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High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology

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Abstract

The Mouse Bioinformatics Research Network (MBIRN) has been established to integrate imaging studies of the mouse brain ranging from three-dimensional (3D) studies of the whole brain to focused regions at a sub-cellular scale. Magnetic resonance (MR) histology provides the entry point for many morphologic comparisons of the whole brain. We describe a standardized protocol that allows acquisition of 3D MR histology (43-micron resolution) images of the fixed, stained mouse brain with acquisition times < 30 minutes. A higher-resolution protocol with isotropic spatial resolution of 21.5 microns can be executed in 2 hours. A third acquisition protocol provides an alternative image contrast (at 43-micron isotropic resolution), which is exploited in a statistically driven algorithm that segments 33 of the most critical structures in the brain. The entire process, from specimen perfusion, fixation and staining, image acquisition and reconstruction, post-processing, segmentation, archiving, and analysis is integrated through a structured workflow. This yields a searchable database for archive and query of the very large (1.2 GB) images acquired with this standardized protocol. These methods have been applied to a collection of both male and female adult murine brains ranging over 4 strains and 6 neurologic knockout models. This collection and acquisition methods are now available to the neuroscience community as a standard web-deliverable service.

Keywords

Mouse; brain; MR; magnetic resonance histology; autosegmentation

INTRODUCTION

The growing use of diverse imaging methods to study the mouse brain has resulted in extraordinary new insight into brain structure and function. In 2000, the National Institutes of Health, National Center for Research Resources (NIH/NCTR) recognized the critical need for infrastructure to collect and organize the flood of data from these studies by establishing the Mouse Bioinformatics Research Network (MBIRN) <<http://nbirn.net/research/testbeds/mouse/index.shtml>>. MBIRN is a consortium of six institutions building tools, creating methods, and collecting data for studying the mouse brain that can be shared globally through the Internet (Martone et al., 2004). MBIRN is currently focused on imaging the mouse brain ranging from the sub-cellular level through the whole brain. One imaging method, magnetic resonance histology (MRH), which was first suggested in 1993 (Johnson et al., 1993) to enhance study of the mouse brain, can now be executed routinely within a reasonable

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acquisition time. MRH complements more traditional methods of histology in four unique ways—MRH is non-destructive; MRH exploits unique contrast mechanisms of water in the tissue; MRH is three-dimensional; and MRH is inherently digital, which makes it possible to readily share morphologic data over the Internet. A number of authors have demonstrated the utility of MRH in the mouse brain (Benveniste et al., 2000; Johnson et al., 2002a; Kovacevic et al., 2005; Ma et al., 2005; MacKenzie-Graham et al., 2004). However, comparing results between these studies is difficult because a wide range of acquisition strategies with varied tissue contrast has been employed. Furthermore, the majority of this previous work has been done at spatial resolution > 40 microns, and in brains removed from the cranium, which can introduce physical distortion. Exceptionally long acquisition times (5–14 hours per specimen) were used to make up for the limited signal available at these resolutions. As part of the MBIRN initiative, we have overcome these barriers so that they might be available to a wider range of researchers. We report here a series of integrated advances that allow a) acquisition of 3D MR images of the mouse brain at spatial resolution of 43 microns in < 30 minutes, b) acquisition of the highest resolution MR images of the mouse brain yet acquired (21.5 microns) in 2 hours, and c) an acquisition method to highlight gray/white matter contrast to support automated segmentation of brain structures.

A standardized MBIRN protocol produces approximately 2 GB of data per specimen. New methods for specimen fixation, acquisition, reconstruction, analysis, and distribution have been devised to handle the volume of data required to make statistical comparisons. The MBIRN protocol and computer infrastructure is designed to make these tasks transparent to the user and is based on an automated efficient workflow required for high throughput.

