Short Communications

Magnetic resonance microscopy: in vivo sectioning of a developing insect

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Summary. The utility of magnetic resonance imaging vis-a-vis insect morphology and development was investigated. MRI is a noninvasive technique that distinguishes between tissues based on proton content and proton 'environment'. At present a resolution of 100 μm is achievable. The technique avoids fixation artifacts and allows the detection of motion within the organism.

Key words. Nuclear magnetic resonance; magnetic resonance imaging; chemical-shift imaging; *Manduca sexta.*

In 1973 Paul Lauterbur demonstrated that signals in a nuclear magnetic resonance experiment could be spatially localized by suitable application of magnetic gradients [1]. The concept has spawned an entirely new discipline of magnetic resonance imaging (MRI). The water in most biological tissues provides an abundant source of protons, which possess a small magnetic moment. The tissue is placed in a strong magnetic field causing the protons to align with and precess about the applied field at the Larmor frequency. A radiofrequency pulse at this same frequency is absorbed by the precessing protons and reradiated yielding the NMR signal. By application of a series of radiofrequency pulses and magnetic field gradients the signal can be mapped from the sample into a digital array (e.g. 256 × 256) yielding an image of a magnetically selected 'slice' of the sample [2].

The intensity of each point in the array reflected by the brightness of that point in the image is dependent on the number of protons at that point as well as the spin lattice and spin-spin relaxation time (T 1 and T 2). These relaxation times are intimately related to the state of the protons (e.g. whether the protons are found in free water, bound water, or are the CH_2 protons in fat). By appropriate timing in the pulse sequence one can emphasize one of these dependencies, producing a wide range of contrast between different tissues. The result is a totally noninvasive imaging technique that enables one to probe the molecular nature of protons in live biological samples [2–4]. The majority of research to date has focused on human imaging. Recently, however, the technique has been extended to a broader range of biological problems through the development of MR 'microscopy' [5–9]. We here illustrate the use of MR microscopy for the noninvasive sectioning of a developing pupal tobacco hornworm, *Manduca sexta* (L.).

Methods and materials. All work was performed on a 2.0 Tesla CSI (chemical-shift imaging) MRI system (GE NMR Instruments, Fremont, CA) which has been modified for MR microscopy. The principal modification is a 3.5 cm diameter by 5.0 cm long radiofrequency coil which yields the higher signal to noise ratio necessary for high resolution studies. The coil is a scaled version of the 'birdcage' design suggested by Hayes et al. [10]. Three-dimensional Fourier spin warp (3 DFT) pulse sequences have been developed that provide efficient signal averaging from a selected volume of the sample [11, 12]. A typical study yields 16 contiguous slices each with an effective thickness of 1.25 mm. In plane pixel dimensions are 100 × 100 μm for the axial images shown and 200 × 200 μm for the sagittal projections.

The pulse sequence timing parameters TR and TE determine weighting of tissue contrast for T 1 and T 2. TR is the cycle time between sets of RF pulses where the first pulse excites the spins and a subsequent series of 1–4 additional pulses causes the formation of radiofrequency spin echoes at differing echo times (TE). This set of pulses is repeated with variation in the gradients for spatial encoding. In this work T 1-weighted images were produced with TR = 400 ms and TE = 20 ms. Images with progressively increased contrast dependence on T 2 were generated in a 3 D Carr-Purcell-Meiboom-Gill (CPMG) series in which a series of spin echoes are produced at TE = 20, 40, 60, and 80 ms. The repetition time, TR, was 1600 ms which is generally sufficient to insure T 1 recovery between the individual pulse cycles, i.e. remove any residual T 1 weighting. The initial (TE = 20 ms) set of 16 images in this CPMG series is the least dependent on T 1 and T 2. Tissue contrast in this image is primarily dependent on differences in proton density. Thus 3 possible types of images are available; T 1-weighted (TR = 400 ms, TE = 20 ms); T 2-weighted (TR = 1600 ms, TE = 40–80 ms); and relative spin densitiy (TR = 1600, TE = 20 ms). A 16-slice T 1-weighted set of images is acquired in 54 min. The 16-slice (4 echo) relative spin density/T 2-weighted set requires 216 min to acquire. We present only relative spin density and T 1-weighted images in this paper.

Each moth pupa was placed in the 3.5 cm coil and positioned in the center of the 30 cm diameter bore magnet. A small quantity (100 g) of dry ice placed in the magnet bore kept the pupa anesthetized during the scans.

Specimens for comparison to the MR images were fixed in Bouin's solution for 48 h prior to processing. They were then rinsed and imbedded in paraffin using vacuum infiltration. The specimens were sectioned (5 μm), mounted, and stained with hematoxylin and eosin.

Results and discussion. The 100 μm resolution in the plane of each pupal cross section allows the recognition of several organ level features. In figure 1 these features are compared to those visible in 5-μm paraffin sections. In this relative spin density image of a female pupa areas with a high proton concentration appear white, e.g. the central rectal fluid (RF) and the lumen of the heart (H), both containing large amounts of free water. Areas of lower proton concentrations such as the muscle bundles (DLM and VLM) and ventral nerve cord (VNC) appear grey and areas of lowest proton concentration, the air sacs (AS), appear black. In a more anterior section (fig. 2 A) a convolution of the intestine (I) is clearly visible and the wing veins (WV) are particularly prominent. A sagittal section near the midline of a male pupa (fig. 2 D) shows a paired testis (T) and the aorta (A) as it loops through the thoracic muscle mass.

