Controlled Microfluidics to Examine Growth-Factor induced Migration of Neural Progenitors in the Drosophila Visual System

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Abstract

Background: The developing visual system in *Drosophila Melanogaster* provides an excellent model with which to examine the effects of changing microenvironments on neural cell migration via microfluidics, because the combined experimental system enables direct genetic manipulation, in vivo observation, and in vitro imaging of cells, post-embryo. Exogenous signaling from ligands such as Fibroblast Growth Factor (FGF) are well-known to control glia differentiation, cell migration, and axonal wrapping central to vision.

New Method: The current study employs a microfluidic device to examine how controlled concentration gradient fields of FGF are able to regulate the migration of vision-critical glia cells with and without cellular contact with neuronal progenitors.

Results: Our findings quantitatively illustrate a concentration-gradient dependent chemotaxis toward FGF, and further demonstrate that glia require collective and coordinated neuronal locomotion to achieve directionality, sustain motility, and propagate long cell distances in the visual system.

Comparison with Existing Method(s): Conventional assays are unable to examine concentration- and gradient-dependent migration. Our data illustrate quantitative correlations between ligand concentration/gradient and glial cell distance traveled, independent or in contact with neurons.

Conclusions: Microfluidic systems in combination with a genetically-amenable experimental system empowers researchers to dissect the signaling pathways that underlie cellular migration during nervous system development. Our findings illustrate the need for coordinated neuron-glia migration in the *Drosophila* visual system, as only glia within heterogeneous populations exhibited increasing motility along distances that increased with increasing FGF concentration. Such coordinated migration and chemotactic dependence can be manipulated for potential therapeutic avenues for NS repair and/or disease treatment.

Key words: Microfluidics, *Drosophila*, Glia, FGF, Visual System

1.0 INTRODUCTION

Neuronal-glia interactions are critical for the development and function of the nervous system (NS) of both vertebrates and invertebrates. Glia and neurons are well-known to migrate to specific target locations during NS development, but the molecular signals that orchestrate this migration remain incompletely understood. While concentration gradients of a variety of growth factors are well-known to determine the positioning and fate of embryonic stem cells (Gomez-Marín and Louis, 2012; Green and Smith, 1991; Kornberg and Guha, 2007), the gradients that mediate the coordinated migration of cells of neuronal-glia lineage are largely unexplored. An understanding of the mechanisms that regulate such coordinated neuronal-glia migration may provide insights into possible therapeutic approaches for the myriad of neurological diseases caused by aberrant migration (e.g. lissencephaly, polymicrogyria, gliomas), as well as advance potential regenerative strategies to aid in NS repair.

Neurological study has increasingly embraced microfluidics-based technologies, with numerous systems used to examine varied aspects of embryogenesis (Levario et al., 2013), neural regeneration and growth (Brunello et al., 2013) and intracellular synaptic activity (Chotard and Salecker, 2004; Kim et al., 2010; Tabata, 2015), among others (Cimetta and Vunjak-Novakovic, 2014; Kim et al., 2015). Previous work from our group has developed microdevices to analyze NS behaviors in response to controlled environments generated without the use of forced flow (Able et al., 2012; Kong et al., 2010; Mishra et al., 2015; Rico-Varela et al., 2015). Such a system is distinctive for study of NS cell migration, as the external pumps and micro-valving routinely used to stream biochemical agents in many existing devices are well-known to alter the responses of cells unaccustomed to physiological flow (Huh et al., 2013; Kim and Tarbell, 1994).

The developing visual system in *Drosophila Melanogaster* provides an excellent model with which to examine neuronal-glial migration via microfluidics, as the combined experimental system enables direct genetic manipulation, in vivo observation, and in vitro imaging of cells, post-embryo.

