The Planar Cell Polarity of Ependymal Cells in the SVZ Neurogenic Niche

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Honors thesis submitted in partial fulfillment of the requirements for graduation with Distinction in Biology in Trinity College of Duke University
Abstract

Ciliopathy and ciliogenesis are important features of nearly every body system, from the respiratory and cardiovascular to the urinary and reproductive systems. Understanding the development of ciliary and basal body planar polarity, and mapping the effects of dysfunction, is important in our understanding of many tissue types, and diseases that affect human health.

Cilia line the surface of ependymal cells along the lateral ventricles of the brain, including the neurogenic sub-ventricular zone (SVZ). Mature ependyma show tissue-level alignment of cilia in a common region on the cell surface, and with a common direction of ciliary beating; this is called planar cell polarity. In this study, we mapped stages in the development of planar cell polarity in 3 ages of infant mice (3, 8, and 11 days post-partum). We found an increasing asymmetry, ventral alignment, and transition from disorganized to organized ciliary beating. Next, we explored the effect of healthy-tissue modulation on the normally developing planar cell polarity. Modulation of previously healthy tissue mirrors the effect of sudden-onset diseases like stroke and brain injury. Both environmental and biochemical modulations were produced in-vitro. We found that healthy developing ependymal cells were sensitive to these changes. Removal of fluid flow led to loss of asymmetrical planar polarity, loss of cilia, and more disorganized ciliary beating. Suppression of FOXJ1, an important biochemical transcription factor, in previously healthy immature cells led to loss of cilia and greater misalignment of basal bodies.
Introduction

The subventricular zone (SVZ) is one of two regions of the brain, along with the subgranular zone of the hippocampal dentate gyrus, that is currently known to house neural stem cells (NSC) in the adult mammal (1). The SVZ is located along the lateral wall of the lateral ventricles in the cerebrum. The rodent subventricular zone serves as a useful experimental model for studying general mechanisms of neurogenesis in the mammalian brain (2). In the mouse, this region is involved in producing and sustaining a niche of neural stem cells, which divide to produce migratory neuroblasts and eventually neurons, astrocytes, and oligodendrocytes (3). These neuroblasts travel on the rostral migratory stream to the mouse olfactory bulb, where they mature and integrate into the established neural circuitry (4). Understanding systems of postnatal and adult neurogenesis is important for learning and memory functions (5), and for producing therapeutic mechanisms after stroke and brain injury (6).

Figure 1. The location of the SVZ is along the lateral wall of the lateral ventricle. Image depicts a coronal section of a mouse brain. (Left) Arrow points to the lateral ventricles. (Right) An enlarged view of the lateral ventricles, with labels for the medial and lateral walls. The sub-ventricular zone is located along the lateral wall of the lateral ventricle. Image adapted from Kuo et. al. (2006)23.
The subventricular zone in rodents is made up of at least four cell types that serve as the precursor for new neurons and glial cells: the quiescent astrocytic neural stem cells (B cells), transient amplifying cells (C cells), migrating neuroblasts (A cells), and ependymal cells (7).

The neurogenic niche of the subventricular zone is modulated, sustained, and protected by the important action of ependymal cells. Ependyma are the multiciliated cells lining the lateral ventricles, and serve as the border between the SVZ neural stem cell niche and the ventricular cerebrospinal fluid (8). Ependymal cells organize on the ventricular surface as pinwheels encircling a neural stem cell (9). These important cells give instructive cues for growth, proliferation, and differentiation of A, B, and C cells in the SVZ (8), and may help indirectly guide neuroblasts to specific areas of tissue damage (10). They support the absorption of neurotoxins to protect the neurogenic niche from damage (11). These cells also mediate the reception, integration, and response of neural stem cell signals from regions within and outside the brain, namely through biochemical cues transmitted in the vasculature (12) and cerebrospinal fluid (13). Some studies also show that, in instances of ischemic stroke, ependymal cells can re-enter the cell cycle to eventually become neuronal precursors that migrate to the mouse olfactory bulb (14).

Notably, ependymal cells form multiple cilia on their ventricular surface. Cilia are hair-like projections that extend from the surface of the ependymal cell into the fluid of the lateral ventricles (15). These projections are anchored to the cell surface by clusters of microtubules called basal bodies (16); they mediate the flow of cerebrospinal fluid through the lateral ventricles (15). A loss of ependymal ciliary beating can lead to a
medical condition of fluid buildup, known as hydrocephalus (17). Thus, the alignment of
ciliary ependymal cells, and an understanding of the effects of physical and biochemical
modulation on ependyma, is important for its applications to medicine, cancer, stem cell,
and neuroscience research.