While in most cases an unmoving specimen is desirable because the NMR signals are averaged over relatively long periods of time, motion artifacts (ghost images) can provide biologically relevant information. In figure 2 B motion artifacts (arrows) indicate that the wall of the air sac momentarily collapsed during image acquisition. This provides direct evidence for active ventilation of the air sacs during the pupal stage.

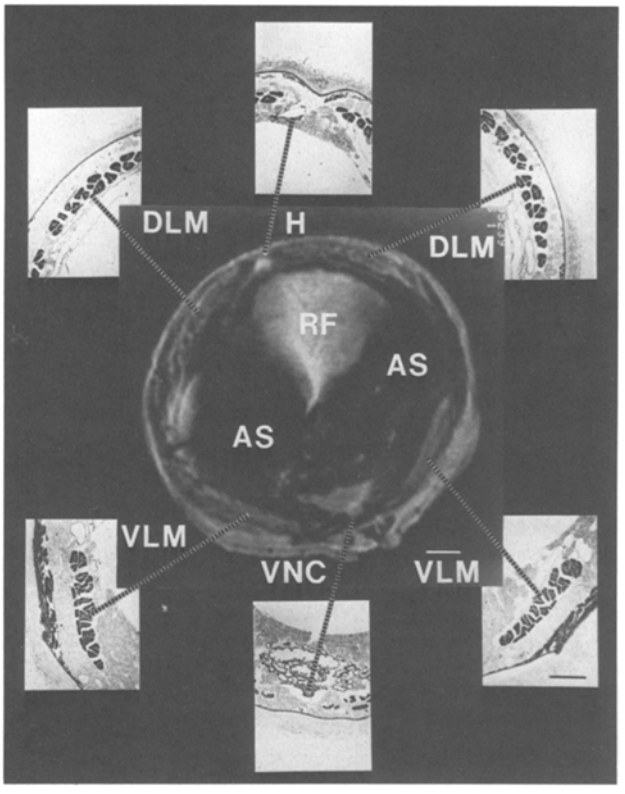

Figure 1. Comparison of relative spin density MR image (female cross section) with standard histological sections (5 µm, hematoxylin-eosin). AS, air sacs; DLM, dorsal longitudinal muscles; H, heart; RF, rectal fluid; VLM, ventral longitudinal muscles; VNC, ventral nerve cord. (Note the ventral nerve cord and associated neural tissue visible in the histological section shrank somewhat during fixation and thus it appears smaller than the MR image.) Scale bars = 1 mm.

In a T 1-weighted image (fig. 2 C) substances with a long T 1 appear black (central rectal fluid with a large amount of free water). Tissue with a short T 1 is accentuated and appears white. An example is the diffuse fat body scattered throughout the body cavity. The fat body was not easily resolved in the relative spin density images. The testis (T) is also apparent with the two vasa deferentia (D) cut in the cross section. Magnetic resonance imaging is rapidly approaching a resolution that will prove useful in insect studies. While it cannot compete at present with standard histological techniques in resolution, it does have significant advantages over them. 1) MRI is a noninvasive technique allowing the study of development within a single living individual. 2) MRI distinguishes between tissues which vary in proton content and proton 'environment'. This is its primary advantage over other imaging techniques. 3) It allows the detection of motion within an organism, and 4) it avoids fixation artifact.

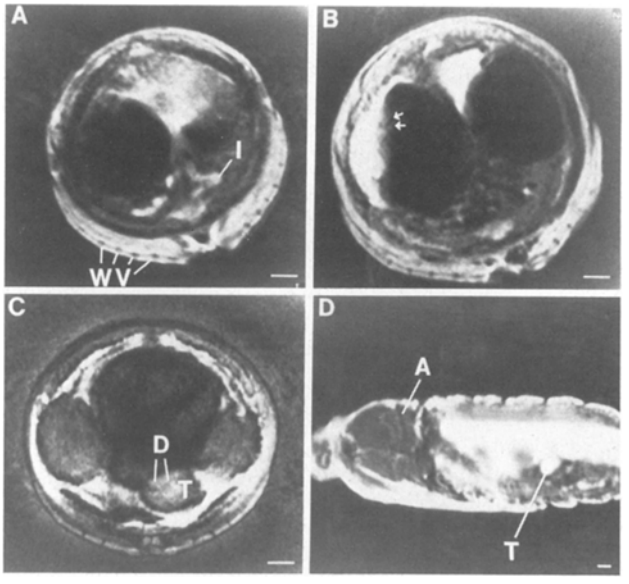

Figure 2. *A* Relative spin density image (female cross section); I, intestine; WV, wing veins. *B* Relative spin density image (female cross section); arrows, motion artifact. *C* T 1-weighted image (male cross section); D, vas deferentia; T, paired testis. *D* Relative spin density image (male sagittal section); A, aorta; T, paired testis. Scale bars = 1 mm.

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