During the development of the *Drosophila* visual system, retinal basal glia (RBG) are generated in the developing brain and migrate into the developing eye imaginal disc. The RBG have been implicated in the targeting of the photoreceptor cell axons to the optic ganglia. While the genes involved and the cellular interactions regulating the development of the photoreceptor R-cells and RBG have been well studied for *Drosophila*, the environmental cues that initiate the coordinated and/or collective migration of these vision-critical cells remain incompletely understood (Choi and Benzer, 1994; Rangarajan et al., 1999). Genetic studies have illustrated that conserved signaling pathways, such as Notch-Delta, Epidermal growth factor receptor (EGFR) and Fibroblast Growth Factor Receptor (FGFR), are central to cell fate specification and pattern formation in the developing visual system (Huang and Stern, 2005; Shamloula et al., 2002). In particular, our group has demonstrated that Notch loss of function disrupts RBG migration (Shamloula et al., 2002), while exogenous EGF signaling has been shown to activate long-distance migration of retinal progenitor cells (Unachukwu et al., 2013). In addition, FGF ligands are known to control glia differentiation, migration, and axonal wrapping central to vision (Silies et al., 2010; Tulin and Stathopoulos, 2010a, b). FGF signaling molecules are believed to be produced at a localized source and then dispersed into targeted tissue. This dispersion occurs via the transient formation of concentration gradients that then dictate the expression of different genes to lead to tissue patterning and morphogenesis (Yu et al., 2009).

In the present study, we examined the extent to which FGF concentration profiles and gradients play a role in regulating critical neuronal-glia migration during development of the visual system. We employed microfluidics to investigate the chemotaxis of RBG cells derived from the third larval instar stage of *Drosophila Melanogaster*. Our results are among the first to illustrate that RBG exhibit concentration-gradient dependent chemotaxis to FGF signaling, and more significantly, these glial cells require coordinated and/or collective neuronal locomotion to achieve directionality, sustain motility and advance large migration distances. These results highlight the importance of coordinated migration between neuronal-glial cells in the developing visual system, as well as reinforce the advantages of microfluidics-based systems for the study of integrated neural cell response.

2.0 MATERIALS AND METHODS

Drosophila Stock, Neural Cell Dissociation, Culture and Viability

Drosophila were cultured on the standard corn meal agar medium and reared at 23° C. To specifically label and mark glia with GFP we used the GAL4-UAS system (Brand and Perrimon, 1994). Briefly, *repo-Gal4/Tb* flies were crossed to *UAS-GFP* flies and the larvae from the progeny of the genotype *repo-Gal4; UAS-GFP* were used for all series of experiments in this study. Flies of this stock express GFP⁺ glial cells that then fluoresce green in the visual system. Flies were mated for 3 days, and subsequent F1 offspring produced 12 days later were dissected in the 3rd instar larvae stage. The procedure for culturing glia was adapted from a widely-accepted method established to culture neural cells from *Drosophila* larvae (Crank, 1979). In brief, third instar larvae were surface sterilized with 70% ethanol and placed in PBS (Mediatech Inc., Herndon, VA). Brain complexes were then dissected out using fine #5 stainless steel tweezers and placed in a solution of 0.5 mg/mL collagenase (Gibco, Grand Island, NY) in phosphate buffered saline (PBS). Brain tissue was then finely chopped, covered and incubated at 25C room temperature for 1 hour with slight manual agitation. The cell suspension was then centrifuged and re-suspended in culture medium consisting of 90% revised Schneider's *Drosophila* medium (Lonza, Walkersville, MD) and 10% heat-inactivated FBS (Mediatech Inc., Manassas, VA) with 50 mg/mL penicillin (Mediatech Inc.) and 50 µg/mL streptomycin sulfate (Mediatech Inc.), as shown in **Figure 1**. Cells were incubated at room temperature at atmospheric CO₂ levels. The brain complexes of 50-75 files were used per experimental condition. Cells were cultured for 5-6 days and examined via MTT assay to verify >93% viability prior to experiments.

Fluorescent Activated Cell Sorting (FACS)

Experiments were performed using glia from two groups of cells extracted from the drosophila NS and sorted via FACS: (A) A heterogeneous neural cell population derived from dissociated brain complexes, which contained cells of both neural and glial lineages; and (B) A homogenous population of GFP⁺ cells of glial lineage, only. FACS was performed using a BD FACS Aria II (BD Biosciences, San Jose CA) with accompanying FACSDiva 6.1.3 software (BD Biosciences, San Jose CA). A 488-nm wavelength laser through a 530/30 filter was used to detect fluorescent signature of GFP (541 nm). The 633 nm laser through a 660/20 filter was used to detect To-pro-3 Iodide. 10 μ L of To-pro-3 Iodide (Invitrogen, Eugene OR) at 25 μ g/mL was added to the sample and incubated for 15 minutes.