Immature ependymal cells begin their development with misaligned cilia that beat
with random uncoordinated directionalities (19). Mature ependyma, however, exhibit a
phenomenon known as planar cell polarity. Planar cell polarity is the tissue-wide and
region-specific intercellular alignment of cellular features and processes, which often
allow groups of cells to carry out specialized functions as a collective unit. Planar cell
polarity is observed in the alignment of ependymal cilia and basal bodies. Over time,
cilia organize their ependymal planar cell polarity in two ways: a translational alignment
of cilia and their basal bodies in a specific location on the cell, and a rotational angular
alignment of the basal bodies that determines the direction that the cilia beats (18). The
biochemical and cellular mechanism for this alignment is unknown, but modulation of
ependymal environment has been found to disrupt planar cell polarity (19). A loss of
planar cell polarity can directly lead to a host of neurological dysfunctions, including fluid
buildup in the brain and a loss of neurogenic function (14).

Prior research shows that the ependymal surface shifts from a disorganized state
to a tissue-wide organized one during postnatal development (27). While this change
has been widely explored using the comparison of an immature mouse with a mature
one, the specific changes that produce tissue-wide polarity are poorly documented over
a broad span of brain development. Spassky et. al. documents changes that occur as
radial glial cells differentiate into ependymal cells from the pre-natal to post-natal day 1
Hirota et. al., through analysis of mice between p5 and p9, documents basal body positions going from broad and disorganized at p5 to organized and polarized at p9. Studies conclude that this polarization occurs, but there is a lack of relevant research that document the specific stages that underlie this alignment across broad age ranges during postnatal development.

While planar cell polarity has been well documented in diseased mutant mice, the instability of ependymal planar cell polarity in modulated conditions is poorly understood, especially when these conditions are introduced to healthy tissues.

Many studies have explored conditions of diseased tissue where polarity is never established. An analysis of relevant literature reveals that mutant mice knocked-out for genes important to cerebrospinal fluid (CSF) flow, or genes important for cilia/basal body functioning, never exhibit the planar cell polarity observed in healthy mice.

But what happens when cilia and basal bodies begin alignment under completely normal conditions in healthy mice, and suddenly are forced to grow in absence of these conditions? What happens when these changes occur during a period when the brain is still developing? These questions are extremely important because, in the real world, changes to the brain often happen suddenly as in the case of stroke and brain injury. These changes can also happen to brains that are immature. Understanding the effect of sudden modulation to healthy developing and/or mature tissue is vastly important to human health and development.

Two in-vivo modulations that ependymal cells may encounter are changes to CSF flow and biochemical signalling -- both features may have disastrous effects to the brain and the organism. Inducing stroke produces dysfunction of CSF flow in organisms
The deletion of genes important for proper CSF flow or basal body alignment, lead to a loss or absence of planar cell polarity, meaning that cilia and basal bodies never align translationally and rotationally in a tissue-level organized fashion (27).

The effects of physical flow and biochemical dysfunction has yet to be explored through an in-vitro model. The extent of planar cell polarity research in these modulations is limited to mutant studies. Thus, it becomes necessary to identify a mechanism to model alterations in-vitro, using healthy animals.

Removal of cerebrospinal fluid flow can be modelled by culturing the ependymal cells in a dish without fluid flow. This system can model the effect of severe hydrocephalus, a disease marked by fluid blockage in the brain completely removes flow over ependymal cells. The effect of this modulation on the development of healthy tissue can be subsequently explored by analyzing changes to planar cell polarity.

Biochemical modulation of healthy tissue in-vitro can occur by enhancing or suppressing biomolecules, such as transcription factors, important for the growth and maintenance of ependymal cells. Ependymal cells can be cultured in the presence of factors that enhance or suppress their stability. An example of such a molecule important for ependymal stability is FoxJ1. FoxJ1 is required for cell differentiation and formation of motile cilia (20). Drugs can enter ependymal cells to increase the expression of this molecule, or block FOXJ1 from its normal function. One drug, IMD0354, is known to suppress FoxJ1 expression in ependymal cells (21). MLN4924, in contrast, enhances FoxJ1 expression (22). We are interested in the effect of biochemical modulation of ependyma, using drugs to enhance or suppress FoxJ1, on the structure and function of the cells, specifically their cilia and basal bodies. This
understanding of ependymal planar cell polarity, and its role both in ependymal function and dysfunction, is critical to our knowledge of tissue development, hydrocephalus, and other neurological disorders that affect human health.

