Internal Motions of Manduca sexta pupae Studied Using Magnetic Resonance Microscopy

K. J. Hallock[1]

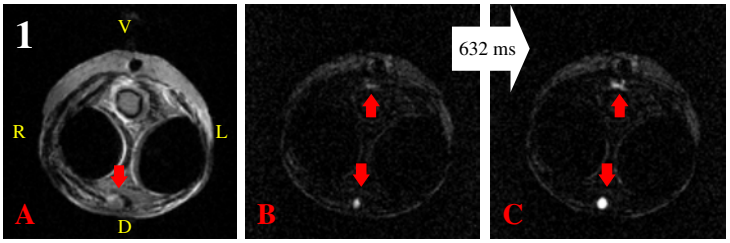
[1]Center for Biomedical Imaging, Boston Medical Center, Boston, MA, United States

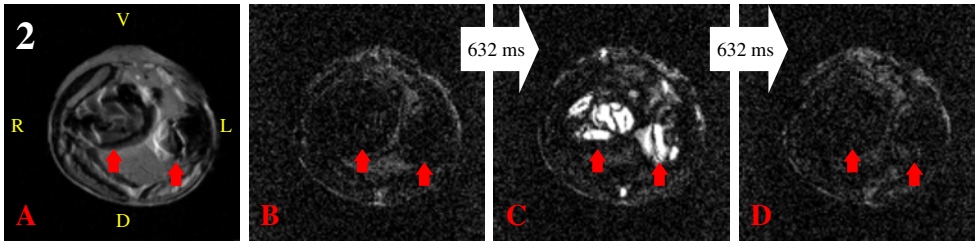
Introduction

Insects are vectors for many diseases, such as malaria, Chagas' disease, and West Nile virus, and cause extensive agricultural damage. To combat this constant threat, many control measures have been used, including ones that are harmful to both humans and the environment. Resistance to pesticides and growing concerns over their use encourages the development of better control strategies. By increasing our understanding of insect physiology, we will improve our ability to develop safer and more effective methods for insect control. To date, studies of insect physiology using magnetic resonance microscopy (MRM) have only been applied to a few insect species and met with mixed success (1-4). One of the noted limitations of MRM is internal motion, which can cause blurring of high resolution structural images (1); however, the fact that images are influenced by dynamic processes suggests MRM would be an excellent tool for studying insect circulation and respiration. An insect's cuticle, open circulatory system, and extensive tracheal system present unique challenges to any experimental technique used to probe them. In this preliminary investigation, we explored the use of balanced FISP in studying respiration and circulation in *M. sexta* pupae.

Materials and Methods

Seven *M. sexta* (Lepidoptera:Sphingidae) pupae were purchased from Carolina Biological (Burlington, NC) and stored at room temperature in the dark for the duration of the studies. MRM experiments were performed using a Bruker 500 MHz instrument operating at a magnetic field of 11.7 T with resonance frequencies of 500.15 MHz (^{1}H). Images were obtained using a commercial Bruker probe fitted with a 20 mm birdcage coil. Proton-density weighted (PDW) images were acquired using a standard spin echo sequence (α=90°, TE=15ms, TR=1s). Each PDW experiment required ≈2 minutes to acquire. Dynamic imaging for the movies was accomplished using a balanced FISP sequence (α = 60°, TE=1.239ms, TR=2.468ms). Each FISP acquisition was preceded by 5000 dummy scans to assure a steady-state was created before data acquisition began. Sixty consecutive image frames were then acquired, with one frame requiring 316ms to complete. Regardless of technique, all images had a field of view (FOV) = 20mm x 20mm, matrix = 128 x 128 (yielding a 156µm in-plane resolution), and slice thickness = 500µm. In the images, "V" is indicates the ventral side, "D" the dorsal side, "L" the left, and "R" the right of the insect.





Results & Discussion

Figure 1 shows representative MRM images obtained from an *M. sexta* pupa, and reveals the air sacs fully inflated with hemolymph flowing through the dorsal vessel and ventral diaphragm. Figure 1A is a PDW image showing an axial slice through the upper abdomen of an *M. sexta* pupa. The dorsal vessel (red arrow) and the gut (light-gray area above the red arrow) are conspicuous. The dark areas on the right and left-hand sides are the inflated air sacs. Figure 1B & 1C are individual frames from a FISP movie that were acquired 632ms apart. In both images, the ventrally-pointing red arrow aims at hemolymph flow, most likely through the ventral diaphragm, while the dorsally-pointing arrow aims at hemolymph flow through the dorsal vessel. These images show that the dorsal vessel regularly brings fresh hemolymph into the image resulting in a bright spot, while the ventral diaphragm exhibits less flow, but is still active. These images represent the most common images acquired from these pupae; the air sacs remain static as hemolymph flows through the dorsal vessel and ventral diaphragm. However, this is not always the case. Figure 2 presents axial images from the upper abdomen of an *M. sexta* pupa. The red arrows point towards the air sacs. Unlike the air sacs shown in Figure 1A, these are not entirely black. Figures 2B-D show a sequence of frames from a FISP movie, each 632ms apart. In Figure 2B, the air sacs are filled with air, then a bright signal appears in the air sac region indicative of hemolymph flow (Fig. 2C), followed by the air sacs returning to their original state (Fig. 2D). The hemolymph clearly flows into the air sac region during deflation, and exits when the air sacs reinflate. The above results, as well as some sagittal and coronal movies (not shown), are consistent with ceolopulses (5). To our knowledge, this is the first direct observation of insect respiration using magnetic resonance techniques and these results demonstrate that MRM is a promising tool for *in vivo* studies of insect circulation and respiration.

Acknowledgements

The author thanks John Vural for useful technical discussions, Karel Sláma for his help with *M. sexta* physiology, as well as James Hamilton for his generous donation of experimental time.

References

1. Hart AG, Bowtell RW, Kockenberger W, Wenseleers T, Ratnieks FLW. Magnetic resonance imaging in entomology: a critical review. Journal of Insect Science 2003;3:5:9 pages.
2. Jasanoff A, Sun PZ. *In vivo* magnetic resonance microscopy of brain structure in unanesthetized flies. Journal of Magnetic Resonance 2002;158(1-2):79-85.
3. Wecker S, Hornschemeyer T, Hoehn M. Investigation of insect morphology by MRI: Assessment of spatial and temporal resolution. Magn Reson Imaging 2002;20(1):105-111.
4. Chudek JA, Crook AME, Hubbard SF, Hunter G. Nuclear magnetic resonance microscopy of the development of the parasitoid wasp *Venturia canescens* within its host moth *Plodia interpunctella*. Magn Reson Imaging 1996;14(6):679-686.
5. Sláma K. A New Look At Insect Respiration. Biological Bulletin 1988;175(2):289-300.