μ Lane Microfluidic System

Experiments used our established microfluidic system, called the bridged μ Lane, whose design and operation has been described previously in greater detail (Able et al., 2012; Dudu et al., 2012; Kong et al., 2010; Rico-Varela et al., 2015). As shown in **Figure 2**, the system is fabricated via elastomeric molding of two layers of conventionally-used Poly dimethyl siloxane (PDMS,) that are bonded to one another, and to a boron silicate glass microscope slide. The first layer includes a 150- μ m diameter by 15-mm long microchannel, as well as a source and sink reservoir, which have volumes of 9- μ L each. The second layer consists of 170- μ L source and sink chambers, and a larger hemispherical bridge channel connecting them. During operation, the biochemical agent of interest, in this case FGF, is inserted into the source chamber, while the remainder of the system is filled with PBS or media. The bridge channel is filled with media last, as its function is to precisely balances the liquid levels in the source and sink chambers to minimize the hydrostatic pressure difference between them (Kong et al., 2010). Transport of

FGF via so-called convective diffusion (Bird and Hassager, 1987; Crank, 1979; Crowe et al., 2000) then establishes a concentration gradient between the source and the sink reservoir that spans several orders of magnitude. This distribution of FGF concentration can be maintained for several days and is modeled computationally and verified experimentally. Concentrations of 10-ng/mL, 100-ng/mL, and 1000-ng/mL of FGF were used to generate concentration profiles for testing of cell migration within the μ Lane. For control experiments, media was added into the μ Lane system in lieu of FGF.

To mimic concentrated biological ECM microenvironments in the NS (Branco et al., 2009; Brandl et al., 2010; Singh et al., 2014; Taylor-Weiner et al., 2013), μ Lane systems were filled with 50 μ g/mL of Laminin (BD Biosciences, Bedford, MA, Cat. No. 354232) and incubated at 37 C for 1 hour. The source of laminin is from Engelbreth-Holm-Swarm murine sarcoma basement membrane. Broadie et. al describe the breakdown of the *Drosophila* ECM molecules specifically two well conserved laminins, Laminin A and a minor Laminin W both sharing common β and γ chains with distinct α , with different tissue distributions (Broadie et al., 2011). Laminin was allowed to gel within the μ Lane for 1 hour at room temperature to generate 2D microenvironments. Laminin was then aspirated from the channel interstitial spaces leaving the protein adhered to the channel surfaces, only. During experiments, the microchannel, sink reservoir, sink chamber, and bridge channel were first filled with PBS solution. As performed previously by our group, fluorescently-labeled FITC-Dextran (20 kDa, Sigma-Aldrich, St. Louis, MO, Cat. No. FD20S-100MG) was used to model FGF-8 due to similar molecular weight. For 2D experiments, Dextran at a concentration of 40 μ g/mL was added drop wise to the source

chamber until the reagent made contact with the PBS in the bridge channel, initiating the experimental system.

Two-dimensional numerical simulations of the bridged μ Lane system were performed in order to model the transport within the entire microsystem, i.e. the microchannel, both the SRR and SKR reservoirs in the 1st layer PDMS, the bridge channel, and both the SRC and SKC chambers in the 2nd layer PDMS. The mass transport within the entire microsystem was modeled using the 2-D continuity equation, convective-diffusion equation, momentum equation, and hydrostatic equation, as described previously in detail by our group. Free solution diffusivities and effective diffusivities of growth factor proteins were determined in the microsystem by modeling the transport within the system microchannel using convective-diffusion, shown below (Yuan et al., 1991):

$$\frac{\partial C}{\partial t} + VB \frac{\partial C}{\partial x} = D \frac{\partial^2 C}{\partial x^2} \quad (1)$$

Where C ($\mu\text{g/mL}$) is concentration, t (s) is time, D (m^2/s) is diffusivity, V_B (m/s) is bulk velocity, and x (m) is position. The time-evolving solution to this equation was modeled computationally via finite-element-analysis (FEM) in Matlab 7.7 (MathWorks, Natick, MA). The boundary conditions fixed the source reservoir ($X_0=0$) at 40 $\mu\text{g/mL}$ and the sink reservoir ($X_L=15\text{mm}$) at 0 $\mu\text{g/mL}$ for all experimental times. The system initial condition was set such that the concentration along the entire length of the microchannel was 0 $\mu\text{g/mL}$ at $t=0$. In order to determine bulk flow velocity, V_B , 1.9- μm -diameter fluorescent beads (Duke Scientific, Palo Alto, CA, Cat. No. G0200) were injected in the system and visualized via fluorescence

microscopy, as done previously. Note that Equation 1 reduces to Fickian diffusion in the absence of convective flow.

