Magnetic Resonance Microscopy of Mammalian Neurons

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INTRODUCTION Procuring images at resolutions high enough to visualize cellular structures in animal and human brain has been a long term goal of MRI. Although large cells—frog ova and Aplysia neurons [1,2]—have been imaged directly, cellular imaging in mammalian neural tissue has so far been elusive. The presence of cells has been detected using exogeneous contrast agents which distort the local MR signal—such as superparamagnetic iron oxide (SPIO) particles [3]—in order to generate contrast between cells and their surrounding tissues. However, these techniques require the use of contrast agents that exert yet unknown effects on normal cell physiology and only offer information about cell position. Because the signal and contrast reported are non-native, they do not provide direct quantifiable information on the cell itself. Although we have previously observed hypointense areas on microimages of neural tissue [4], histological correlation was not feasible, and thus it could not be ascertained if these structures were cells or other features: such as iron deposits, blood vessels or artifacts. In the present work, this challenge is solved by cutting tissue slices with thicknesses less than that of the thinnest MR slice permissible using our current gradients and pulse program setup and using diffusion weighting to eliminate the confounding perfusate signal. In this way, the exact same tissue is visualized in the MR and optical images making the direct correlation of cellular structures unambiguous.

METHODS All imaging was performed on a 600MHz Bruker spectrometer interfaced with microsurface coils developed by Bruker Instruments Inc. [5] and shown in Figure 1. Perfusion-fixed (4% formaldehyde) spinal cord sections (25μm thick) were bisected to permit placement into the coil's tissue well. The slice was secured in place using a retention system developed in-house consisting of a circular, nylon mesh screen with a viewing window cut into its center held in place by a nylon ring. Once secured, PBS was placed into the tissue well to prevent drying of the sample and the well was sealed using adhesive PCR film (ABgene, AB-0558). Diffusion-weighted images (b = 4025 s/mm^2, Δ = 17ms, δ = 6ms, slice thickness = 80μm, res. = 7.8μm in-plane, acquisition time = 7h7min) were acquired. Although the nominal slice thickness for the excitation pulse was set to the minimum value possible within the boundaries of our scan parameters (80μm), heavy diffusion weighting resulted in total abolition of signal from the PBS surrounding the sample yielding an effective slice thickness to be that of the tissue sample itself: i.e. 25μm. Following MR imaging, slices were Nissl stained to delineate cell bodies and photographed using a digital camera (QImaging, Retiga 4000R Fast 1394 Color) attached to a Zeiss microscope (Axioplan 2, Zeiss) and processed with software (QCapture Pro 6.0) available from QImaging. Several tissue samples were successfully analyzed using the methods described.

RESULTS Alpha motor neurons and axon bundles are visible in both the histological and MRI data sets. The boundary between gray and white matter at the ventral horn of the spinal cord is also evident in both images. Tissues imaged in the MRI experiment correlate spatially and morphologically with those in the histology making the assignation of tissue types unambiguous.

DISCUSSION and CONCLUSIONS This paper represents the first MR imaging of individual neurons in mammalian tissue achieved using a combination of high magnetic fields and microsurface coils. Corresponding optical histology clearly shows the cell bodies that appear as hypointense areas using diffusion-weighted MR microscopy. Future studies include quantitation of the MR signals and the development of a microperfusion chamber. This will facilitate the investigation of cells undergoing pathophysiological perturbations—such as ischemia—and therapeutic interventions, so that the microstructural and physiological origins of MR signals in tissues may be understood. The goal is to use this data to develop meaningful and realistic working mathematical models of tissues that can be used to interpret lower spatial resolution MRI, including human imaging, and then use these models to improve the sensitivity and specificity of clinical MRI.

REFERENCES and ACKNOWLEDGEMENTS 1) Aguayo, J.B., *et al.* Nature 1986 322:190-1 2) Schoeniger, J.S., Aiken, N., Hsu, E., & Blackband, S.J. *J Magn Reson B* 1994 103(3):261-73 3) Shapiro, E.M., *et al. Proc Natl Acad Sci* 2004 101(30):10901-6 4) Flint J.J., *et al.* 2007 *Proceedings of the ISMRM, Berlin, 19th-25th May* 5) Massic, C., *et al. Sensors and Actuators A: Physical* 2002 97:280-8 Funded by the NIH, the NHMFL, the KTI, DNRF, D-AF, DMF, Julie von Müllen's Foundation and the Oticon Foundation.

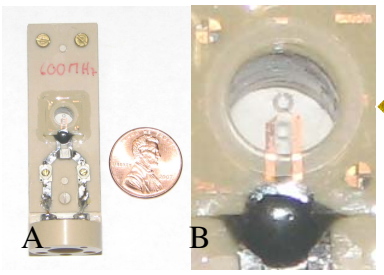

Figure 1. (A) Photograph of the 500μm surface microcoil developed by Bruker, Switzerland (Z76409). The four-turn coil sits inside a 5mm diameter, 500μm deep tissue well (B). A 200μm diameter coil was also used

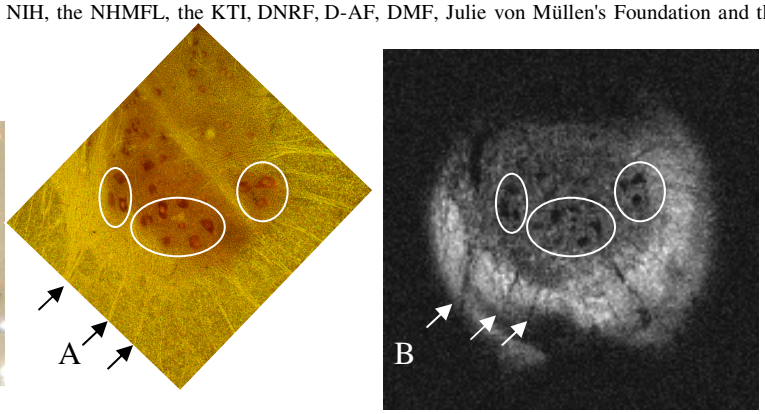

Figure 2. MR microscopy of α-motor neurons in the ventral horn of the rat spinal cord with corresponding histology. (A) Histological section showing α-motor neurons (red) and axonal projections at the tissue boundary of the ventral horn (arrows). (B) MRM image of cell bodies and projections that correlate spatially and morphologically with those seen in our histological analysis.