Standardizing Interoperability of MR/Histology Atlases of the Mouse Brain

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Introduction

Magnetic resonance histology (MRH) was first suggested in 1993. MRH provides several attributes that complement more traditional histology. Most notable among them is the potential for truly three-dimensional imaging providing much more accurate morphometry of brain structure than is possible with traditional techniques. Over the last 10 years, increasing efforts have exploited this attribute to develop 3D digital atlases of the mouse brain. Each atlasing group has developed their own approach and standards, but there is a compelling need to rationalize these efforts to do the following:

a) make the data from all groups more uniformly available to the neuroimaging community
b) allow comparison among atlases
c) provide a framework that would encourage cooperation in future efforts

The "best practices" described here are not necessarily the "best" practices and certainly are not the only approach. Rather, this document will provide a starting point – a point from which we fully expect more sophisticated and complete approaches will be developed.

Background

Magnetic resonance microscopy (MRM) provides a unique tool to facilitate comparisons of brains and brain atlases. MRM is non-destructive, so the intact tissue is completely undistorted by shrinkage or cutting. Data can be visualized along any axis without distortion or loss of spatial resolution (Figure 1a,b). But, as with conventional optical histology, an abundance of imaging methods each produce unique tissue contrast. Figure 1b,c shows representative images from the same specimen with two differing sets of acquisition parameters. Layers in the hippocampus seen in (b) are not apparent in (c). But, cortical layering and subthalamic nuclei in (c) are not evident in (b). The widely varying contrast in the cytoarchitecture is clear. In order for registration software to work effectively, there must be some common structures visible in the target and sample datasets.

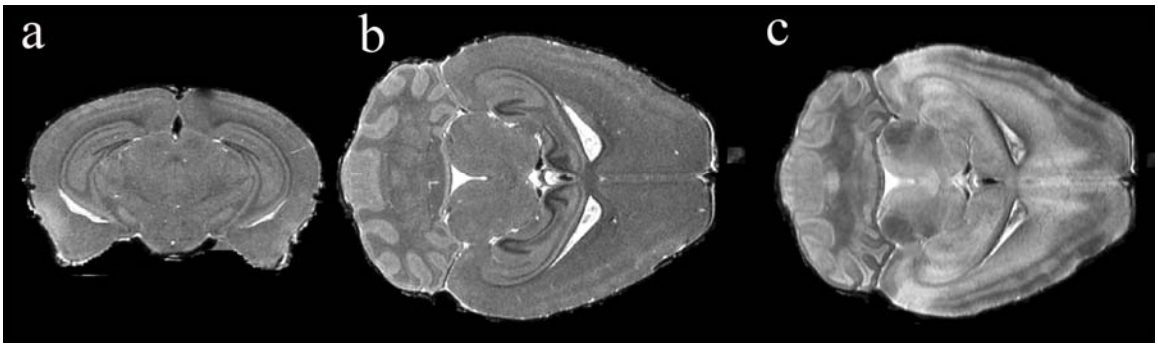

Figure 1: a) Coronal section from a 3D MR microscopy image of an actively stained mouse brain; b) dorsal plane from the same datasets showing isotropic resolution. Since the brain is fixed in the skull, there is limited shrinkage and no physical distortion from sectioning. c) Dorsal plane of the same specimen acquired with different acquisition protocol demonstrates completely different cytoarchitecture.

Goal

The global goal of the best practices document is to provide methodology that will allow comparison of digital atlases of MR and conventional histology. More specifically, we will articulate a common set of methods that will allow groups to prepare digital MR data with minimal distortion and common contrast so that existing algorithms will permit registration based on maximization of mutual information.

Previous work

A number of talented researchers have developed MR atlases of the mouse brain [1-3]. Table 1 from [4] summarizes some of the more recent acquisition methods. These atlases are all subject to the fact that ongoing technical improvements continue to provide higher resolution and contrast.

Reference	Contrast	Resolution (microns)^3	Volume (pico l)	Time (min)	Relative Efficiency
Clinical MRI	T1	1000^3	1	10	0.32
Benveniste et al [5]	T2*	39×39×156	240	840	144
Johnson et al [6]	PD	20^3	8	840	4312
MacKenzie-Graham,[1][a]	T2	60×60×120	400	819	87
Ma et al [2] [b]	T2*	47^3	100	330	550
Kovacevic et al [3]	T2	60^3	220	555	192
T1 Hi ThruPut[4]	T1	43^3	80	27	2436
T2 Hi ThruPut* [c][4]	T2	86^3	630	49	226
T1 Hi Res*[4]	T1	21.5^3	10	123	9220
T2 Hi Res*[4]	T2	43^3	80	246	807

[a-c]See Figure 2

As is evident from the table, all of the groups have chosen different approaches. Data are acquired at vastly different spatial resolution. And more importantly, there is a diverse range of imaging methods leading to widely varying contrast in the cytoarchitecture. Finally, it is clear that the acquisition time is generally quite long and the higher the spatial resolution, the longer the acquisition time. The one notable exception is the T1-weighted Hi ThruPut protocol described by Johnson et al. [4]. The spatial resolution (voxel volume) at 80 pico l is 20% smaller than the next closest method (with the exception

of the other work from Duke), yet the scan time is more than 12-times shorter. The key to this simultaneous high spatial resolution and rapid acquisition is the use of active stains to reduce the spin lattice relaxation times (T1) [7]. Figure 2 shows comparisons among the methods of Ma, MacKenzie-Graham, and Johnson.