Two technical issues make MRH of the mouse brain challenging. The signal is very weak and the data arrays are very large. A mouse at 25 grams is ~3000-times smaller than a human. We must acquire images at 3000-times higher spatial resolution to achieve anatomic definition in the mouse comparable to that in a routine clinical exam. If the voxels are 3000-times smaller, the MR signal will be 3000-times weaker. We have addressed this problem of sensitivity by a series of novel approaches that allow us to routinely image at spatial resolution more than 100,000-times higher than clinical exams. But when one uses these approaches to image the whole brain, the data arrays become exceptionally large. A typical 3D clinical exam is acquired with 256^3 image arrays and isotropic spatial resolution of 1 mm^3 , i.e. each voxel in the 3D image represents the signal from a $1 \times 1 \times 1\text{ mm}$ cube of tissue ($1\text{ }\mu\text{l}$). By comparison, the mouse brain image shown in Fig. 4d is from a $512 \times 512 \times 1024$ image array with each element of the image representing the signal from a 21.5-micron (10 pl) isotropic voxel. The arrays are 16-times larger and the voxels are more than 100,000-times smaller than the voxels in a typical clinical image. Even a limited study of 12 animals can result in nearly 24 GB of data. Here, we describe the MBIRN computational infrastructure that enables investigators from any place on the globe to access quantitative mouse brain anatomy in a routine and standardized fashion.

MATERIALS AND METHODS

Fig. 1 provides a schematic of the workflow and integration of the methods discussed in this paper. All animal studies were performed under protocols approved by the Duke Institutional Animal Use and Care Committee. The images presented here are of adult (9–12 week) C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME; Charles River, Raleigh, NC). Active staining—perfusion with a contrast agent to enhance the MR signal—is combined with a novel (partial Fourier) acquisition strategy that amplifies the high-frequency information (extended dynamic range), while simultaneously reducing the acquisition time.

Fixation/Staining

Active staining, first described by Johnson et al. in 2002 (Johnson et al., 2002b) uses a contrast agent mixed with a fixative to simultaneously preserve the tissue, while preferentially enhancing the signal by reducing the spin lattice relaxation time. A range of fixatives (buffered formalin, paraformaldehyde, glutaraldehyde) and MR contrast agents (MnCl₂, Magnevist [gadopentetate dimeglumine] [Berlex, Montville, NJ], ProHance [gadoteridol] [Bracco Diagnostics, Princeton, NJ]) have been explored with wide variation of application methods (immersion, transcardial perfusion) and varied concentrations. The method of choice for the mouse brain uses a series of perfusing solutions administered via a transcardial approach. Animals are anesthetized with Nembutal. A catheter is inserted into the left ventricle of the mouse heart. The animal is perfused with a peristaltic pump first with a mixture of 0.9% saline and ProHance (10:1, v:v), then followed by a mixture of 10% buffered formalin and ProHance (10:1, v:v). This perfusion method simultaneously fixes the tissue, so that conventional histology can be performed, while preferentially reducing the spin lattice relaxation time (T₁) of the tissue.

All images are acquired using a 9.4 T vertical bore Oxford magnet with shielded coil providing gradients of 950 mT/m. The system is controlled by a GE EXCITE MR imaging console, which is nearly identical to those used in the clinical domain. An additional stage in the transceiver chain converts the transmit and receive signals via a mixer driven with an external 400 MHz oscillator. Specimens are imaged in a solenoid radiofrequency coil constructed from a single sheet of microwave substrate (Hurlston et al., 1997). Fig. 2 compares a brain fixed with formalin (unstained Fig. a–d) and a (stained Fig. e–h) brain fixed with the formalin/ProHance mixture. The 1 mm-thick transverse slices through the hippocampus were acquired with short 2D scans at TR ranging from 20–160 ms to allow quantitative measure of T₁ and to demonstrate the change in signal and contrast. TR, the repetition interval for the scan, must be short to acquire the large arrays in a short period of time. As shown in Fig. 2, there is virtually no signal in the unstained specimen at the shorter TR (Fig. a–d), and there is an 8.3-times increase in SNR at TR=80 ms for the stained specimen (Fig. e–h). But, equally important is the significant increase in contrast between many important structures in the stained brain.