Well Plates

Experiments utilized 96 well plates (Corning Inc., Corning, NY) coated with Laminin as described above, and 100- μ L of cell solutions per well to image migration of both cell populations to defined concentrations of FGF. Concentrations of 1-ng/mL, 10-ng/mL, and 100-ng/mL of FGF were used for testing, while no FGF was used for controls.

Microscopy and Imaging

Dissected eye brain complexes from larvae were cleaned and mounted with Vectashield medium (Vector Laboratories, Burlingame, CA). Images were taken with a Zeiss CLSM-510 (Zeiss, Jena, Germany) and a 40X objective. An argon laser with excitation of 488 nm was used activate the GFP+ cells. Images were obtained as Z-stacks and processed with LSM software, which converted the Z stacks to a projection. Imaging within the μ Lane system was performed using a Nikon TE2000 inverted microscope with a 20x objective and a cooled CCD camera (CoolSNAP EZ, Photometrics, Tucson, AZ) with Nikon software (Nikon Instrument Element 2.30 with 6D module, Morrell Instrument Company Inc., Melville, NY).

Fluorescent images of the μ Lane were taken every hour for 72 hours at the points $x=4\text{mm}$, $x=8\text{mm}$, and $x=12\text{mm}$, where the origin was defined as the point where the source reservoir abutted the microchannel. Imaging was performed using a Nikon TE2000 inverted microscope with a 20x objective and a cooled CCD camera (CoolSNAP EZ, Photometrics,

Tucson, AZ) with Nikon software (Nikon Instrument Element 2.30 with 6D module, Morrell Instrument Company Inc., Melville, NY).

Data Analysis and Statistics

Data were analyzed using the Chemotaxis and Migration Tool (Ibidi, Verona, WI) in ImageJ(NIH). Cells were individually tracked using the manual tracking plug-in. Cell trajectories were plotted, along with accumulated distance and motility. In addition, the Chemotactic index, CI, was used as the measure of directed cell migration, as defined in Equation 2 (Kong et al., 2011):

$$CI = \frac{x}{d} \quad (2)$$

Where x is distance toward gradient and d is total accumulated distance. Values approach 1 as cells move more directly toward the gradient, are negative for cells moving away from the gradient. CI approaches zero in the absence of a gradient.

Statistical Analysis

Statistical significance between groups was evaluated using a student's t-test. In the gradient experiments, the Raleigh test was used to assess uniformity of the cell distribution.

3.0 RESULTS

This study examined how the chemical cues from the microenvironment influence coordinated migration of cells of glial and neuronal lineages. In general, during *Drosophila* neural development glia have been shown to provide both attractive and repulsive guidance cues to NS neurons as well as require their associated axons for trophic support. Parker and Auld (Parker and Auld, 2006) have shown specifically that the midline glia are crucial for the proper formation of horizontal commissural axon tracts in the *Drosophila* CNS. In a review by Lemke (Lemke, 2001), reciprocal interactions between differentiating glial cells and neurons define the course of nervous system development even before the point at which these two cell types become definitively recognizable. Glial cells control the survival of associated neurons in both *Drosophila* and mammals, but this control is dependent on the prior neuronal triggering of glial cell fate commitment and trophic factor expression. The first set of experiments used FACS to generate two groups of cells from the *drosophila* CNS: (A) A heterogeneous population derived from dissociated brain complexes, which contained cells of both neural and glial lineages; and (B) A homogenous population of GFP+ cells of glial lineage, only. As seen in **Figure 3A**, *Drosophila* brain complexes are imaged after utilizing the Gap4-Repo system to genetically introduce GFP+ glia into live *Drosophila*. As shown, glia are seen to migrate from the optic stalk (~250 microns in cross-section) to prescribed locations in the eye upon being ushered by concentration gradients of key growth factors (Silies et al., 2010). These eye complexes were then removed and FACS sorted for GFP+ glia, as seen in **Figure 3B**. Here, analysis of FACS revealed less than 10% of the viable cells derived from larvae NS presented as GFP+ glia, requiring increased sample sizes to insure that both the heterogeneous and homogeneous populations retained recovery of 10^5 cells for each experimental test. Furthermore, viability tests

were performed on all FACS sorted populations to insure that live cells (>90%) were used for migration testing.