**Project Aims**

Ciliopathy and ciliogenesis are important features of nearly every body system, from the respiratory and cardiovascular to the urinary and reproductive systems. Understanding the development of ciliary and basal body planar polarity, and mapping the effects of dysfunction, have applications that extend far beyond the confines of the brain; they can apply to development and disease in multiple tissue types.

The goals of this project are 1) to explore and understand planar cell polarity as a feature of post-natal ependymal development 2) to modulate healthy developing ependymal tissue physically and biochemically 3) to understand effects of healthy tissue modulation on planar cell polarity and ependymal stability. We will modulate the physical and biochemical environments of ependymal cells in-vitro, and explore the effects on the expression, localization, and planar cell polarity of ependymal cilia and ciliary basal bodies. The generalized hypothesis is that physical and biochemical modulation will lead to changes in the expression, location, and tissue-level polarity of ependymal cilia. Removal of normal fluid flow, and suppression of FoxJ1 will lead to a reduction in planar cell polarity and cilia length, while FoxJ1 enrichment will increase ciliary length and stabilize planar cell polarity.

We began by exploring age and region-specific development of polarity by analyzing acutely fixed ependymal cells from three different infant ages (3, 8, and 11 days post-natally), and from two different regions (caudal and mid-section). We
modulated physical environment by culturing ependymal cells for 24 and 48 hours in absence of CSF fluid flow. We also modulated the biochemical environment by culturing ependymal cells using media infused with FoxJ1 drug enhancers and suppressors. Immunohistochemical staining of ependymal cells with fluorescent antibodies allowed for imaging of cilia (acetylated tubulin), basal bodies (gamma-tubulin), and ependymal cell borders (phalloidin, beta-catenin). Imaging was performed on a Leica S5 confocal microscope. Comparative analysis was conducted through ImageJ image analysis software, and MatLab numerical computing software.

Methods

Part 1: The Development of Ependymal Planar Cell Polarity


Preparation of Solutions (performed by laboratory technician)

1 molar PBS: 8g of NaCl, 0.2 g of KCl, and 1.44g Na2HPO4, 0.24 g of KH2PO4 were mixed into 800mL deionized water. The pH was adjusted to 7.4 via titration with HCl and pH meter. Deionized water was subsequently added to produce a 1 liter stock solution.

Live Imaging Media: A 50mL stock solution of live imaging media for wholemount ependymal culturation experiments was prepared from a 39.4mL cell culture media (Gibco DMEM/F12 11330-032), 10mL horse serum (Gibco), 250 µL L-glutamine, D-glucose, NaHCO3, CaCl2, MgSO4, 358mg HEPES, 4.8 µL ascorbic acid, and 12.5 µL insulin (Sigma 15500). Solution was adjusted to pH 7.28. Solution was filtrated in 0.2 µm filter tube and kept in a sterile 15mL conical tube.
Acute (Uncultured) Dissection

Three CD-1 wild type mice were sacrificed by a trained research scientist approved under the The Institutional Animal Care & Use Committee. The ages were as follows, in days after birth: 1 p3, 1 p8, 1 p11. The brain was removed and wholemounts of the lateral ventricular walls were freshly dissected in 2mL1x phosphate buffered saline (PBS). A ventriculotomy was performed on each hemisphere to expose the walls of the lateral ventricle from their anterior to posterior extent. The exposed walls were then directly immersed in 4% paraformaldehyde at 4°C overnight.

Figure 2. An overview of the SVZ wholemount dissection and immunohistochemical staining.
(Top) A lateral wholemount depicting shape and orientation of the SVZ ependymal surface from a coronal view.
(Bottom) Antibody markers in our experiment stain for ependymal cilia (α-tubulin, gray), basal bodies (γ-tubulin, red), and cell borders (phalloidin or β-catenin, black)
Part 2: The Ependymal Culture Experiment

**Purpose:** To explore the effect of flow removal on the development of planar cell polarity in a previously healthy developing mouse ependymal tissue.

