Research Article

Insights of Clinical and Medical Images

Why Does *Papilio Bianor* Pupae Proton Magnetic Resonance Imaging Show Similar Results In T1-weight Image And T2 -weight Image?

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Author Contributions Statement

S. I., K. N., Y. O., and K. K. operated instruments. T. A. I. prepared the butterfly pupae, conducted the study, and prepared the manuscript. M. Y., K. N., K. H., Y. O., and K. K. supervised this study. T. F. and S. I obtained the fund used for this study. All the authors reviewed the manuscript.

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1. Abstract

To establish a noninvasive technique for visualizing *Papilio bianor* pupae, we devised parameters for Proton Magnetic Resonance Imaging at 9.4 T. We attempted five imaging modes: T1-weighted without fat suppression, T1-weighted with fat suppression, T2-weighted without fat suppression, T2-weighted with fat suppression, and T2-weighted with water-signal suppression. Among these, only T2-weighted imaging with water signal suppression mode was useful for detecting fat bodies located in the brain, abdominal fluid, and wing margins of the pupae. We believe that this method is useful for detecting fat bodies in butterfly pupae. In contrast, no significant differences were observed between these imaging methods, except for T2-weighted images with water signal suppression. We believe that further improvements are required to accommodate insect observations.

2. Keywords

Papilio bianor, pupae, fat body, Proton Magnetic Resonance Imaging (PNRI), adult differentiation

3. Introduction

Most insects undergo metamorphosis. In particular, as the difference between larvae and adults is quite remarkable in butterflies, many researchers and individuals have been interested in methods to differentiate their pupae to clarify the morphological and physiological changes made secretly under the cover of the epidermis. Adult differentiation in pupae of *Drosophila melanogaster* (Diptera: Brachycera) was studied anatomically[1]. In this way, however, a large number of pupal samples have to be prepared, and each examination results in the termination of the life of thesample; thus, a sequential observation of the same individual is not possible. Moreover, from the viewpoint of protecting the rights of laboratory animals, such observational methods are somewhat controversial. After 2000, observations have been conducted using synchrotron X-ray photography [2]. Lowe et al. [3] described the adult differentiation of *Vanessa cardui* into pupae. However, because such ionizing radiation causes serious damage to the pupae, four out of nine pupae died during the process of photography. In addition to these

radiation methods, observations using Proton Magnetic Resonance Imaging (PMRI) have also begun. Unlike X-rays, PMRI is free from ionizing radiation and is harmless to pupae. Rowland et al [4] described adult differentiation during late larval and late pupal stages in *Manduca sexta*. The application of PMRI to insect observation has not spread quickly because the observation volume of the probes used in scanners designed for clinical use is too large to obtain high-quality images of a small subject such as an insect. Even when combined with an additional probe in small animals, gradient systems equipped with clinical scanners are insufficient for this purpose. In this situation, some research groups have installed self-built imaging probes for smaller subject on an NMR spectrometer (e.g. http://www.bk.tsukuba. ac.jp/~mrlab/). Alternatively, some vendors have manufactured products for the dual use of spectroscopy and microscopic imaging. One such instrument was installed at Tokai University. During our observation of adult differentiation in pupae using this microimaging system, we found some characteristic spatial distribution of fat signals inside the pupae of *P. bianor*. Therefore, in this study, we attempted to visualize suitable imaging conditions. In addition, we attempted to localize the water and fatbodies using this system. In Figure 1, we showed one sagittal slice plane position and four transverse slice plane positions those we observed.