Large 3D arrays

Because the staining with the MR contrast agent permits use of short TR, one can spatially encode using a 3D radiofrequency refocused spin echo encoding method originally suggested by Johnson et al. (Johnson et al., 1983) and later extended to “large arrays” by Suddarth and Johnson (Suddarth et al., 1991) in 1991. The use of the term “large array” in 1991 to describe 256³ image sets has subsequently been replaced in this work by arrays of 512 × 512 × 1024, which are 16-times larger. The use of such large arrays introduces two problems—the dynamic range of the digitizers on all MR imaging systems is not adequate for image arrays much larger than 256³ (Maudsley, 1987), and the acquisition time for 3D arrays is the product $xres \cdot yres \cdot TR$ (where $xres$ and $yres$ are the resolution along the two phase-encoding axes), so even for TR=50 ms, the acquisition time for these large arrays is ~4 hours, too long for high throughput.

Several approaches have been suggested previously to extend the dynamic range of the system (Conturo et al., 1990; Wedeen et al., 1988). Our approach applies the frequency-encoding gradient along the long (1024) axis and selectively alters the receiver gain during the phase-encoding steps. The schematic in Fig. 3 depicts the two phase-encoding axes. A script calls the image acquisition sequence, which advances from the edge of Fourier space toward the center. The more peripheral parts of Fourier space are acquired with the analog gain set as high as necessary to fill at least 15 bits of the digitizer. Towards the center of k-space, to avoid saturation of the digitizer, the analog gain is decreased in 6 dB-steps. For each 6 dB-step, the data sampling is doubled. This not only matches the signal level to the signal levels of the more

peripheral parts of Fourier space, but also increases the dynamic range towards the center. Although three or four steps are routinely used, as many as six gain steps are possible, with each step tailored to the magnitude of the signal in that region of Fourier space. Separate experiments have been performed to verify that the changes in receiver gain do not produce phase discontinuities where the receiver gain is changed.

Several authors have suggested using partial Fourier acquisition. These methods make use of the conjugate properties of Fourier space and correct for phase errors by iterative computation (MacFall et al., 1988; Stenger et al., 1998). Xu and colleagues demonstrated the method in a 3D acquisition similar to our needs (Xu et al., 2001), providing a 2-fold reduction in acquisition time. Since the periphery (of one side) of Fourier space is sampled with expanded gain, we have been able to zero fill the opposite high frequency volume of Fourier space and maintain the resolution defined by the Nyquist limit on the side that has been sampled.

The workflow depicted in Fig. 1 shows the acquisition strategies that have been implemented on our GE EXCITE imaging console (acquiring the Fourier data for T1 and T2 images). The raw data files are very large. The T1 raw data file ($384^2 \times 768$) is 864 MB. The reconstructed image is 500 MB. While the raw data for a single echo in the T2 sequence is smaller (102 MB), there are 8 individual 3D images at TE=7–56 ms, resulting in a raw dataset of 1.15 GB. A total of $8 \times 256^2 \times 512$ arrays are archived for each T2 acquisition, plus a composite image derived from a 4th dimension of Fourier transform (along TE) (Sharief et al., 2006). This results in 640 MB of reconstructed data per T2 image set, which when combined with the T1 image, results in an aggregate of ~1.2 GB of reconstructed image data per study. The reconstruction process involves a number of complex steps, each reading and writing multiple very large files. The entire process has been automated on dedicated 64-bit Silicon Graphics (Origin 3000) processors with high internal bandwidth, allowing routine reconstruction in < 2 hours. A second pipeline derives the weighted T2 image (Sharief et al., 2006) by performing the 4th dimension of Fourier transform (along TE range) and archives this with the original composite images. The images are then added into the archive (currently ~2 TB) and tied to the original animal metadata via an Oracle database.

