Magnetic Resonance Microscopy of Discreet Microstructures in 5µm-Isotropic Images of the Excised Rat Striatum

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INTRODUCTION

During the past decade, the field of MRI has seen tremendous growth in its ability to resolve microstructural details of tissue. Thanks to the increasing field strengths of superconducting magnet systems coupled with advances in RF microcoil design, we are closer than ever to realizing the resolution gains needed to make non-invasive imaging of cellular structure in mammalian tissues possible [1,2]. While single-cell imaging research has been conducted on excised cells from marine organisms as a means of overcoming resolution limitations, such studies are unable to accurately replicate *in vivo* tissue architecture [3,4]. Likewise, all studies examining *in vivo* preparations of mammalian cells require the addition of exogenous agents such as supraparamagnetic iron oxide as a means of overcoming contrast and resolution limitations [5,6], and detect the presence of single cells rather than provide information on cell shape and internal composition.

In this study, we demonstrate the ability to routinely resolve discreet microstructures in excised slices of the rat striatum. Although positive identification of these structures remains the focus of future experiments, it should be noted that said structures are only visible at the upper limits of resolution deemed theoretically capable of resolving mammalian cells.

METHODS

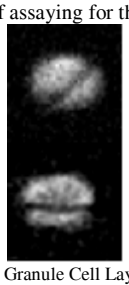
Rat striatum was excised in 500µm thick slices and immersion fixed in a 4% formaldehyde solution made in phospho-buffered saline (PBS) for no less than 24h. Following fixation, samples were washed in PBS overnight as a means of removing formaldehyde prior to our imaging experiments. Washed tissue was placed inside the sample well of a 200µm diameter micro surface coil (Bruker, Z76412) and MR data were acquired using a 600 MHz, 51mm vertical-bore spectrometer (Oxford Instruments) interfaced to a Bruker console. Three dimensional gradient-echo images were acquired at 4.7µm isotropic resolution [TE=10ms, TR=150ms, matrix=128^3, 0.6mm FOV, acquisition time=22h]. Following imaging, samples (n=6) were snap-frozen in cryosectioning medium (Richard-Allan, 6502), sliced in 25µm increments, and affixed to poly-L-coated microscope slides. After allowing tissue slices to adhere overnight, slides were Nissl stained (0.5% cresyl violet, 0.3% acetic acid) in order to visualize the location of cell bodies contained within each slice.

RESULTS

Figure 1A shows representative images taken by the 200 µm microcoil of the granule (top-DWI) and CA1 (bottom-MSME) cell layers in the rat hippocampus. Both contrast mechanisms result in hypointense signal regions in areas of densely-packed cell bodies. Figure 1B is a three dimensional segmentation reconstruction (AMIRA, Mercury Computer Systems, Inc.) of multiple 4.7µm-thick frames (70µm total depth) detailing the semi-spherical, discreet nature of the structures in the striatum. The non-contiguous nature of these structures allowed us to rule out vasculature as a potential histological correlate to the microstructures. Figure 2A is a representative image (4.7 µm isotropic, TE=10ms, TR=150ms) exhibiting a large volume of the microstructures. Figure 2B is a Nissl-stained cryosection (25µm) which allows for visualization of both descending white-matter tracts (arrow) and cell soma (arrowheads) in rat striatal tissue.

DISCUSSION and CONCLUSIONS

The data demonstrate our ability to produce, to our knowledge, the highest resolution MR images of neural tissue known to date. Clearly, as demonstrated by our findings, there are soft-tissue structures present in biological samples that can be visualized only thanks to the vast increases in resolution offered by contemporary spectrometer and micro RF coil design. When comparing the histology and MR images, it is tempting to conclude that the dark spots on the MRI are cell bodies; however, this has yet to be confirmed. Histological studies are being performed that will identify the nature of the image heterogeneity. For example, because iron deposits are known to accumulate in the basal ganglia and because iron manifests itself as areas of low signal intensity in MR images, we cannot rule out that the dark spots originate from iron deposits. Thus, our histological regimen will include Perls stain as a means of assaying for the presence of iron in our samples.



Top: Granule Cell Layer Diffusion Weighted Image Bottom: CA1 Cell Layer T2-weighted MSME Image

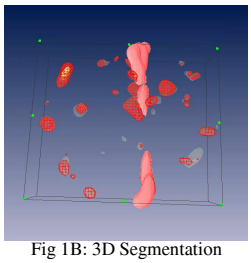


Fig 1B: 3D Segmentation reconstruction (70µm depth) of 15 separate image frames (1 frame pictured in Fig 1A)

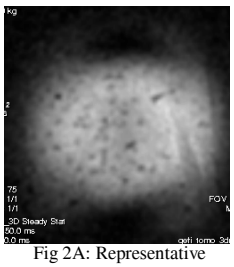


Fig 2A: Representative image (4.7µm res) showing large numbers of the structures (quadratic filter applied)

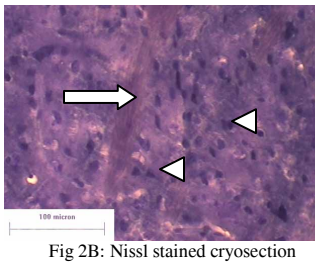


Fig 2B: Nissl stained cryosection (25µm depth) of the rat striatum illustrating both a white matter tract (arrow) and cell bodies (arrowheads)

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