Avicenna Veterinary Research
Vol. 1, No. 1, Winter 2025
Bu-Ali Sina University

ORIGINAL RESEARCH PAPER

Histological Characterization of the Brain and Eye in Drosophila Melanogaster: A Model for Neurodegenerative Research

M. Hallaj Salahipour[a,*], **B. Ghorbanzadeh**[b]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
[b] Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article info

Article history:
Received 2025-05-10
Received in revised form 2025-05-28
Accepted 2025-06-01

Keywords:
Drosophila melanogaster
Eye
Brain histology
Retinal morphology
Hematoxylin-eosin staining

Abstract

Background: Drosophila melanogaster, a key model in genetic and neurological research, offers advantages due to its short life cycle (∼10 days), high fertility (400–500 offspring per cycle), and ethical feasibility for experiments. It has been widely used in studies of Alzheimer's, diabetes, and Parkinson's disease, yet limited histological analysis of its head structures has been conducted in Iran. This study aims to characterize its brain and ocular histology to enhance its application in neurodegenerative disease modeling.
Material & Methods: Drosophila melanogaster specimens were anesthetized at temperatures below 4°C, then sex-separated under a dissecting microscope. Heads were dissected from the thorax, ensuring optimal penetration of Carnoy's fixative. Samples underwent fixation, dehydration, paraffin embedding, and serial sectioning. Histological staining was performed, and imaging was conducted using Dino-Lite digital microscopy, processed via Dino Capture software (Version 2).
Results: Histological analysis showed that the eye's external surface consists of mosaic-shaped photoreceptor cells, beneath which lies the retinal layer. Below the retina, the lamina was identified. The brain contains two hemispheres, each with lamina, medulla, lobula plate, and lobula. Lobular architecture revealed dense neuronal nuclei surrounding each lobe, with interspersed fibers and centrally distributed neuroglial matrix. Neuronal fiber degeneration led to vacuolated spaces, mainly within lobular cores, indicating neurodegenerative changes.
Conclusion: Considering the distinct histological characteristics of Drosophila melanogaster especially the prominent staining properties of its head structures it appears to be a highly suitable laboratory model for studying neurodegenerative diseases and evaluating the impact of various factors on disease induction and recovery.

1. Introduction

Drosophila melanogaster, a species within the Drosophila genus and Drosophilidae family, is commonly known as the fruit fly or vinegar fly (Sang, 2001). Wild-type Drosophila exhibits yellow-brown coloration, brick-red eyes, and black transverse bands on the abdomen. This species demonstrates sexual di-

*Corresponding author: M. Hallaj Salahipour
E-mail address: Mahsa.Hallaj14@gmail.com
http://dx.doi.org/10.22084/avr.2025.31018.1005

morphism, where females reach approximately 2.5 mm in length, while males are slightly smaller with a darker dorsal surface. Males can be distinguished from females by coloration differences, as well as the presence of a distinct black patch on the abdominal surface and sex combs_a row of dark bristles on the forelegs (Steven *et al.*, 2016).

Drosophila melanogaster has a diploid chromosome number (2n = 8), making genetic investigations more straightforward. The species exhibits XY sex determination, with males being the heterogametic sex. This organism is widely used in laboratory research due to its short life cycle (∼10 days), high reproductive rate (400–500 offspring per cycle), and ethical feasibility for experimentation. Moreover, its double-stranded DNA structure enables robust molecular analyses (Adams *et al.*, 2000).

The pioneering geneticist Thomas Hunt Morgan introduced Drosophila melanogaster as an essential model for genetic research in 1933 (Sang, 2001). Since then, Drosophila has been extensively used to study Alzheimer's disease, diabetes, and Parkinson's disease, revealing mechanistic insights into neurodegeneration (Huang *et al.*, 2019). Histological sections of Drosophila brains have also facilitated the modeling of Parkinson's disease (Drobysheva *et al.*, 2008). Additionally, neurodegenerative effects such as neuronal deterioration in Alzheimer's disease have been studied using Drosophila (Sunderhaus and Kretzschmar, 2016).