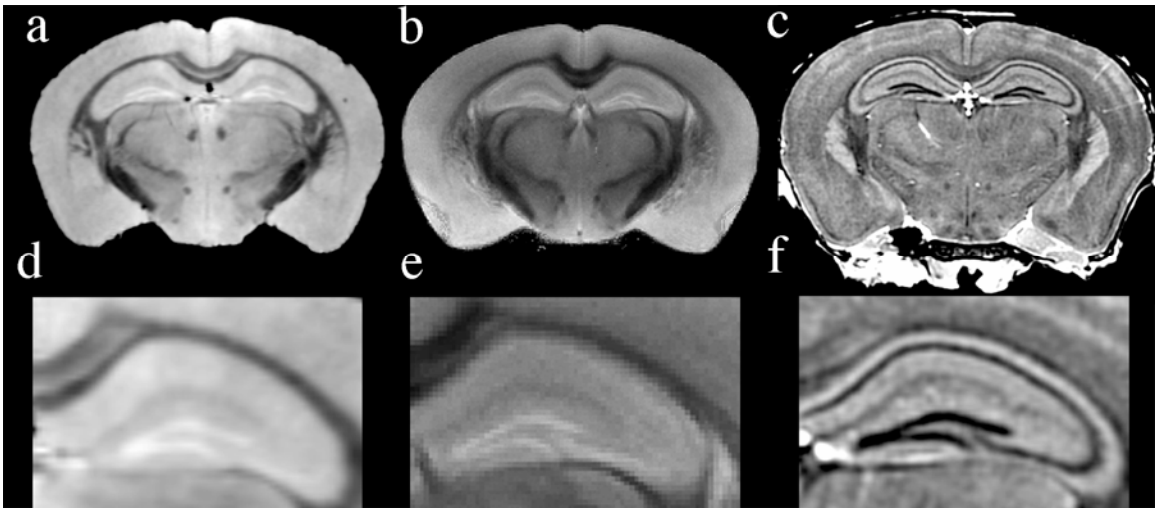

Figure 2: Representative images from (a)(d) Ma [2], (b)(e) MacKenzie-Graham [1], and (c)(f) Johnson [4]. (d-f) show 3X magnification of the hippocampus. While the granular layer is visible in all three images, the pyramidal layer, dentate gyrus, CA1, CA2, CA3, molecular layer, and oriens layer are only visible in (c) and (f).

Specimen Preparation

One of the most important elements of these methods is the active staining of the specimen in the skull. Note in Figure 2, the substantial difference in morphology between (a), (b), and (c). Both MacKenzie-Graham and Ma have removed the brain from the skull. Many histology studies remove the brain from the skull to facilitate penetration of the fixative. We suggest the use of an internal access, i.e., a transcardial perfusion that leaves the brain intact in the skull, thereby reducing distortion.

The method for fixation and staining is provided below.

INSTRUMENTS NEEDED:

- Fine tissue forceps, 12 cm Adson 1X2, tip width 1 mm
- 2 Fine dressing forceps, 12 cm Adson fine serrated, tip width 1.2 mm (optional)
- Halsted - Mosquito hemostatic forceps 12 cm, straight
- Surgical scissors 12 cm sharp-blunt, straight
- Surgical scissors 13 cm sharp-blunt, straight
- Spring scissors 8.5 cm sharp, angled

OTHER SUPPLIES NEEDED:

- Gloves
- tape
- 20-gauge catheter

Note: The catheter is made from a 20-gauge stub adapter cut off from the hub. The catheter is inserted into PE 100 tubing with about 5 mm extending from the end of the tubing. The tip is beveled to give it a cutting edge for puncturing the left ventricle.

- Vacuum aspirator for removing the fluids, scavenger system for removing formaldehyde fumes.

PERFUSION APPARATUS:

- Fluid reservoirs
- Two peristaltic pumps (one for saline and one for formalin)
- Double perfusion pumping station
- Plastic tubing
- Heating device
- Three-way solenoid inlet valve
- Pressure transducer

The two peristaltic pumps are operated by one double-perfusion pumping device for easy switching between the saline flush and the fixative. The saline is pumped through tubes that also wind through a warming device before reaching the animal. The formalin is kept at room temperature. Both the saline and formalin lines then go through the three-way valve and into a single outlet line. One end of a pressure transducer is connected to the valve outlet and the other to the PE tubing and catheter.

DRUGS, SOLUTIONS:

- Isoflurane, Nembutal and Butorphanol
- Saline flush - 1:10 solution of ProHance in 0.9% saline plus 0.1 % heparin, warmed to 37°C (1 part contrast agent and 9 parts 0.9% saline).
- Fixative - 1:10 solution of ProHance in 10% phosphate buffered formalin

PROCEDURE:

This is a conventional transcardiac perfusion fixation with inflow to left ventricle and outflow from right atrium. The pumps are set at a flow rate of 9 ml/minute. This setting is for an adult mouse between 20 and 40 grams. Modifications will be needed for animals outside this range.

