Histological Confirmation of *Aplysia californica* Neuron Structure Observed Using MR Microscopy

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INTRODUCTION

Since the first application of magnetic resonance microscopy (MRM) on large single cells (1), MRM has developed over the last 25 years as a complementary microimaging technique with a variety of applications ranging from materials to biological tissues (2). Several large single cells have been successfully imaged (3). Recently, using new surface microcoils to improve sensitivity, we performed the first MRM of neurons in mammalian tissue (4). Although it offers the potential to study tissues *in vivo*, the inherently low sensitivity of nuclear magnetic resonance (NMR) has limited MRM to the detection of only two cellular compartments—the cytoplasm and nucleus—in relatively large cells: i.e. frog ova (~1mm diameter) (1) and *Aplysia californica* neurons (~ 300-350 µm diameter) (5). However, it has so far been assumed that the structures observed in the MRM correspond to the nuclear and cytoplasmic compartments. In this work, we report the extension of the new microcoil technology to improve MRM of *Aplysia californica* neurons, and—for the first time—present correlative light microscopy including Nissl and fluorescent staining methods to label specific cellular substructures. These studies are also a precursor to the examination of the effects of collagenase on the MR signals in these neurons.

METHODS

MRM was performed using commercially available 500µm diameter (Serial # B6370) microcoils provided by Bruker Biospin. The MR studies were conducted on a 600 MHz (14.1T) Bruker Biospin system. In the standard protocol, extracted ganglia are placed in a collagenase solution to soften membranes thus aiding cell extraction (3). However, in the present study, collagenase digestion was omitted (see discussion). After isolation by gross dissection, L7 neurons (n=2) of *Aplysia californica* were fixed in a 4% Formaldehyde solution for no less than 24h, and then washed in PBS buffer (137mM NaCl; 2.7mM KCl; 10mM Na_2HPO_4; 1.8mM KH_2PO_4: pH 7.4) to remove fixative before imaging so that T_2 relaxation is restored to pre-fixation levels for improved SNR. The slices were then embedded in 3% of agarose. Neurons were sectioned into 50 µm thick slices using a Vibratome. MRM was performed using a 2D diffusion-weighted image sequence (7.8 µm in-plane resolution, 200µm slice width, b= 1500 s/mm^2, TR/TE = 2000/20 ms, NEX=100, acquisition time = 7hrs8min). Following MRM, slices were stained with PKH 67 for detection of the plasma membrane, and DAPI for detection of the nucleus. Additionally, a separate slice adjacent to the MR imaged slice was stained with Nissl (0.5% cresyl violet, 0.3% glacial acetic acid, in ddH_2O) to delineate the cytoplasm through labeling of the endoplasmic reticulum (ER).

RESULTS

Figure 1 shows an example diffusion-weighted image of an *Aplysia californica* neuron, where contrast is observed between the cell nucleus and cytoplasm (3). Figure 1 also shows optical images using different stains highlighting the cytoplasmic material (A), nuclear material (E) and the plasma membrane (D). A clear correlation is observed between the hypointense nucleus and the hyperintense cytoplasm in the MR image when compared with the optical images. Additionally, a large number of small cells are observed on the surface of the L7 cell, contributing to the hyperintense signal observed in the MR image at the L7 cell's surface.

DISCUSSION and CONCLUSIONS

Although it would be difficult to imagine what the structures observed in the MRM would be assigned to were they not the nucleus and cytoplasm, these data, for the first time, verify their identity via direct correlative histology. This is an important validation as we move forward to develop working mathematical models of tissues based on the observed MR signals in these samples and how they vary with perturbations (6). These data also represent the highest spatial resolution MR images of single *Aplysia californica* cells reported to date. Additionally, a large number of smaller cells surrounding the neuron are confirmed when collagenase is not used in the cell extraction procedure. Future studies will compare collagenase and non-collagenase extraction techniques, and their potential impact on quantitative MR studies in isolated single cells. Ultimately, these data will influence quantitative models of MR signals in tissues and how they improve our understanding of MR signals and their changes in human MRI.

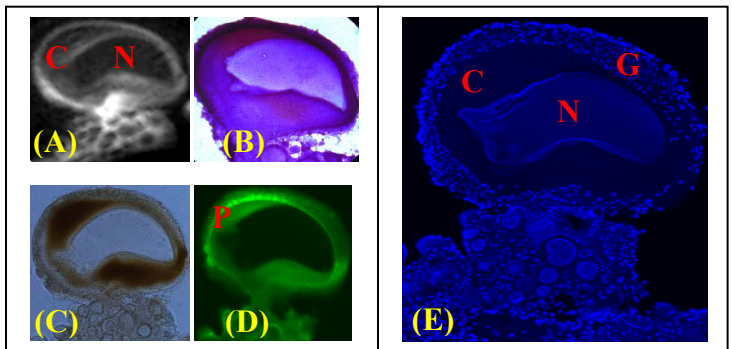

Figure 1. (A) Example diffusion-weighted MR microimage of an *Aplysia californica* neuron. (B) Nissl-stained histology of a slice adjacent to that in A. (C) light microscopic image and (D) PKH 67 green fluorescent-stain of the same tissue section as in (A). (E) Fluorescent image of DAPI-stained nucleic acid. N = nucleus, C = cytoplasm, P = plasma membrane, G = Connective tissue layer with embedded cells.

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