

Establishing Primary Neuronal Cultures from *Drosophila melanogaster* Pupae: A Proposal for Neurobiological Assay Development

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Abstract

The fruit fly, *Drosophila melanogaster*, is a long-standing model organism in biomedical research due to its genetic tractability, short life cycle, and evolutionary conservation of key biological pathways. The purpose of this study was to develop primary neuronal cultures from *Drosophila* pupae aged 55–78 hours to explore their potential as a neurobiological assay for synaptic development. Brains were dissected from late-stage pupae and processed following a modified version of the protocol described by Sicaeros et al. (2007), using papain-based enzymatic dissociation and DDM2 culture medium. While the cultures failed to produce viable neuronal growth—primarily due to contamination and low cell yield—the experiment highlights methodological challenges such as maintaining sterility, ensuring sufficient sample size, and optimizing centrifugation and plating steps. These findings underscore the importance of procedural precision in establishing viable neuronal cultures and lay groundwork for improved techniques in future neurodegenerative disease modeling using *Drosophila*.

Introduction

The fruit fly, *Drosophila melanogaster*, has long served as a powerful model organism for the biomedical sciences. Its advantages include low cost, ease of maintenance, a small genome, and well-characterized genetics (Tolwinski, 2017). *Drosophila*'s wide range of applications makes it particularly valuable in neurobiological research. For brain disease modeling, most *Drosophila* models successfully replicate aspects of human disorders and are suitable for high-throughput pharmacological assays (Jeibmann & Paulus, 2009).

With the advent of the GAL4/UAS system, *Drosophila* has become an ideal candidate for genetic screening tools. The present study aims to develop neuronal cultures from *Drosophila* pupae aged 55–78 hours. At this stage, pupae are developmentally ready for optic lobe dissection and exposure of the central nervous system. By generating pupal brain cultures, we sought to identify factors influencing neural synapse formation.

Cell culture methods in neurobiology are advantageous due to their capacity for controlled manipulation and homogeneity, allowing researchers to investigate the cellular microenvironment. However, they also pose challenges, such as chemical or microbial contamination and deviations from natural physiological conditions.

This study investigates the feasibility of creating a neurobiological cell culture assay using *Drosophila* pupae. The culture system was intended to enable exploration of neuronal connectivity and synaptic structure, providing a foundation for future research in neurodegenerative disease modeling. Despite challenges in culture viability, our findings inform methodological improvements for future attempts.

Materials and Methods

Our protocol was adapted from Sicaeros, Campusano, and O'Dowd (2007) (*Journal of Visualized Experiments*, doi:10.3791/200).

Specimen Selection and Preparation.

We selected 10–15 golden-brown *Drosophila* pupae (55–78 hours post-pupariation) and placed them on a petri dish. Using forceps to hold each pupa, we removed the cuticle with a 27-gauge syringe needle and severed the neck to extract the head. Heads were transferred into a drop of dissecting solution on the petri dish.

Dissection and Enzymatic Digestion.

While the protocol recommends dissection under sterile laminar flow, our first trial was conducted in non-sterile conditions. Brains were extracted by creating a vertical incision through the forehead and pushing the brain out using a needle. Brains were transferred with a 20 μ L pipette into an enzymatic papain solution (0.8 mg 100X cysteine solution, 35.7 μ L papain, 150 μ L DS cysteine, and 5 mL DS buffer).

After removing the optic lobes, brains were placed in 1 mL of sterile papain solution and incubated on a rotator at 60 rpm for 10 minutes. Samples were then centrifuged at 1600 rpm for 3 minutes. The resulting material was placed in a CO₂ incubator for four days. Fungal contamination was observed, and the procedure was repeated under sterile conditions.

Second Trial Adjustments.

In the second run, six brains were dissected and processed as above but washed less extensively. One set of three brains was incubated for 10 minutes, and another set for 5 minutes, followed by two washes with DDM2 media.

Culture Media Composition.