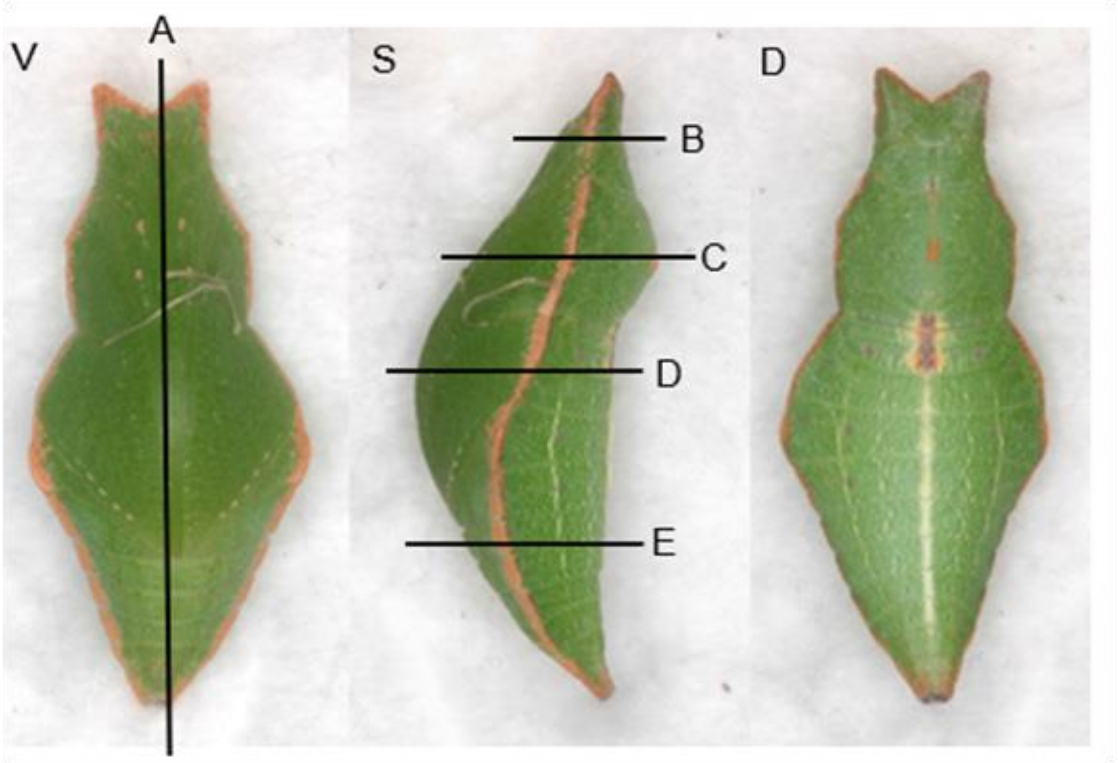

Figure 1: Ventral (V), Side (S), and Dorsal (D) views of *P. bianor* diapause pupae. Five lines (A-E) denote cross slices of the proton magneticresonance imaging shown in Figure 2.

4. Material and Methods:

Preparation of butterfly pupae

The pupae used in this study was collected from Ôiso, Kanagawa, Japan, on November 1, 2019, in the form of final instar larvae. This individual became a pupa in diapause on November 4. We started imaging according to the protocol described below from November 06, and repeated the same protocol every 3 or 4 days until December 7, 2019. Among these data, those taken on December 7, 2019, were used for this morphological study. On May 27, 2020, a female butterfly emerged from the pupae.

4.1. Instruments and condition of imaging

Instruments and protocols were as previously described by Ikegami et al [5]. A 9.4 T micro-imaging system operating at 400 MHz for proton resonance (Ascend 400WB with Topspin Ver. 2.0 and Paravision Ver. 5.1, Bruker Biospin, Billerica, Massachusetts, US). The pupal samples were fixed on a homemade cradle and mounted on a radiofrequency coil with an effective diameter of 25 mm (M81112-07, Bruker BioSpin) combined with a microimaging probe unit (T119618, Bruker BioSpin). After the gradient coil system (1P T23369; Bruker BioSpin) was inserted into the main magnet, a probe unit was inserted into the gradient system. The entire system was controlled using a console operating on Linux. Since this device can cool the gradient magnetic field coil, the electrical characteristics can be stabilized over a long period of time, so stable images can be obtained even during long-term imaging. The image data were saved in DICOM format. Slice images, 3D volume-rendered views, and MIP views were reconstructed using the DICOM Viewer software, OsiriX DM, Horos 4.0, Onis 2.5, and 3D-Slicer 4.11.0.

Insights of Clinical and Medical Images

5. Results

Figure 2 depicts T1-weighted images without fat suppression (row 1), T1-weighted images with fat suppression (row 2), T2-weighted images without fat suppression (row 3), T2-weighted images with fat suppression (row 4), and T2-weighted images with water suppression (row 5).