RESULTS

Fig. 4 shows the results of an imaging experiment designed explicitly to demonstrate the increased resolution at reduced scan time compared to previous methods. Two scans were both acquired at relatively long TR (100 ms), (i.e. the repetition interval between spatial-encoding steps). While our routine protocol uses a shorter TR to keep the acquisition time down, we have used a longer TR in this series of experiments to ensure sufficient signal in the higher-resolution structures to allow comparison of high-resolution structure. The total imaging time is given by $x_{res} \times y_{res} \times TR$, where x_{res} and y_{res} are the acquisition dimensions usually along the two smaller encoding axes of the acquisition. Fig. 4a shows a 21.5-micron transverse section from a 3D image showing the sub-region of the hippocampus we have chosen as a focal point for comparison. Fig. 4b shows an image of the specimen acquired with a $256^2 \times 512$ array at 43-micron isotropic resolution, comparable (and in most cases superior) to the resolution of references (Benveniste et al., 2000; Kovacevic et al., 2005; Ma et al., 2005; MacKenzie-Graham et al., 2004). The raw data for this image was acquired with full sampling of Fourier space without the use of extended dynamic range. Fig. 4d shows a 21.5-micron slice of the same specimen acquired at 21.5-micron resolution using the extended dynamic range method. The acquisition time for this $512^2 \times 1024$ array (full k-space acquisition) was 7.28 hours. Fig. 4c shows the same level, reconstructed from the same data using only part of Fourier space ($384 \times 384 \times 768$), which can be completed in 4.1 hours. Note the small vessels visible (arrows 1 and 2) in Fig. 4c and Fig. 4d, which are not visible in Fig. 4b. The granular layer of the dentate gyrus (GrDG) is visible in all three images, but a thin layer (arrow 3) between the granular

layer and the polymorph layer (PoDG) is visible in Fig. 4c and Fig. 4d. Finally, the stratum lucidum (SLu) (arrow 4) is visible in Fig. 4c and Fig. 4d, but not in Fig. 4b. All labels are according to (Paxinos et al., 2001). Our standard protocol is executed with TR=50 ms, which allows us to acquire images with effective spatial resolution of 21.5 microns, more than 8-times the spatial resolution of previous work in 2 hours—more than 2-times faster than any previous work. Interested readers can review all of the data that has been made available in our publicly accessible archive <<http://www.civm.duhs.duke.edu/pubs/supplemental/NeuroImage200701/index.html>>.

One of the advantages of MR histology is the wide range of soft tissue contrast that can be derived from the same tissues by appropriate adjustment of the acquisition parameters. Tissue can be differentiated based on differences in proton density, spin lattice relaxation time (T1), spin-spin relaxation time (T2), and diffusion, to name but a few of the mechanisms. The interested reader can find an expanded discussion of contrast in (Callaghan, 1991;Haacke et al., 1999). We have coined the term “proton stain” with analogy to more traditional chemical treatments (such as H&E, cresyl violet) (Johnson et al., 1993). The contrast in the images in Fig. 4 is heavily dependent on the density of protons in each tissue and T1, i.e. the proton stain is T1-weighted. We have recently implemented a 3D method to highlight contrast differences due to T2, i.e. a T2-weighted proton stain (Sharief et al., 2006). Fig. 5 shows the same transverse slice imaged with both proton stains. The T2-weighted acquisition (Fig. 5b) requires a longer TR (200 ms) than the T1-weighted acquisition (TR=50 ms) in Fig. 5a). A protocol that can be executed routinely with a reasonable acquisition time is the focus of our effort. To limit acquisition time, we have reduced the resolution in the T2-weighted sequence to 43 microns, using a $256^2 \times 512$ array. Using the same partial Fourier methods with expanded dynamic range (an acquisition matrix of $192 \times 192 \times 384$), this T2-weighted image can be collected in 4 hours. Fig. 5 demonstrates the complementary nature of the two sequences. In the T1-weighted image at 21.5 microns in Fig. 5a, contrast and spatial resolution are sufficient to resolve the pyramidal cell layer in the hippocampus (arrow 1) and the outer boundary of the caudate putamen (arrow 2). But, the complementary contrast of the T2-weighted sequence in Fig. 5b does a far superior job in defining the inner boundary between the caudate putamen and the globus pallidus (arrow 3). While cortical layers are visible in Fig. 5a, these layers are much more clearly defined in Fig. 5b (arrow 4). Subcortical gray matter (e.g. laterodorsal nucleus of the thalamus shown by arrow 5) and the hypothalamus (arrow 6) are not visible at all in Fig. 5a, but are well differentiated in Fig. 5b. Thus, the protocol combining the T1-weighted and T2-weighted acquisition provides both high spatial resolution for delineating cellular layers and high contrast resolution for defining the boundaries of subcortical white matter.