Two CD-1 wild type mice were sacrificed by a research scientist trained under The Institutional Animal Care & Use Committee. The mice were 8 days old. The brain was removed from the skull and wholemounts of the lateral ventricular walls were freshly dissected in 2mL live imaging media, warmed to 37 degrees centigrade in an incubator before use. A ventriculotomy was performed on each hemisphere to expose the walls of the lateral ventricle from their anterior to posterior extent. The exposed walls were then cultured for either 24 or 48 hours on a cell culture membrane insert in a 6 well plate and left for the allotted time in an incubator set to 37 degrees centigrade. Dissected tissues were fixed in 4% PFA overnight after culturing, at 4 degrees centigrade.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>P3 Acute</th>
<th>P8 Acute</th>
<th>P8 24 hr</th>
<th>P8 48 hr</th>
<th>P11 acute</th>
<th>P15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissection.</td>
<td>2mL PBS</td>
<td>2mL PBS</td>
<td>2mL culture media</td>
<td>2mL culture media</td>
<td>2mL PBS</td>
<td></td>
</tr>
<tr>
<td>Post Dissection.</td>
<td>4% PFA Overnight</td>
<td>4% PFA Overnight</td>
<td>Cultured 24 hours in culture media then 4% PFA Overnight</td>
<td>Cultured 48 hours in culture media then 4% PFA Overnight</td>
<td>4% PFA Overnight</td>
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</tr>
</tbody>
</table>

**Table 1:** Summary of Part 1 and 2 experimental conditions for analysis, including age, conditions for dissection and post-dissection manipulation of tissues.
Part 3: The FOXJ1 Culture Experiment

Purpose: To understand whether FOXJ1 has protective effects against changes to planar polarity in healthy developing ependymal tissue, by modulating expression and exploring effects on cilia, basal bodies, and tissue-level planar polarity.

FoxJ1 Wholemount Culture

Three p15 mouse wholemounts were cultured and fixed as previously described. Media was infused with the FOXJ1 enhancer MLN, and the FOXJ1 suppressor IMD.

FoxJ1 Ependymal (E1) Culture

The sub-ventricular zone ependymal cells were separated from all SVZ cellular components and cultured individually as previously described, forming a monolayer at the bottom of a glass cell culture plate. Cells were cultured as previously described.

Immunohistochemistry

Wholemounts were removed from 4% paraformaldehyde after 12 hours, and transferred to separate wells of a 24 well plate. Wholemounts were washed 6 times, for 10 minutes each, in 1M PBS with 0.5% triton X-100 to remove excess paraformaldehyde. Wholemounts were permeabilized in 10% donkey serum diluted in 1M PBS for 1 hour. Primary antibodies used were rabbit gamma-tubulin (basal body marker) and mouse acetylated-tubulin (cilia marker). Primary antibodies were diluted 1:1000 in a solution of 10% donkey serum in 1M PBS. Wholemounts were incubated in primary antibody for 12 hours.

Wholemounts were washed in 1M PBS with 0.5% triton X-100 6 times for 10 minutes each, to remove excess primary antibody. Secondary antibodies used were 1D
(anti-mouse, red fluorescent staining), 4D (anti-rabbit, green fluorescent staining), 5D (anti-rabbit, red fluorescent staining), phalloidin (cell borders, far-red fluorescent staining altered to gray). Wholemounts were incubated in secondary antibody for 2 hours, covered with tin foil to preserve fluorescence. Wholemounts were washed 3 times, for 10 minutes each, in 1M PBS with 0.5% triton X-100. DAPI was added at a concentration of 1:1000 (blue fluorescent staining).

**Imaging**

Images were taken on a Leica SP5 Confocal Microscope using a 20x oil objective. Region for analysis was the caudal (posterior) end for p3 and p8 ages, and dorsal mid-section for p11 age (see Figure 2). Images were recorded on Leica Confocal Imaging Software.

![Region for analysis](image)

**Figure 3: Orientation of the lateral wholemount in our experiments.** A wholemount taken from the lateral wall of the lateral ventricle in the left hemisphere of the mouse brain. Blue squares indicate region of analysis for each age cohort: posterior ventral section for p3 and p8 groups, and dorsal mid-section for the p11 age group.