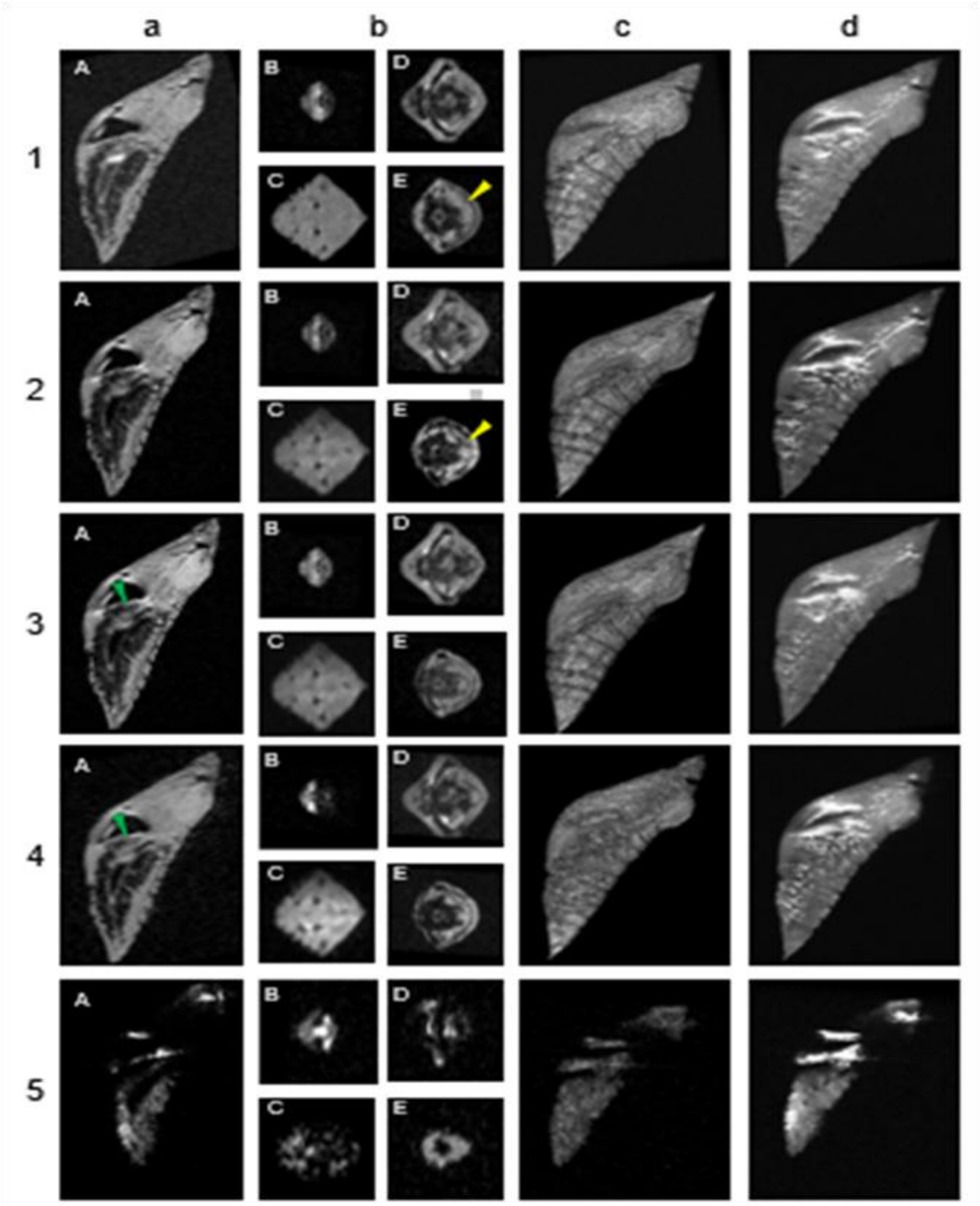

Figure 2: Proton magnetic resonance imaging from in *P. bianor* pupae. Column a shows sagittal images at line A delineated in Figure 1. Column b shows axial images at lines B (upper left), line C (lower left), line D (upper right), and line E (lower, right) delineated in Figure 1. Column c shows three-dimensional (3D) volume rendered views. Column d shows maximum intensity projection (MIP) images. Images in row 1 are T1-weighted images without fat suppression, while those in row 2 are the T1-weighted images with fat suppression. Those in row 3 are T2-weighted images without fat suppression, while those in row 4 are the T2-weighted images with fat suppression. Images in row 5 are the T2-weighted with water suppression.

