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Technical Note

Magnetic resonance microscopy of human and porcine neurons and cellular processes

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ABSTRACT

With its unparalleled ability to safely generate high-contrast images of soft tissues, magnetic resonance imaging (MRI) has remained at the forefront of diagnostic clinical medicine. Unfortunately due to resolution limitations, clinical scans are most useful for detecting macroscopic structural changes associated with a small number of pathologies. Moreover, due to a longstanding inability to directly observe magnetic resonance (MR) signal behavior at the cellular level, such information is poorly characterized and generally must be inferred. With the advent of the MR microscope in 1986 came the ability to measure MR signal properties of theretofore unobservable tissue structures. Recently, further improvements in hardware technology have made possible the ability to visualize mammalian cellular structure. In the current study, we expand upon previous work by imaging the neuronal cell bodies and processes of human and porcine α-motor neurons. Complimentary imaging studies are conducted in pig tissue in order to demonstrate qualitative similarities to human samples. Also, apparent diffusion coefficient (ADC) maps were generated inside porcine α-motor neuron cell bodies and portions of their largest processes (mean = $1.7 \pm 0.5\ \mu m^2$/ms based on 53 pixels) as well as in areas containing a mixture of extracellular space, microvasculature, and neuropil ($0.59 \pm 0.37\ \mu m^2$/ms based on 33 pixels). Three-dimensional reconstruction of MR images containing α-motor neurons shows the spatial arrangement of neuronal projections between adjacent cells. Such advancements in imaging portend the ability to construct accurate models of MR signal behavior based on direct observation and measurement of the components which comprise functional tissues. These tools would not only be useful for improving our interpretation of macroscopic MRI performed in the clinic, but they could potentially be used to develop new methods of differential diagnosis to aid in the early detection of a multitude of neuropathologies.

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Introduction

Owing to the fact that the technique does not rely on endoscopy or ionizing radiation, MRI is now arguably the most dominant non-invasive, non-destructive imaging modality of biological systems, with utility in both medical and material sciences. Despite finding widespread use in the clinic for identifying macroscopic features of a limited number of pathologies—e.g. tumors (Quadery and Okamoto, 2003), ischemic stroke infarct (Moseley et al., 1990)—MRI's utility for differential diagnosis is limited by a lack of specificity and fundamental understanding regarding the nature of how pathologies alter the MR contrast of microscopic tissue structures. Moreover, because the hallmarks of many diseases are often first detectable at the cellular level, understanding the characteristics of these changes at resolutions sufficient to observe them using MR is integral to creating new methods for detecting diseases at their earliest stages of development. Thus, to investigate such tissue contrast in the microscopic regime, we propose using magnetic resonance microscopy (MRM) as a means of characterizing MR contrast properties in cellular elements of human and porcine central nervous system tissue.

Although there were early concerns regarding its feasibility (Mansfield and Morris, 1982), MRM has evolved into a *bona fide*

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imaging regime of MR in the same sense that optical microscopy is a regime of optical imaging (Callaghan, 1991). As predicated in the literature, MR microscopy is loosely referred to as imaging (two-dimensional mapping) conducted at resolutions below 100 μm in at least one of the two spatial dimensions (Benveniste and Blackband, 2005; Tyszka et al., 2005). True three-dimensional MRM may have isotropic resolution, but most often the in-plane dimensions are equal, while the third, through-plane dimension is larger. Such resolutions are employed to take advantage of certain samples' inherent symmetry which, when oriented so as to achieve structural homogeneity in the through-plane dimension, allows for larger slice geometries and increases the signal to noise ratio (SNR) without loss of structural delineation due to volume averaging.

MRM offers unique challenges as an imaging modality. The SNR is, in the majority of cases, the single greatest factor to consider in terms of attaining the highest spatial resolution. Increased spectral line widths associated with employing higher magnetic field strengths are another important consideration for microscopy studies as they have the potential to limit resolution (Cho et al., 1990; Ciobanu et al., 2003). Moreover, for the spatial resolution implied by the voxel size to be real, said voxel size must dominate potential blurring imposed by both the intrinsic transverse relaxation time (T2) and diffusivity (D) of the nuclear species being imaged. In summation, generating accurate MRM data requires sufficient SNR and strong gradients, limiting most experiments to those imaging the hydrogen contained in water of relatively small samples at high magnetic field strengths.

The first such works published on MRM (Aguayo et al., 1986; Eccles and Callaghan, 1986; Johnson et al., 1986) appeared in 1986: over a decade after the first publication on MRI (Lauterbur, 1973). The Eccles and Callaghan study employed a plant stem, achieving an in-plane resolution of 20 μm, and—taking advantage of the sample's symmetry—a relatively large (1.5 mm) slice thickness. The study by Johnson et al. was the first published work conducted on animal tissue, achieving a resolution of $50\times50\times1000$ μm in the rat brain. Experiments conducted by Aguayo et al. were the first to visualize single animal cells—frog ova, ≈ 1 mm diameter—which was achieved using an image resolution of $10\times13\times250$ μm. Later studies explored the utility of *Aplysia californica's* L7 neuron as an alternative model for cellular imaging (Hsu et al., 1997). Such aquatic animal models became popular because their cell size allowed for excellent accessibility; however, the diameters of such cells are two to ten times greater than even the largest examples of mammalian cells (100–150 μm) and orders of magnitude greater than the majority of mammalian cells (5–15 μm). This size disparity was not trivial regarding the early limits of cellular visualization using MRM because, while the cells of certain aquatic organisms could be visualized using relatively thick slice geometries, the through-plane volume averaging associated with such scans precluded the detection of mammalian cellular structures by these means.