Despite the wide application of Drosophila in neurological research, histological investigations of its head structures remain limited, particularly in Iran. This study aims to characterize the histology of the brain and eye in Drosophila, establishing its potential as a laboratory model for neurodegenerative disease research.

2. Materials and Methods

2.1. Sample Preparation

Prior to specimen collection, Drosophila melanogaster flies were anesthetized by exposure to temperatures below 4°C. Male and female flies were carefully separated under a dissecting microscope before regaining consciousness. The heads were dissected from the thorax, ensuring optimal penetration of the fixative solution through the connective passage.

2.2. Fixation & Tissue Processing

Samples were fixed using Carnoy's solution, prepared as follows:

60 mL absolute ethanol
30 mL chloroform
10 mL glacial acetic acid

Specimens were immersed in Carnoy's fixative for 4 hours to ensure proper tissue preservation. Subsequent dehydration was performed using two ethanol baths (100%), each for 30 minutes. Following dehydration, specimens were air-dried at room temperature, then immersed in methyl benzoate for 1 hour before being embedded in molten paraffin and processed into paraffin blocks.

3. Sectioning & Staining

Histological sections were prepared using a rotary microtome (DS4055), generating serial sections of 7 m thickness. Staining was performed following standard histotechnical procedures (Morovvati and Kalantari-Hesari, 2019).

3.1. Imaging

Images of stained sections were captured using a Dino-Lite digital microscope and processed using Dino Capture software (Version 2).

4. Results

Histological examination of Drosophila melanogaster head sections revealed distinct structural correlations between ocular and cerebral components.

4.1. Eye Morphology

The external surface of the eye comprises mosaic-shaped photoreceptor cells, each functioning as an independent visual unit. Beneath these mosaic cells, the retinal layer was identified, with bundles of nerve fibers connecting to individual photoreceptor units (Fig. 1(B)).

4.2. Brain Organization

Directly beneath the retina, the lamina layer was observed. Generally, the Drosophila brain consists of two hemispheres, each subdivided into:

- Lamina
- Medulla
- Lobula plate
- Lobula (Fig. 1(A), 1(C)).

5. Neurohistological Features

The lobular structures exhibited a dense aggregation of neuronal nuclei, primarily surrounding each lobule, while bundles of neural fibers and glial matrix were concentrated centrally. Degeneration of neuronal fibers

resulted in the appearance of vacuolated spaces, particularly within central lobular regions (Fig. 1(C), 1(D)).

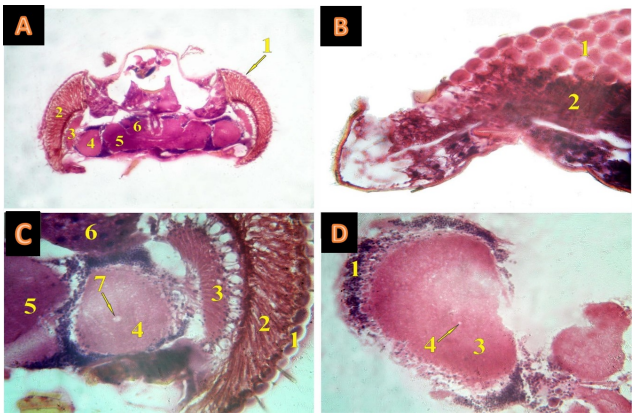

Fig. 1. Histological Sections of the Eye and Brain in Drosophila melanogaster. Hematoxylin & Eosin (H& E) staining, magnification 100X and 1000X. A) Mosaic-shaped photoreceptor cells (1), retina (2), lamina (3), medulla (4), lobula plate (5), lobula (6). B) Mosaic-shaped photoreceptor cells (1), retina (2). C) Mosaic-shaped photoreceptor cells (1), retina (2), lamina (3), medulla (4), lobula plate (5), lobula (6), vacuolated spaces due to neuronal degeneration (7). D) Aggregation of neuronal nuclei (1), neural fibers and neuroglial matrix (2), vacuolated spaces due to degenerative changes (3)

