.024	1538
2007	(Hjornevik et al., 2007)	3.0	T_1	0.195	0.195×0.195	0.007	449
2010	(Nie et al., 2010)	7.0	T_2	0.3	0.14×0.14	0.00588	377
2010	(Lu et al., 2010)	9.4	T_2	1.0	0.137×0.137	0.0187	1199
2011	(Veraart et al., 2011)	7.0	T_1, DTI	0.088	0.088×0.088	0.00068	44
2012	This work	7.0	T_2*, DTI	0.025	0.025×0.025	0.0000156	1

[a] The resolution is normalized to this work by dividing the encoding voxel volume by 0.0000156, i.e. the volume of the voxels in our gradient recalled echo (GRE) image.