Spring 4-2-2017

A Drosophila Model to Examine Collective Migration during Retinogenesis

Caroline Pena
CUNY City College

Stephanie Zhang
CUNY City College

Mildred Kamara
CUNY City College

Tadmiri Venkatesh
CUNY City College

Maribel Vazquez
CUNY City College

Recommended Citation

Beck C, Singh T, Farooqi A, Venkatesh T, Vazquez M., 'Controlled microfluidics to examine growth-factor induced migration of neural progenitors in the Drosophila visual system,' J Neurosci Methods. 2015 Dec 29;262:32-40.

A Drosophila Model to Examine Collective Migration during Retinogenesis

The City College of New York

Caroline Pena*, Stephanie Zhang*, Mildred Kamara+, Dr. Tadmiri Venkatesh+, Dr. Maribel Vazquez*

160 Convent Avenue, Grove School of Engineering*, ST-508 and Department of Biology+, MR 526

New York, NY 10031

Abstract

Retinal dysfunction is often caused by aberrant neural cell migration during development. In this study, we observed the migration of neural cells of the *Drosophila melanogaster* after marking cells of the 3rd instar larvae with the GAL4-UAS expression system when exposed to a concentration gradient of FGF-8 through the use of a microfluidic device. The glial and neuronal cell ratio in the developing brain was determined through immunofluorescent staining and observation. In future studies, a microfluidic device that mimics the developing *Drosophila* brain and retina will be designed in order to better understand the biological factors that affect the migration and differentiation of the cells.

Introduction

- In *Drosophila melanogaster,* the development of the retina occurs when populations of neural progenitor cells differentiate into photoreceptors and project their developing axons through the optic stalk into the brain lobe. [1]
- Neuronal and glial cell precursors migrate collectively, a mechanism that remains incompletely understood. [2]
- Fibroblast growth factor (FGF) has been shown to be a major component in cell fate specification and migration in the developing retina of vertebrates. [3]
- Previous work from our group has shown that *Drosophila* glial and neuronal cell clusters in a microfluidic system exhibited increased motility with an increase in FGF concentration. [4]
- Investigation of the cooperative mechanisms and associated signaling molecules behind glial and neuronal movement would allow for better understanding of the underlying factors behind neuronal migration disorders.

Materials and Methods

***Drosophila* Stock, Dissection/Dissociation**

- Drosophila stocks were kept on standard cornmeal agar at 25°C
- Third instar stage larvae were collected and washed (Figure 1)
- Fifteen larvae were dissected and the brain complexes were obtained.
- Brain complexes were dissociated into glial and developing neuronal cells in and resuspended in supplemented Schneider's medium.

UAS-GFP x Repo-GAL4 Genetic Crosses

- Repo-GAL4 males were mated with virgin UAS-GFP females for 5 days to produce GFP+ glial cells.
- Third instar larvae that were produced were collected 8-11 days later. (Figure 1)
- Dissection and dissociation protocols were followed to obtain neuronal cells and GFP+ glial cells

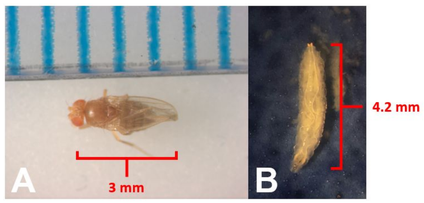

Figure 1. (A) Adult Drosophila fly under light microscope at 16X magnification. (B) Third instar larvae under light microscope at 16X magnification

Microfluidic Device

- A microfluidic device was created through photolithography and utilizing Polydimethylsiloxane (PDMS) bonded to a coverslip for evaluation of external chemotactic and cluster movement.
- Dissociated neuronal and GFP+ glial cells were inserted into the microfluidic device through injection

Materials and Methods

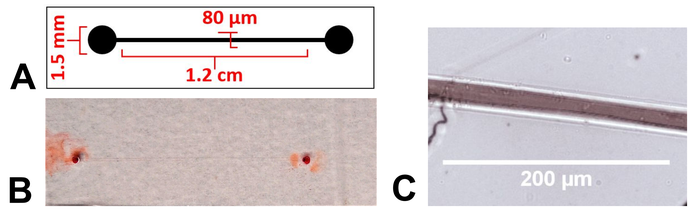

Figure 2. (A) Schematic of microfluidic device (Channel with a length of 1.2 cm, width of 80 µm and an injection port with a diameter of 1.5 mm). (B) Physical microfluidic device with red dye in order to ensure complete flow through channel. (C) Microfluidic device under brightfield microscope at 10x magnification.