The pressure at this setting and for the size of the tubing and stub adapter is about 85 mmHg, as read out from the transducer. Set up the pumps and fill the tubes, making sure there are no air bubbles in the line and that the PE tubing and catheter are filled with saline to start.

1. Have ready nembutal injection equivalent to a dose of 100mg/kg and butorphanol at 2mg/kg.
    a) Make sure everything is set up properly and ready to go.
    b) The perfusion has to follow quickly after the injections, since it is a fatal dose of nembutal.

2. Place a small amount of isoflurane (about 2 ml) on a stack of gauze in a jar or large beaker with a cover.
    a) There is a screen barrier over the stack of gauze to keep the animal from direct contact with isoflurane. (An induction chamber can be used for this. We typically use a large beaker or a ziploc bag and a gauze pad with isofluorane).
    b) Place the animal in the beaker for a few seconds, just until it stops moving around.

c) Remove from beaker and inject nembutal and butorphanol intraperitoneal. Watch animal closely.
d) When the animal reaches surgical plane anesthesia (no reflexes), tape it down on a platform.
e) Incise skin at xyphoid process and cut cranially to the manubrium. Snip the diaphragm and cut the chest open enough to expose the heart. Retract and hold back the rib cage with a hemostat.

3. If this will make it easier, use one pair of dressing forceps to gently hold the heart and the other to hold the catheter.
    a) In quick succession, insert the catheter into the apex of the left ventricle, snip the right atrium and start the saline flow.
    b) Perfuse with saline for 4 minutes. Switch to formalin flow. Observe muscle contractures as the formalin reaches the heart. This is an indication that fixation is taking place.
    c) Continue formalin perfusion for 4 minutes.

4. After perfusion, cut the head off close to the shoulders.
    a) Store head in a small jar of buffered formalin (with no ProHance).
    b) Within 24 and 48 hours, trim head and image. Other internal organs can also be removed for MR imaging.

Scanning Protocol

There are an enormous number of possible scanning protocols. The specific one we have outlined below is not magic. We have considered several elements. The first is that T1 is generally very field-dependent. So the contrast one might get at 4.7T could be considerably different than that at 9.4T. However, the staining process outlined above effectively reduces the T1 of all the tissue types to <100 ms. Thus the protocol we have chosen, even with the relatively short TR (for high-speed acquisition), will provide relatively consistent contrast across many different magnetic fields.

The second important decision for our best practice scan is the spatial resolution and associated 3D image array (43 μm isotropic resolution; 256 x 256 x 512). The canonical image array we have provided for Waxholm Space is 8X larger (512 x 512 x 1024) and the spatial resolution is 8X greater (21.5 μm isotropic resolution). But, this resolution and the ability to routinely handle these large arrays are currently unique to the Duke Center for In Vivo Microscopy. The smaller array should be readily achievable in most laboratories, and the spatial resolution is an integer multiple of the higher resolution canonical atlas, which makes upsampling (or downsampling) an integer multiplication/division. Finally, the entire acquisition is relatively fast (<2 hours) with sufficient signal that spectrometers (at varied magnetic field) that are not tuned to their peak performance should provide reasonable results.

Best Practice Acquisition Protocol

Magnetic field	1.5-11.7 T
Solenoid rf coil	Internal diameter: <14 mm Length: 20-30 mm
Specimen should be placed in container and surrounded with fomblin (or similar perfluropolyether) to limit susceptibility artifact	
Sequence	3D rf refocused spin echo
TR/TE	50 ms/5 ms
Number of excitations	2 (more can be used if required)
Bandwidth	< ±32 kHz
Field of View	11 x 11 x 22 mm
Spatial resolution	0.043 x 0.043 x 0.043 mm

References

1. MacKenzie-Graham, A., et al., A multimodal, multidimensional atlas of the C57BL/6J mouse brain. J Anat, 2004. 204(2): p. 93-102.
2. Ma, Y., et al., A three-dimensional digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy. Neuroscience, 2005. 135(4): p. 1203-1215.
3. Kovacevic, N., et al., A three-dimensional MRI atlas of the mouse brain with estimates of the average and variability. Cereb Cortex, 2005. 15(5): p. 639-45.
4. Johnson, G.A., et al., High-throughput morphologic phenotyping of the mouse brain with magnetic resonance histology. NeuroImage, 2007. 37(1): p. 82-89.
5. Benveniste, H., et al., Magnetic resonance microscopy of the C57BL mouse brain. Neuroimage, 2000. 11(6 Pt 1): p. 601-611.
6. Johnson, G.A., et al., Magnetic resonance histology for morphologic phenotyping. Journal of Magnetic Resonance Imaging, 2002. 16(4): p. 423-429.
7. Johnson, G.A., et al., Morphologic phenotyping with magnetic resonance microscopy: the visible mouse. Radiology, 2002. 222(3): p. 789-793.