**Analysis**
Confocal images were transferred to FIJI-ImageJ software for processing. Images were max-projected, such that tissue slices were overlayed to form one final image. We overlayed the image of basal bodies with cell borders to determine translational position of basal bodies within the cell. A vector was drawn from the cell center to the cell border, pointing anteriorly. A second vector was drawn from the cell center to the basal body, and its distance was recorded. Using a proportion between the basal body vector and cell border vector, adjustment was made to account for variability in cell size in the final distance calculation. Rotational angle was measured by measuring the angle between Vector 1 (taken with anterior-pointing direction as 0 degrees) and the vector pointing towards the basal body. Distance and angle were tabulated.

These two features of basal body translational polarity: distance from the center, and angular location, were reconstructed in MatLAB software. Statistical analysis involved measurement of mean distance of basal bodies from the cell center, including standard deviations to measure variability within the wholemount, and the mean rotational angle of basal bodies from anterior. Quantification of basal body translational position for the imaged region was mapped on a polar plot.

An overview of the MatLab code used to produce Polar Plots:

```matlab
>>Rng (0, ‘twister’)
>>M=randn(20,20);
>>Z=eig(M);
>>Figure
>>compass(Z)
>>angle = [dataA1 dataA2 dataA3…dataAN];
>>dist = [dataB1 dataB2 dataB3… dataBN];
>>rdir=angle*(pi/180);
>>[x, y]=pol2cart(rdir,dist);
>>compass(x,y)
```
Figure 4. The orientation of the SVZ surface (Top Left) The orientation of the ependymal wholemount for aid in visual analysis. Left image adapted from Gonzalez et. al. (25). Right image adapted from Nature

Figure 5. An example of our analysis. Green staining is gamma tubulin (basal body marker) and gray is beta-catenin (cell border marker). Red arrow depicts a vector of the basal body distance from the cell center and rotational position on the cell surface. White arrow depicts the cell radius and points dorsally for angle 0 degrees in our polar plot. The red arrow’s length, and angle, were measured and mapped on a polar plot (Figure 6-7). Ependyma were excluded from analysis if the cell border or basal body could not be identified with certainty.
Results

Part 1: Mapping the Normal Postnatal Development of Planar Cell Polarity (p3-p11) in healthy developing tissue

**Purpose:** An age-comparative analysis of stages in the development of normal SVZ ependymal planar cell polarity, using immature non-transgenic mice of ages p3, p8, and p11.

**Rationale:** We wanted to understand how planar cell polarity develops in healthy mouse ependymal tissue from the sub-ventricular zone.

**Method:** We stained the brain of a healthy, normal non-transgenic mouse 3, 8, and 11 days old, without any modulation from its healthy state. We observed basal bodies and mapped planar cell polarity across each stage of brain development.

**Summary of results:** At p3, we observed development of cilia and basal bodies. At p8, we observed disorganized cilia and basal bodies in a majority of cells. By p11, we observed the development of planar cell polarity by increasing migration away from the cell center (asymmetry), alignment in the ventral region of the cell, increased flux of mis-aligned basal bodies, and more organized ciliary beating.
Figure 6. Part 1 Results. The Development of Planar Cell Polarity  

Polar Plot for p3, p8, and p11 acute ependymal wholemounts constructed by mechanism depicted in Figure 5. Arrows depict translational position of basal bodies on the ependymal surface, including regional location of the basal body within the cell, and the distance of the basal body from the cell center. A confocal image taken for Gamma tubulin (red) and beta-catenin (green) were used for the construction of polar plots. Note p11 polar plot has a scale extended to 15 microns in order to accommodate larger distance estimates.
1. Ependymal Planar Cell Polarity Development is Marked by Basal Body Asymmetry

Basal body asymmetry is marked by increasing distance away from the cell center. The furthest basal body in the p3 mouse was 7.7 microns from the cell center. The furthest basal body in the p8 mouse was 9.8 microns from the cell center. In the p11 mouse the furthest basal body was 11 microns from the cell center (Figure 7). The furthest basal body in the p11 wholemount was much further than that of the p3 and p8. This indicates that basal bodies are migrating significantly further away from the center.