Our goal has been the development of routine quantitative morphometry. Volume measurement requires a robust segmentation method, which also has been developed and validated (Ali et al., 2005). The method is based on the dual-contrast data derived from registered T1- and T2-weighted images described previously, along with statistical location priors describing the structures to be identified. The T1 and T2 data are registered to the reference frame of the priors using automated image registration (AIR) (Woods et al., 1998). Automated anatomical image segmentation is executed as part of the processing pipeline and calculates 3D label volumes for the 33 brain structures listed in Table 1. Fig. 6 shows color-coded brain structures embedded in the 3D T1 image. The labeled volumes are linked to the original component images and the associated animal metadata in the database archive.

To this point, this description has focused on the protocol for high-resolution scans. But, a higher-throughput protocol has also been developed. Table 2 summarizes both the high-resolution and the high-throughput protocols. The high-throughput T1 acquisition at 43 microns is higher resolution than what many have published to date. More importantly, it is

acquired on mouse brain specimens that remain in the cranium to limit physical distortions that are inevitable when the brain is removed from the cranium. For higher throughput, the use of contrast agents and the extended range partial Fourier acquisition allows us to reduce the scan time to 27 minutes. A lower-resolution T2-weighted scan can be accomplished in 49 minutes. Fig. 7 compares registered images from the lowest-resolution scan (Fig. 7a), the fastest scan (Fig. 7b), and the highest-resolution scan (Fig. 7c).

The final component of the MBIRN infrastructure, shown in Fig. 1, is the distribution of these large images to the neurosciences community. These images are up to 10-times larger than previously published work. MBIRN has developed distribution mechanisms on Internet II with particular attention to high-bandwidth access. Data is supplied in a number of formats. Fig. 8 shows one such format, a composite image from the mouse brain atlasing tool (MBAT) <<http://www.nbirn.net/downloads/mbat/>>. Data are all registered to a common reference frame and labels are all referenced to a common ontology (Paxinos et al., 2001). MBAT is a four-dimensional tool that shows the three spatial planes of the isotropic data. The 4th dimension is accessed from a volume manager that allows the user to switch between the volumes providing the T1-weighted image, the T2-weighted image, and the labels. Composite images from multiple volumes can be compared by using a feature of MBAT that renders multiple levels simultaneously with user-selected transparency. A representative dataset, along with the MBAT tool and viewing instructions, are available at <<http://www.civm.duhs.duke.edu/pubs/supplemental/NeuroImage200701/index.html>>.

DISCUSSION

A number of major efforts have been undertaken by MBIRN participants, and others to characterize the morphology in the mouse brain using both conventional and MR histology. The Mouse Brain Library <<http://www.mbl.org>> at the University of Tennessee provides an extensive collection of digitized optical histology images with low-resolution MR. The Allen Brain Atlas <<http://www.brain-map.org>> provides an extensive collection of histologic and gene expression data. In the present effort, we have shown how MR histology can complement these tools, since it is now possible to acquire a full 3D morphologic dataset in 2 hours. Table 3 summarizes the work from a number of investigators using MR histology in the mouse brain. The studies employ a wide range of acquisition protocols with varied proton stains dependent on proton density (PD) T1, T2, T2*. The resolution is equally varied, ranging from a low of 60 × 60 × 120 microns (400 pl) to a high of 20.0³ microns (8 pl)—a difference of 50-fold. Acquisition times range from a high of 14 hours down to < 30 minutes. With such a wide range of acquisition protocols and such long acquisition times, MR histology cannot be a routine tool for the neuroscience community. A high-field MR microscope can easily cost more than \$1 million. Moreover, the specialized expertise to apply MR histology and the large infrastructure we have described here create additional barriers to routine use. Access of users to the computational infrastructure required for these very large image arrays is yet another barrier.