Once cell samples were sorted into two populations, their migratory responses to exogenous FGF signaling at different concentrations were examined using 96 well plates. As seen in **Figure 4**, glia from homogeneous populations migrated less than 20 microns in response to increasing FGF concentrations of 1-, 10-, and 100-ng/mL. By contrast, glia of heterogeneous populations migrated steadily larger distances of up to 3-fold higher than that of homogeneous glia when exposed to the same FGF concentration fields. Furthermore, **Figure 4** illustrates that migration of glia from both populations was non-directional and radially semi-uniform within each plate at all concentrations examined. In addition, experiments measured the average accumulated distances traveled and average motilities of glia from both the heterogeneous and homogeneous populations when exposed to different concentrations of FGF, shown in **Figure 5**. As seen, glia from heterogeneous populations migrated statistically-significant larger distances in response to increasing FGF concentrations, and demonstrated increased average motilities in response to signaling from increasing concentrations of FGF. By contrast, populations of homogeneous glia illustrated no statistically-significant differences in migration distances or average motilities in response to signaling from increasing FGF concentrations.

The final set of experiments used the μ Lane system to examine migratory responses from glia of both homogenous and heterogeneous cell populations to signaling from defined concentration gradients of FGF. Here, the molecular transport within the microchannel was, first, validated experimentally to characterize the concentration distribution within the system. Bulk flow within

the system was examined using fluorescent beads with and without the use of the bridge channel, as described previously (Kong et al., 2010). Beads were seen to migrate with different velocities in the microsystem with and without the bridge channel. An average bulk velocity of $V_{\text{avg}}=92.3\pm1.4\text{ }\mu\text{m/s}$ was measured within the μLane system in the absence of the bridge channel ($n=12$ channels), but an average velocity of $V_{\text{avg}}=0.27\pm0.06\text{ }\mu\text{m/s}$ was measured within the bridged μLane system ($n=16$ channels). In addition, no bulk flow was measured within the bridge channel when beads were used, confirming that transport within the bridge was diffusion-dominated and did not significantly affect transport within the microchannel nor alter concentrations of the large volume SRC and SKC chambers over experimental time scales of up to 72 hours.

The spatial and temporal patterns of FGF profiles generated within this system were rigorously analyzed by solving the convective–diffusion flux between the system reservoirs and channels, exactly. As seen in **Figure 2**, the μLane was able to generate multiple concentration gradient fields within one experimental setting. The spatial distribution of ligand within the microsystem generated a wide range of gradients along the 12-mm-long channel length that were sustained for 5-7 days. Here, some gradient fields were generated via small changes in absolute concentration of attractant, and were therefore shallow and approximately linear, while other gradient fields (Rico-Varela et al., 2015) were created via much greater changes in attractant concentration, and were thus steeper and highly non-linear. Lastly, as seen in **Figure 2**, the experimentally-obtained data were within 2% of the analytically-predicted value.

With a validated μ Lane system, heterogeneous and homogenous populations of neural-derived cells were then inserted into the μ Lane to examine glia viability, morphology and migration in response to different concentration gradients of FGF. Raw data images illustrate that glia do not modify their rounded morphology within any portion of the channel when exposed to varied FGF concentrations and gradients. Further, continued GFP expression from glia denoted that cells remained viable throughout the experimental test period of 72 hours. **Figure 6** illustrates sample trajectories of glia derived from both homogenous and heterogeneous populations in response to signaling from different concentration gradient fields of FGF. As seen, glia from homogeneous samples did not exhibit statistically-significant differences in accumulated distance or motility, with values of each remaining approximately constant for the different FGF profiles studied. By contrast, the trajectories of glia from heterogeneous populations illustrate that cells increased their migration distance up to 6-fold when responding to concentration gradients of increasing FGF concentration. Further, glia from heterogeneous populations illustrated optimal CI values at gradient fields of intermediate FGF concentration of 10-ng/ml. Lastly, average motility of these cells was seen to increase in a statistically significant manner in response to gradient fields of increasing FGF concentration.