Most recently however, improvements in MR hardware technology—higher fields, micro surface-coils (Badilita et al., 2010; Ehrmann et al., 2007; Massin et al., 2003; Weber et al., 2011), and strong, rapidly-switching imaging gradients (Weiger et al., 2008)—have enabled the first reported instance of mammalian cell imaging using MRM (Flint et al., 2009). Perikarya of α-motor neurons in the ventral horn of the rat spinal cord were observed ($7.8\times7.8\times25$ μm) using diffusion-weighted microimaging methods. Identification of the specific microstructural elements within such MRM images can be achieved using light microscopy-based correlative histology techniques (Meadowcroft et al., 2007; Nabuurs et al., 2011). In the present study, we extend these experiments to include the first MRM images of individual human cells. Cell bodies and neural processes of human α-motor neurons are visualized with native MR contrast in excised portions of intact, fixed spinal cord tissue. MRM studies on porcine tissue are also reported as a means of demonstrating its similarity in morphological and MR signal characteristics to human tissue.

Methods

Tissue preparation

All procedures involving procurement, imaging, and disposal of human tissue specimen were reviewed and approved by the University of Florida's Internal Review Board (IRB # 129-2010). Immersion-fixed (4% Formaldehyde) spinal cord sections of 1.5 cm length were excised by gross dissection from both cervical and lumbar enlargements. Histological cross sections (50 μm) were cut in an ice-cold bath of phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4:pH 7.4) using a vibratome (Ted Pella, Lancer series 1000). Prior to imaging, slices were washed overnight in PBS to reduce fixative levels in the samples.

MRM

Imaging of human α-motor neuron perykaria

All MR imaging was performed using a 600 MHz Oxford spectrometer interfaced to a Bruker Biospin console. An area of the spinal cord's ventral horn ($3\text{ mm}\times3\text{ mm}\times50$ μm) containing α-motor neurons was identified and excised from each spinal cord cross section ($n=3$) prior to being secured in the tissue well of a 500 μm diameter micro surface-coil (Bruker Biospin, B6370) as described previously (Flint et al., 2010). Briefly, ventral horn tissue was excised from intact slices by hand and secured over the micro surface-coil using a nylon mesh insert of 50 μm pore size (Small Parts, CMN-0053-C) held in place by a retention ring of 300 μm thickness fashioned from a nylon washer (Small Parts, WN-M02-C). Sample selection and placement were achieved with the aid of a dissecting microscope (Zeiss, OPMI 1-FC). After securing the sample, additional PBS was added to the microcoil's tissue well and a piece ($1\text{ cm}\times1\text{ cm}$) of polymerase chain reaction (PCR) film (ABgene, AB-0558) was used to seal the well and prevent sample dehydration. Diffusion-weighted, 2D images (TR/TE = 2000 ms/23.5 ms, res = 7.8 μm × 7.8 μm × 50μm, temp = 23 °C, $\Delta=8.36$ ms, $\delta=2$ ms, b = 2000 s/mm^2, Avg = 40, scan time = 5 h 40 min) were employed to visualize cell bodies of human α-motor neurons. A linear smoothing algorithm (Bruker Biospin, ParaVision 3.0.2) helped define the boundaries of cell bodies present in our MR images.

Imaging of human and pig α-motor neuron processes

Spinal cord samples ($n=3$) were prepared and secured as described above. In experiments targeting processes, a micro surface-coil of 100 μm diameter was employed (Bruker Biospin, B6372) and three-dimensional spin-echo images (TR/TE = 2000 ms/12.75 ms, res = 6.25 μm isotropic, Avg = 14, scan time = 63 h 43 min) were collected. One such dataset was analyzed using an automated segmentation tool (Amira 3.1.1) to visualize cellular structures from multiple vantage points and display the close spatial arrangement of processes extending from adjacent neurons. In order to investigate intracellular diffusivity, three-dimensional diffusion-weighted images (TR/TE = 2000 ms/6.2 ms, res = 6.25 μm isotropic, temp = 23 °C, $\delta=0.31$ ms, $\Delta=2$ ms, Avg = 14, scan time = 63 h 43 min) were collected at two b values (b = 300 s/mm^2; b = 600 s/mm^2) and used to map the apparent diffusion coefficient (ADC) in the sample.

Correlative histology

Nissl stain for neuronal perikarya

Following diffusion-weighted, 2D MRM in the ventral horn of the human spinal cord, samples were removed from the micro surface-coil and stained for neuronal cell bodies. Samples were immersed in a solution of Nissl stain (0.5% cresyl violet acetate, 0.3% glacial acetic acid, 99.2% ddH_2O) for 2 min prior to receiving quick rinses in baths