The MBIRN consortium has addressed these barriers. The protocol described here that combines fixation with active staining, along with novel methods for acquisition and reconstruction, has resulted in an enormous gain in resolution and efficiency. An estimate of the efficiency of this protocol, relative to previous efforts can be made by assuming comparable signal-to-noise ratio (SNR) for all the studies. Under this assumption, one can derive the following expression relating scan time (T) and voxel volume (Δv) to a metric of relative efficiency (E):

$$E \propto \frac{SNR}{\Delta v \sqrt{T}}$$

We include a reference in Table 3 to a clinical MR scan at $1 \times 1 \times 1$ mm, which is readily accomplished in < 10 minutes. From this comparison, one can see that the Hi Res T1 protocol is more than 100-times more efficient than the least efficient method of the previous studies. When examining comparable contrast methods, the Hi Res T2 protocol is nearly 10-times more efficient than some of the previous work.

The computational tools (for example, partial Fourier reconstruction, T2 Fourier image, AIR, autosegmentation) have been linked together via the BIRN infrastructure. More than 2 TB of image archive is now online and more than 100 mouse brains have been scanned. Our representative population of C57BL/6J data is now publicly available at <<http://www.civm.duhs.duke.edu/pubs/supplemental/NeuroImage200701/index.html>>. Additional data are online, published as supplementary material (Badea et al., 2007), (Badea, A., et al., Computational anatomy in the C57BL/6J mouse brain, submitted to NeuroImage 2007). The staining and scanning protocols have been streamlined. The autosegmentation protocols have been validated against manual segmentations. An atlas of variability of the C57BL/6J with the brain in the cranium has been developed (Badea, A., et al., Computational anatomy in the C57BL/6J mouse brain, submitted to NeuroImage 2007). The entire process is now available to the neuroscience community as a service to provide a routine and standardized method for morphologic phenotyping of the many new mouse models (collaboration link from <www.civm.duhs.duke.edu/>). We believe these methods will be a measurable step forward for the neuroscience community, which can now receive very high-resolution MR histology data, registered to a common reference space with quantitative measures of volume for 33 mouse brain structures, all defined with a standardized naming convention.

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Figure 1.

The schematic describes the process and infrastructure developed for high-throughput MR histology of the mouse brain. As the brain is fixed and stained, the critical metadata is entered into the database. A novel acquisition strategy encodes Fourier (k) space for both T1-weighted and T2-weighted images. Dedicated computers reconstruct the very large (1.2 GB) image arrays and automatically archive image data and critical acquisition/reconstruction parameters in the Oracle database. Post-processing steps, including T2 enhancement and autosegmentation, provide additional derived data, all of which is assimilated into the database with the animal metadata. The entire database is made available via the BIRN infrastructure providing online access to over 100 high-resolution MR images of the mouse brain.

Figure 2.

Perfusion fixation with contrast agent reduces the spin lattice relaxation time, so that there is substantial increase in signal-to-noise; **a—d**): formalin-fixed specimen at TR=20–160 ms, SNR (unstained)=1.9, 2.6, 4.8, 10.2; **e—h**) actively stained specimen at TR=20–160 ms, SNR (stained)=11.0, 21.8, 36.7, 51.5.

Figure 3.

Spin warp encoding is used with a traditional Cartesian sampling pattern. A script breaks Fourier space into separate volumes with the gain sequentially decreased as the acquisition proceeds from the periphery of Fourier space (where the signal is small) to the center of Fourier space (where the signal is large). Since the gain has been increased in the periphery, the conjugate side of the acquisition is not required, allowing us to limit the acquisition to the area bounded by the dotted line.

Figure 4.

a) A representative 21.5-micron slice shows a region through the hippocampus (in the inset) for comparison; **b)** reconstructed at 43 microns; **c)** reconstructed at 21.5 microns with expanded range partial Fourier method (scan time 2.05 hours). The arrows represent: (1) and (2) small blood vessels; (3) thin layer between the granular layer and polymorphic layer of dentate gyrus; (4) stratum lucidum; **d)** reconstructed at 21.5 microns with full Fourier scan (scan time=7.28 hours).

Figure 5. Transverse images of the identical level demonstrate the complementary nature of the **a)** T1-weighted sequence at 21.5 microns ($TR=50$ ms, $TE=5$ ms), total acquisition time=2 hours; **b)** T2-derived image at 43 microns ($TR=400$ ms, $TE_{eff}=56$ ms), total acquisition time=4 hours. The arrows represent: (1) boundary of pyramidal cell layer in the hippocampus; (2) boundary of caudate putamen; (3) boundary between caudate putamen — globus pallidus; (4) cortical layers; (5) laterodorsal thalamic nucleus; and (6) hypothalamus.