Immunofluorescent Staining of Glial and Neuronal Cells

- Glial and neuronal cells obtained from uncrossed UAS-GFP or Repo-GAL4 strains were cultured for at least 12 hr in a petri dish.
- Cell suspension were pipetted on microscope slides and were coated in Concanvalin-A in order to allow for adhesion of cells.
- Antibodies utilized: Rat-Elav-7E8A10 anti-elav (Neuronal cells) and 8D12 anti-Repo (Glial cells) obtained from Developmental Studies Hybridoma Bank

Results

GFP+ Glial Cells in Obtained Brains Complexes and Dissociated Cell Culture

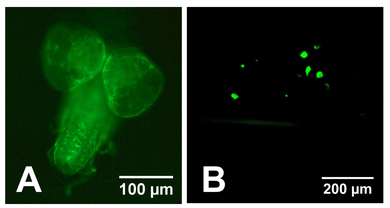

Figure 3. *GFP+ Glial Cells*. (A) The brain complexes indicate highlighted green areas, which prove that the glial cells are GFP+. These were imaged under 20x magnification. (B) The brain complexes were then dissociated through collagenase and centrifuging down. These were imaged under 40x magnification.

Cell Migration Observed in Microfluidic Device

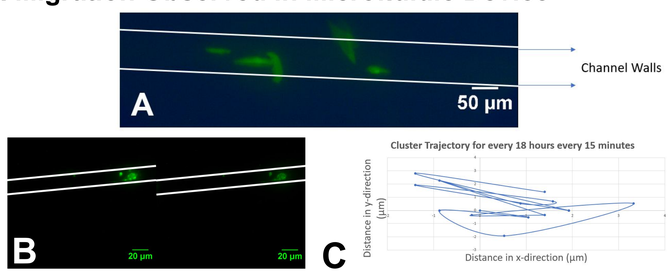

Figure 4. *GFP+ Glial Cells Adhered within Microdevice*. After the neural cells were injected into the microdevice with the PLL (poly-L-lysine), selections of the channel were imaged under 40x magnification with a fluorescent microscope. (A) The adhered cells within the microdevice prior to the addition to FGF-8 (100ng/mL). (B) These images denote the migratory pattern of the GFP+ glial cells moving in the channel. Images were taken consecutively every 15 minutes for 18 hours. (C) Plot of cluster trajectories normalized to the origin. Each data point was collected every 15 minutes for 18 hours. The total distance traveled was 25.287 µm.

Results

Glial to Neuron Ratio is about 1:8 in Third Instar Developing Brains

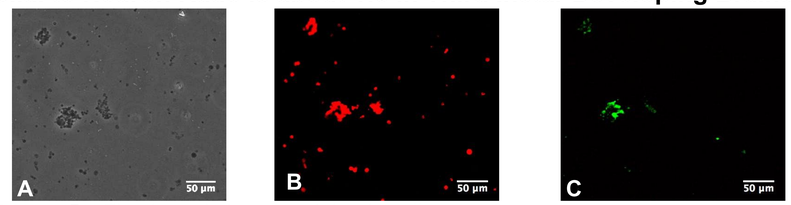

Figure 5. Fixed and immunostained cells under bright field and fluorescence and resulting glial to neuronal cell ratio. (A) Section of glial and neuronal cells under bright field. (B)Section of fluorescently labeled neuronal cells. (C) Section of fluorescently labeled glial cells.(D) Comparison of total cells (10^5), glial cells ($8.8*10^4$) and neuronal cells ($1.2*10^4$) obtained from 15 brains. Taken under 10x, scale bar 50 µm and ImageJ used as cell counter

Discussion

GFP+ Glial Cells

- The success of the genetic crosses UAS-GFP virgin female flies and Repo glial male flies were demonstrated by the dissections of the brain complexes and imaging through a fluorescent microscope under 40x magnification

Migratory Pattern of GFP+ Glial Cells in Microdevice

- Data has shown clusters of neural cells migrating towards FGF concentration.
- Further investigation will determine the influence of increasing FGF concentration on size and collective movement of neural progenitor clusters.

Glial to Neuron Cell Ratio

- In the adult Drosophila CNS, glia are outnumbered by neurons in a 10:1 ratio, while in mammals glia outnumber neurons 10:1. [6]
- In the *Drosophila* embryo, about 60 glial cells and 700 neurons are found per abdominal neuromere. [6]
- Immunostaining has shown that in our sample of third instar larvae brains the glial cell to neuronal cell were in a ratio of about 1:8.

Future Direction

Future work will be to design a microfluidic system that mimics the Drosophila optic-stalk in order to evaluate collective migration after genetic modification. Results will demonstrate the mechanisms and signaling pathways directly rooted in progenitor needed during retinogenesis.

Acknowledgements

We would like to thank the members of the Venkatesh and Vazquez Lab for all their contributions.

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