4.0 DISCUSSION

Coordinated neuronal-glia interactions are known to be critical for the development, function and response of the visual system. Yet, how the collective but finely-tuned migration of these cell populations is regulated by their microenvironment remains incompletely understood. Results of the current study illustrated distinct migratory behaviors between homogenous populations of glia, and glia from heterogeneous populations containing cells of both glial and neuronal lineages. Using conventional well technology, glia of the homogeneous populations were seen to migrate radially, and with constant motility and distance independent of the FGF concentration used. By contrast, glia within heterogeneous populations exhibited increasing motility along migration distances that increased with increasing FGF concentration. We propose that these differences can be attributed to glia-neuronal interactions that are absent in the homogeneous glial population. We note that while FACS is known to influence cell viability (Arun et al., 2005; Freer and Rindi, 2013; Herzenberg et al., 2002) glia from both populations expressed comparable intensity levels of GFP and their migration remained constant over multiple experiments for several days. Our migration data is significant because RBG glia were initially only believed able to migrate into the eye disk via physical mechanisms of haptotaxis, i.e. crawling along a substrate (Haeger et al., 2015; Ricoult et al., 2015). However, more recent work suggested abilities of RBG to migrate towards differentiating photoreceptors without using a continuous physical substratum (Rangarajan et al., 1999). The data from our study support this later phenomenon, which point to short-range diffusible signals as regulators of RBG-neuron

interactions, in addition to hypothesized contact-mediated processes that require physical interactions between cell types. Such neuronal-glia coordinated migration has been previously unstudied, and points to the importance of examining so-called collective migration (Kopf, 2015; Kumar et al., 2015; Pocha and Montell, 2014; Riahi et al., 2015; Stonko et al., 2015) for potential therapeutic avenues for NS repair and/or disease treatment.

We next utilized a microfluidics system, the μ Lane, to examine the migration of RBG in the presence of defined concentration gradients of FGF. While traditional benchtop technologies such as boyden chambers and agarose assays have contributed greatly towards identifying combinations of cytokines that generate directed cell movement (Reviewed in (Hulkower and Herber, 2011; Kramer et al., 2013; Okada, 2012; Sackmann et al., 2014)), these systems cannot be used to measure mechanistic parameters of migration, such as motility and directionality. By contrast, our quantitative and tunable μ Lane system empowers researchers to examine the migration of cell populations as a function of the ligand(s), concentration(s), and concentration gradient field(s) used. While it is well-established that no in vitro system can physiologically replace the in vivo environment, microfluidics offer unique advantages of biological scale that enable closer approximation to in vivo conditions. For instance, the μ Lane device creates a microenvironment for RBG that is 100 μ m in diameter, which approaches dimensions of the in vivo optic stalk (Edwards et al., 2012). This creates an experimental test system where cells experience diffusible signals, matrix interactions, and cell to cell contacts on physiological scales. Thus, in this work, we used FGF as a diffusible signal because of its well-established role in signaling transduction critical to eye development (Silies et al., 2010), laminin as ECM because it is ubiquitous in the developing visual system of *Drosophila* (Singh et al., 2014), and

neuronal-glia cell densities that approach physiological numbers based on in vivo spacing and dimensions (Li et al., 2015).

Using FGF as a model cytokine in this work, the μ Lane was able to generate multiple concentration gradient fields within one experimental setting that were sustained for 5-7 days. This exploits the analytical nature of microfluidic devices as the μ Lane can examine which ranges of concentration and gradient (i.e. concentration distribution over specified distances) stimulate migratory responses from cells. Such a tunable system facilitated the experimental tracking of 25–50 distinct cells for each FGF gradient field. Three different concentrations of FGF were used to generate gradient fields within the μ Lane. Glia of the homogenous population were not observed to migrate appreciable distances (less than 1 cell diameter) in our system, although cells remained viable within the device for several days as evidenced by their GFP expression and unchanged morphology. Amazingly, glial cells of the heterogeneous population were seen to migrate in highly directed trajectories along increasing FGF gradient, and with increasing motility. These results point to an obvious chemotactic dependence on FGF concentration gradient, as cell trajectories and distances traveled were much larger for cells exposed to signaling from FGF gradient fields than from concentration alone. Our results further underscore the presence and significance of coordinated neuronal-glia migration in the development of the *Drosophila* visual system. Cells of the homogeneous glial population did not exhibit measurable movement, while glia of the heterogeneous population illustrated directed migration within gradient fields, as well as sensitivity to specific FGF concentration. These data underscore the impact of the coordinated migration of neuronal-glia cells requisite for development of the visual system.