In T1-weighted images without fat suppression (row 1) and with fat suppression (row 2), the signal intensities in the tissues between the hindwing and ventral abdominal surface and inside the abdomen were slightly higher in the sagittal image without fat suppression than in those with suppression (as indicated by the yellow arrows). In the axial images on line E, some structures were observed between the dorsal surface and the alimentary tract only in the image with fat suppression (yellow column b, lower right arrows). These differences are also visible in the maximum intensity projection (MIP) views shown in column d. On T2-weighted images without fat suppression (row 3) and with fat suppression (row 4), the signals between the hindwing and ventral abdominal surface and inside the abdomen were higher in the fat-suppressed group than in the non-suppressed group (green arrows). This contrast appeared to be inverted in the case of T1-weighted because of the difference in contrast mechanisms between T1 and T2. The axial images on line B, showing tissue likely to be the brain, were remarkably different (red arrows). Almost half of the tissue region was suppressed in the fat-suppressed images. This is also evident in the MIP images in column (d). A similar trend was observed in the T1-weighted images, although the difference between the fat-suppressed and non-suppressed images was somewhat ambiguous. Water-suppressed T2-weighted images (row 5) exhibit spatial distribution of non-water components, such as fat. The images in this row show negative/positive reverted images compared with those in row 4. We can observe the lipid contained probably in the nervous system in the brain, as well as the fat contained around the alimentary tract and in the wing margin. The MIP view in this row shows the entire distribution of fat.

6. Discussion

According to the imaging conditions used in this study in T1-weighted, generally, fat, melanin, or protein-rich fluid should be imaged as a high-signal area because the T1 values of protons are relatively short in these tissues. Diamagnetic compounds, such as Mn and Cu, shorten the water protons short in T1-weighted. In contrast, in T2-weighted images, low-viscosity water signals were high. From this point of view, in our images of P. bianor pupae, the similarity of T1-weighted with fat suppression images and T2-weighted without fat suppression images were reasonable, because melanin and its precursor is rich in the exoskeleton of insects, and protein is rich in hemolymph. The water-suppressed T2-weighted images revealed tissues in the brain around the alimentary tract in the abdomen and the wing margin with high signals. Both brain and eye tissue are rich in fat bodies. The outer region of the alimentary tract is filled with hemolymph, which is rich in fat bodies and proteins [6]. There are many mechanical sensory hairs on the margins of the wings [7~10]. The water-suppressed T2-weighted images corresponded well with these facts; thus, the imaging technique was useful for detecting fat bodies in pupae. The question is whether T1-weighted without suppression and T2-weighted images without suppression, which have a negative-positive relationship, at least when the human body is imaged (e.g., https://en.wikipedia.org/wiki/Magnetic_resonance_imaging), were almost the same in the *P. bianor* pupae images. Other images did not show any significant differences, except for T2-weighted with water suppression. This may be due to the fact that the images were taken at a less appropriate time during the adult differentiation stage, but in any case, these imaging methods likely still need to be improved according to the actual conditions of insects. In this study, we used protocols established for vertebrates and believe that this may have been the cause of this failure.

Table 1: Parameters of each Proton Magnetic Resonance Imaging.

	T1-weighted	**T2-weighted**
TE (ms)	8.5	33
TR (ms)	2185.5	5232.6
Slice thick (mm)	0.25	0.25
Slice gap (mm)	0	0
Number of slice	48	48
FOV (mm^2)	27 x 27	27 x 27
matrix	256 x 256	256 x 256
average	4	4
Bandwidth (MHz)	800	800
Flip Angle	90	90

7. Conclusions

The present work demonstrated the usefulness of PMRI for depicting the morphological information of the pupa with 100 μm in-plane resolution. Although the extent of tissue differentiation in the present sample was not clear, and thus the true diagnostic powers of the T1- and T2-weighted imaging were not fully evaluated, the water-suppressed T2-weighted imaging clearly exhibited spatial distribution of low-water-content tissues such as fat in the pupa of *P. bianor*. Thus, the present work established a non-invasive methodology to visualize the decomposition, differentiation, and composition of the tissues in the pupae of Lepidoptera. We are currently working on further methodological development of magnetic resonance imaging and spectroscopy suitable for pupal visualization.

8. Funding

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9. Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

10. Acknowledgments

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