![Graph showing the distance of the furthest basal body at 3 age points (3, 8, and 11 days post-natally). The furthest basal body is 7.7 units at p3, 9.8 units at p8, and 11.4 units at p11. At p11, the furthest basal body is significantly further from the cell center, which provides evidence for increasing asymmetry.](image)

**Figure 7. Planar Cell Polarity is the movement of basal bodies further from the cell center.** Graph shows the distance of the furthest basal body at 3 age points (3, 8, and 11 days post-natally). The furthest basal body is 7.7 units at p3, 9.8 units at p8, and 11.4 units at p11. At p11, the furthest basal body is significantly further from the cell center, which provides evidence for increasing asymmetry.
2. Ependymal Planar Cell Polarity is Marked by Ventral Basal Body Alignment

Ventral alignment increased with age between p8 and p11. At p8, basal bodies could be found spanning an entire 360 degrees of locations within the cell. The confocal images in Figure 8 reveal random direction. 58% of basal bodies were dorsal, and 41% ventral. However, it was much easier to see the ventral skew of basal bodies in the p11 ependymal cells. 83% of basal bodies in the p11 animal were in the ventral-most region of the cell (Figure 8). Thus, we see basal bodies which were once aligned across the span of 360 degrees, were now aligning in a common ventral region of the cell.

Angular Position of Basal Bodies as a Percentage Dorsal (Orange) or Ventral (Blue)

Figure 8. Planar Cell Polarity is the development of angular alignment in the ventral region of the cell. Confocal image shows ependymal cells (beta catenin, green) and their basal bodies (gamma tubulin, red). Arrow shows the direction of the vector pointing to the basal body’s angular location in the cell. Arrows pointing “up” are ventral, arrows pointing “down” are dorsal. At 8 days, 46% of cells have their basal body in the dorsal region of the cell, and 54% have their basal body in the ventral region of the cell (left pie chart, N=51 cells). Random direction of arrows reveals a lack of intercellular coordination. At 11 days, 16% of cells have their basal body in the dorsal region of the cell, and 84% of cells have their basal body in the ventral region of the cell (right pie chart, N=46 cells).
3. Ependymal Planar Cell Polarity is Marked by Ventral Vangl2 Alignment and Basal Body Co-localization

Vangl2 alignment followed ventral localization of basal bodies. Vangl2 is a known planar cell polarity protein in mammals. In other tissues, Vangl2 localizes to specific asymmetrical cell-cell boundaries during development, and is involved in the asymmetrical anchoring of other proteins (31).

In our study, we mapped the location of Vangl2 in a p8 and p15 ependymal tissue (caudal region). At p8, vangl2 showed very little localization; it could be found in random locations along the cell boundary. At p15, vangl2 was highly localized in the ventral region of the cell. This was consistent with the ventral localization of basal bodies.

Figure 9. Vangl2 Transitions from Unlocalized to Localized in the Anterior-Ventral Cellular Region. Red staining shows Vangl2, a protein with a known role in planar polarity. At p8, Vangl2 can be found throughout the cell, indicating lack of localization. At p15, Vangl2 has localized in the anterior-ventral region of the cell. This result is consistent with our gamma-tubulin localization. This result may indicate vangl2-gamma tubulin colocalization, whereby vangl2 may play a role in the observed ventral flux of basal bodies.
4. Ependymal Planar Cell Polarity is Marked By Increased Basal Body Flux at P11

There is a significant increase in tissue-wide variability at p11 (Figure 10, bottom graph), as well as the existence of basal bodies that appear closer to the cell center (Figure 10, top graph). Tissue wide variability is consistent with our observation in the polar plot (Figure 6) that p11 tissues had basal bodies very close and very far from the cell center.

When paired with our observation of ventral alignment (Figure 8), this variability may indicate that the dorsal basal bodies are in flux towards the ventral region of the cell. Increased variability might be a sign that basal bodies oriented incorrectly at p8, are now migrating inwards towards the cell center, then back outwards towards the ventral region.

**Figure 10-11. Basal bodies are in flux towards their final position at p11.** Top graph shows the distance of the basal body closest to the cell center. At p8, the closest basal body was 3.8 microns. At p11 the closest basal body was 2.4 microns from the center. Bottom graph shows the variability in distance estimates, by standard deviation of our average distance estimate. Standard deviation of the p11 tissue-wide distance average was 2.4 microns, indicating high variability.
5. Ependymal Planar Cell Polarity is Marked by Greater Cilia Organization

We see from qualitative analysis of Figure 12 that planar cell polarity is marked by ciliary development (p3), followed by the appearance of disorganized cilia that are oriented sporadically. The p8 image shows cilia floating with very little clustering or coordinated direction. p11 image, in contrast, shows that the tips of cilia are tightly clustered; these clustered tips seem to be pointing in a common direction which might be the direction of flow. Cilia seem to be curved, with tips pointed ventrally.