DDM2 solution contained:

- 0.134 g DMEM (50-003-PB)
- 10 mL DI water

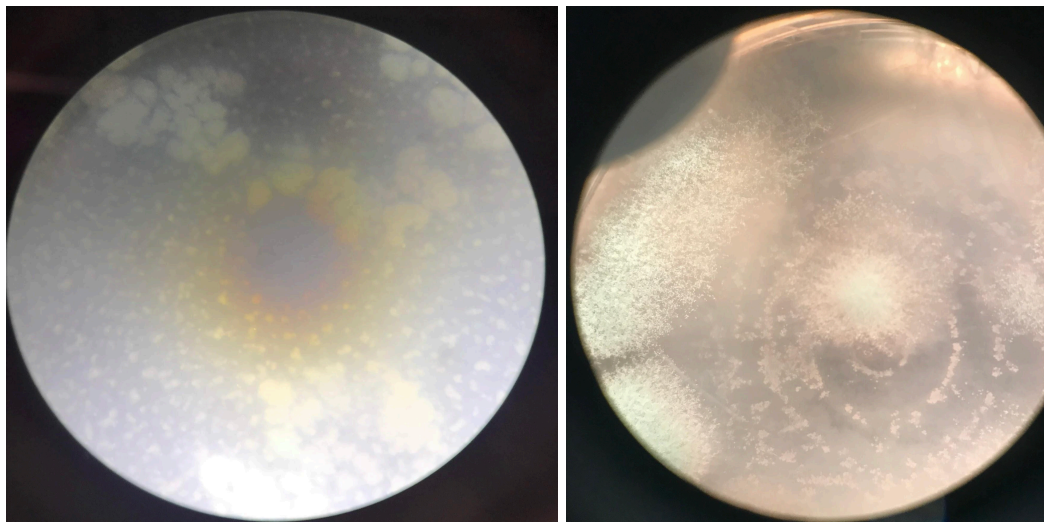
- 1 mL insulin-transferrin-selenium
- 10 μ L hydrocortisone (1 mg/mL)
- 1 mL progesterone (10 ng/mL; 2000 mg/mL stock)
- 15 μ L putrescine (666 $\times$ stock)

Cultures were incubated in CO₂ for eight days before observation.

Results

In the initial trial, fungal contamination appeared as green, fibrous growths on the plates. The culture medium exhibited color changes and opaque regions consistent with microbial contamination. Consequently, the cultures were discarded.

In the second attempt, fungal contamination persisted. The first plate showed a central green mound with brown discoloration and scattered small circular bodies beneath the surface. The second plate contained multiple green clumps and a lighter red medium. Though small, white cellular aggregates were visible, they likely represented nonviable debris rather than neurons. Neither trial yielded evidence of viable neuronal growth.



The above images were photographed on a phone through the lens of a dissecting microscope each at 4X magnification. The image to the left is the first plate from our second round of conducting the protocol, and the image on the right is the second plate from our second round of conducting the protocol.

Discussion

The failure to culture viable *Drosophila* neurons likely stemmed from a combination of technical and procedural factors, including insufficient sample size, ineffective centrifugation and plating, and inadequate sterile technique.

First, too few brains were collected for plating. The reference protocol calls for 10–15 brains per sample, but only 7–9 were used per trial. Reduced input likely limited neuronal yield. In addition, separation of optic lobes from central mushroom bodies may have led to further neuronal loss.

Second, centrifugation appeared insufficient for pelleting viable cells, as no clear pellet was observed. Without a concentrated cell mass, proper washing and plating were compromised. Optimizing centrifugation parameters could significantly improve cell viability and plating success.

Finally, sterility played a crucial role. Conducting dissections outside of a laminar flow hood and failing to sterilize between steps introduced fungal contamination. All subsequent dissection and culture steps must occur under sterile conditions to ensure reproducibility.

Future iterations of this experiment should prioritize (1) full sample collection (10–15 brains), (2) visible pellet formation post-centrifugation, and (3) strict maintenance of sterility during all procedures. These adjustments will likely improve the probability of establishing viable *Drosophila* neuronal cultures suitable for neurobiological research.

Conclusion

This study explored the feasibility of generating primary neuronal cultures from *Drosophila melanogaster* pupae as a foundation for neurobiological assay development. Although viable neuronal cultures were not successfully produced, the experiment illuminated several procedural limitations that hindered successful cell growth, including insufficient sample size, ineffective cell pelleting, and inadequate sterile technique. These findings emphasize the need for methodological refinement and consistency when adapting *in vivo* brain tissue for *in vitro* culture.

Future research should focus on optimizing dissection under controlled laminar flow conditions, standardizing enzymatic digestion timing, and confirming neuronal viability through staining or electrophysiological assays. With these improvements, pupal-derived neuronal cultures could become a reliable platform for studying synaptogenesis, neural circuitry, and the cellular mechanisms underlying neurodegenerative diseases.

References

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