6. Discussion

The brain structure of Drosophila melanogaster consists of distinct layers, including mosaic-patterned photoreceptor cells, retina, lamina, medulla, lobula plate, and lobula. The histological examination conducted in this study using light microscopy confirms findings previously reported through electron microscopy, demonstrating the consistency of Drosophila's neuroanatomical organization (Huang *et al.*, 2019; Drobysheva *et al.*, 2008; Sunderhaus and Kretzschmar, 2016).

One of the most striking observations in this study was the clear structural separation between the retinal layers and underlying neuronal compartments . The mosaic-shaped photoreceptor cells function as individual visual processing units , each connecting to nerve fiber bundles. Degenerative changes within the lobular regions resulted in vacuolated spaces , a characteristic commonly associated with neurodegenerative diseases (Sunderhaus and Kretzschmar, 2016).

Given Drosophila melanogaster's short life cycle (∼10 days), high reproductive rate (400–500 offspring per cycle), and genetic accessibility, it is widely used as a model organism for studying neurodegeneration, aging, and genetic disorders. Previous studies have successfully applied Drosophila to model Alzheimer's disease, diabetes, and Parkinson's disease, utilizing its high tissue staining affinity and rapid phenotypic manifestation (Huang *et al.*, 2019; Drobysheva *et al.*, 2008). The present findings further validate its suitability for histopathological studies on disease progression, neuronal degeneration, and therapeutic interventions.

7. Future Directions

To expand upon these findings, future research should focus on:

1. Advanced neuronal mapping techniques, such as immunohistochemistry and confocal microscopy, to assess apoptotic and oxidative stress markers.
2. Comparative studies with mammalian models, investigating parallels between Drosophila's retinal degeneration and human neuropathologies.
3. Potential neuroprotective interventions, evaluating how drug candidates influence Drosophila's brain structure and neuronal integrity.

8. Conclusion

In conclusion, Drosophila melanogaster's distinct histological characteristics, genetic flexibility, and staining affinity establish it as an ideal experimental model for investigating neurological disorders. These findings strengthen its relevance in neurodegenerative disease modeling, facilitating mechanistic studies and therapeutic advancements.

References

[1] Morovvati, H. Kalantari-Hesari, A. Comprehensive Histotechnology and Laboratory Tissue Processing Management. 1st ed. Tehran University Press. p. 100-175. 2019.

[2] Sang JH. Drosophila melanogaster: the fruit fly. InEncyclopedia of genetics 2014 Jan 14 (pp. 157-162). Routledge.

[3] Marygold SJ, Crosby MA, Goodman JL, FlyBase Consortium. Using FlyBase, a database of Drosophila genes and genomes. Drosophila: Methods and Protocols. 2016: 1-31.

[4] Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA. The genome sequence of Drosophila melanogaster. Science. 2000 Mar 24; 287(5461): 2185-95.

[5] Huang Y, Wan Z, Wang Z, Zhou B. Insulin signaling in Drosophila melanogaster mediates Aβ toxicity. Communications biology. 2019 Jan 8; 2(1): 13.

[6] Drobysheva D, Ameel K, Welch B, Ellison E, Chaichana K, Hoang B, Sharma S, Neckameyer W, Srinakevitch I, Murphy KJ, Schmid A. An optimized method for histological detection of dopaminergic neurons in Drosophila melanogaster. Journal of Histochemistry & Cytochemistry. 2008 Dec; 56(12): 1049-63.

[7] Sunderhaus ER, Kretzschmar D. Mass histology to quantify neurodegeneration in Drosophila. Journal of visualized experiments: JoVE. 2016 Dec 15(118): 54809.