Figure 6. Volume-rendered image of C57BL/6J mouse brain showing structures that have been automatically segmented: Brstm (brainstem), Cb (cerebellum), fi (fimbria), GP (globus pallidus), Hc (hippocampus), IC (inferior colliculus), SC (superior colliculus), VS (ventricular system).

Figure 7.

a) Lower-resolution T2-weighted scan at 86 microns can be acquired in 49 minutes; **b)** an 8-times higher resolution, high-throughput T1-weighted scan can be acquired in 27 minutes; **c)** the 64-times higher resolution T1 scan at 21.5 microns can be accomplished in 2 hours.

Figure 8.

Composite figure from MBAT shows **a—c**) orthogonal planes from the T1-weighted volume; **d—f**) registered orthogonal planes from the T2-weighted volume; and **g—i**) combined T1 image with color-coded labels derived from the autosegmentation routine. The entire volume is available at full resolution for download as supplementary material at: <http://www.civm.duhs.duke.edu/pubs/supplemental/NeuroImage200701/index.html>

Table 1

Segmented structures and abbreviations for the actively stained mouse brain as defined by Paxinos (Paxinos et al., 2001).

Structure	Abbreviation	Structure	Abbreviation
Nucleus Accumbens	Acb	Lateral lemniscus	ll
Amygdala	Amy	Olfactory bulb	OB
Anterior commissure	ac	Optic tract	opt
Brainstem	Brstm	Periaqueductal gray	PAG
Caudate putamen	CPu	Pontine nuclei	Pn
Cerebellum	Cb	Septal nuclei	Spt
Cerebral cortex	Cx	Substantia nigra	SN
Cerebral peduncle	cp	Superior colliculus	SC
Cochlear nuclei	CN	Spinal trigeminal tract	sp5
Corpus callosum	cc	Thalamus	Thal
Fimbria	fi	Ventricular system	VS
Globus pallidus	GP	Anterior pretectal nucleus	APT
Hippocampus	Hc	Mesencephalic nuclei	MRN
Hypothalamus	Hy	Thalamic Nuclei	
Inferior colliculus	IC	Laterodorsal thalamic nuclei	LD
Internal capsule	ic	Geniculate nuclei	GN
Interpeduncular nucleus	IP	Ventral thalamic nuclei	VT

Table 2

Summary of protocols for high-throughput and high-resolution magnetic resonance histology for automated morphologic phenotyping.

Protocol	TR/TE	Array	Resolution	Time
T1 Hi ThruPut	25/5 ms	$256^2 \times 512$	43.0 μm	27 min
T2 Hi ThruPut	200/50 ms	$128^2 \times 256$	86.0 μm	49 min
T1 Hi Res	50/5 ms	$512^2 \times 1024$	21.5 μm	123 min
T2 Hi Res	400/50 ms	$256^2 \times 512$	43.0 μm	246 min

Table 3

Comparison of resolution, scan time, and efficiency for MR histology of the mouse brain. Note that the T1 Hi Res scan is more than 100-times efficient than reference (MacKenzie-Graham et al., 2004). *See Methods section.

Reference	Contrast	Resolution (microns) ³	Volume (pico l)	Time (min)	Relative Efficiency
Clinical MRI					
(Benveniste et al., 2000)	T1	1000 ³	1	10	0.32
(Johnson et al., 2002a)	T2*	39×39×156	240	840	144
(MacKenzie-Graham et al., 2004)	PD	20 ³	8	840	4312
(Ma et al., 2005)	T2	60×60×120	400	819	87
(Kovacevic et al., 2005)	T2*	47 ³	100	330	550
T1 Hi ThruPut*	T2	60 ³	220	555	192
T2 Hi ThruPut*	T1	43 ³	80	27	2436
T1 Hi Res*	T2	86 ³	630	49	226
T2 Hi Res*	T1	21.5 ³	10	123	9220
	T2	43 ³	80	246	807