Conclusion

Our studies presented here illustrate the efficacy and power of microfluidic systems in combination with a genetically amenable experimental system to dissect the signaling pathways that underlie cellular migration during nervous system development. Taken together, these findings illustrate the significance of the coordinated neuron-glia interaction for migration in the development of the *Drosophila* visual system. Our results show that glia within heterogeneous populations exhibited increasing motility along migration distances that increased with increasing FGF concentration. Such coordinated migration and chemotactic dependence of these cells can be manipulated for potential therapeutic avenues for NS repair and/or disease treatment.

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Figure 1: Derivation of Drosophila-derived neural cell populations. (A) Illustration of GAL4-UAS system. Repo-Gal4 flies are mated with UAS-GFP. Offspring express GFP in glia only. Drosophila were bred and grown in house and brain complexes of those in the larva stage were extracted for cell testing. (B) Confocal image of Drosophila whole brain complex, confirming presence of GFP+ glial cells.

Figure 2: Description of Bridged μ Lane System. A) Schematic of microfluidic system used in the current study. The reservoirs hold a 9 μ l volume and both chambers hold a 170 μ l volume each. Images of μ Lane system B) First layer of PDMS: Two reservoirs connected by a 15-mm-long microchannel C) Second layer of PDMS: Sink and source chambers connected by a bridged channel D) GFP+ glial cells within the microchannel at 0, 24, and 48 hrs, respectively E) FGF distribution within laminin-coated μ Lane system, predicted analytically and measured

experimentally. Experimental data confirms that a steady-state FGF distribution occurs within the entire length of the channel before 24 hours.

Figure 3A: Derivation of Drosophila-derived neural cell populations.

Drosophila larvae. Panel (A) shows an early third instar developing eye imaginal disc attached to the brain lobe. Arrow points to the glia migrating from the brain through the optic stalk into the developing eye. Panel (B) shows a late third instar eye imaginal disc with glia migration in to the eye disc complete. The glia migrate and stop at the Morphogenetic Furrow (MF) [Arrow]. Ahead of the MF dividing undifferentiated cells can be seen labeled with the mitotic marker, anti- Phospho-Histone 3 antibody. The developing photoreceptors are not labeled.

Figure 3B: Selection of homogeneous and heterogeneous neural cell samples. FACS analysis of cells derived from brain tissue of third instar larvae drosophila. A) A four quadrant distribution (Q1-Q4) of cells stained with a To-pro-3 Iodide as a viability marker (APC-A) against a GFP signature (FITC-A). Cells in Q3 and Q4 are considered viable cells, while cells in Q2 and Q4 are denoted as GFP positive. Points in Q4 represent cells that are both alive and GFP positive. B) A representative FACS sorting for cells that are GFP positive/negative only. Points denoted in P3 denote cells that are GFP+.

Figure 4: Trajectories of homogeneous (sorted) and heterogeneous (unsorted) neural cell samples in response to FGF signaling. Plots illustrate representative trajectories from cells of homogenous population exposed to (A1) media only (control), (A2) 1-ng/ml (A2) 10-ng/ml and (A3) 100ng/ml of FGF. Plots of representative trajectories of cells from heterogeneous populations of (B1) media only (control), (B2) 1-ng/ml (B2) 10-ng/ml and (A3) 100ng/ml of FGF. Each plot contains 10 paths, and each path contains 1 data point every 2 hours for 48 hours. Concentrations shown are uniform; 10 cells were tracked for each concentration.

Figure 5: Accumulated distances and motilities of heterogeneous and homogeneous neural cell populations. (A) Average accumulated distance traveled and (B) Average motility of cells from both populations. Values reported are mean with error bars (+/-) standard deviation. An * Indicates $p < 0.05$ compared to the control.

Figure 6: Migration of heterogeneous cell populations to concentration gradients of FGF.

A) Plot of cell trajectories normalized to the origin. Each plot contains 10 trajectories generated using data points gathered every 2 hours. Gradient increases from left to right as indicated by the arrows. Each line on the grid is 20 microns. B) Values of chemotactic index for all experimental conditions. C) Values of average motility for all experimental conditions. Reported values are mean data with standard deviation error bars. An * indicates $P < 0.05$ compared to control.

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