This result should be taken as a preliminary study of ciliary planar cell polarity; not as strong evidence. Strong conclusions about “coordinated direction of movement” cannot be established from fixated cilia, because ciliary beating cannot be observed once the tissue is fixed in paraformaldehyde. Future researchers should conduct high-speed live imaging studies to track the direction of beating in freely-moving, unfixed cilia, to establish stronger conclusive evidence that they are moving in a coordinated fashion in the direction which we are proposing here.
Figure 12. **Planar Cell Polarity is the development of coordinated ciliary beating.** Figure shows cilia (acetylated-tubulin, gray) in the caudal region of the wholemount. Most cells have short or undeveloped cilia at p3. By p8 most cells have acquired cilia that beat in uncoordinated directions. At p11 cilia appear to be more tightly clustered, and appear to beat in more coordinated directions. The tips appear to be curved towards the ventral region of the wholemount. Orange arrows map the curvature and direction of observed ciliary beating.

**Part 2: The Effect of Flow Disruption on the Developing Planar Cell Polarity of Cilia and Basal Bodies**

**Rationale:** We wanted to understand how healthy developing ependymal cells react to the sudden loss of fluid flow.

**Method:** We cultured p8 (immature) ependymal cells in absence of fluid flow for 24 and 48 hours. We stained cilia and basal bodies, and compared the cultured ependymal cells to a healthy control to map any changes in planar cell polarity.

**Summary of results:** Culturing developing ependymal cells 48 hours in-vitro led to a significant loss of basal body asymmetry, indicating that ciliary basal bodies are moving closer to the cell center in absence of fluid flow. Qualitative analysis of cilia shows shorter length, weaker expression, and more disorganized direction of ciliary beating within the cell.
Figure 13. Results for Part 2. The Ependymal Culture Experiment. Polar Plot for p8 uncultured ependymal wholemount (left), cultured 24 hours (middle), and 48 hours (right). Arrows depict translational position of basal bodies on the ependymal surface, including angular position of the basal body and the distance of the basal body from the cell center. A confocal image taken for Gamma tubulin (green) and phalloidin (gray) were used for the construction of polar plots. Example of analysis can be seen in Figure 4.
7. 48 hour Culture Leads to Loss of Asymmetrical Planar Polarity

Culturing ependyma 48 hours in absence of CSF flow significantly reduced the distance of basal bodies from the cell center (3.60 ± 1.39) compared to the acute p8 non-transgenic mouse (6.78 ± 1.20). A decrease in basal body distance from the cell center was also observed for 24-hour cultured ependymal cells (4.12 ± 1.49), but this was not statistically significant enough to establish correlation. Reduced distance from the cell center is evidence that asymmetrical planar polarity is lost.

Figure 14. Loss of planar cell polarity is the movement of basal bodies towards the cell center after 48 hour culturing. The mean distance of basal bodies from the cell center is mapped at 3 different time points after culturing in absence of CSF flow. The mean distance is 6.78 for uncultured ependyma. At 24 hours, mean distance of basal bodies from the cell center is 4.703. After 48 hours, mean distance of basal bodies from the cell center is 3.599.

8. 48 hour Culture Leads to Loss of Coordinated Intracellular Ciliary Beating and Potential Loss of Cilia

Qualitative observation reveals that 48 hour cultured ependymal cells show greater reduction of ciliary expression, as revealed by weaker acetylated tubulin signaling, cilia which appear less clustered and more dispersed. Weaker acetylated tubulin signaling could potentially also be experimental variation in our imaging. As previously stated in Outcome #6, very few conclusions can be drawn about the organization of ciliary beating, because we are observing a fixed tissue with immotile cilia.
Figure 15. Loss of Planar Cell Polarity after 48-hour culturing is observed via reduced acetylated tubulin expression, reduced clustering (high dispersion) of cilia. Figure shows cilia in the caudal region of the wholemount of uncultured and 48-hour cultured ependymal cells. Staining uses acetylated tubulin as a marker for cilia (gray). Uncultured cells have have coordinated beating and strong cilia expression. 48 hour culturing results in shorter cilia with more random and sporadic directionality of ciliary beating, with weaker acetylated tubulin expression (weaker acetylated tubulin staining is potentially confounded by variability in our procedure).
Results for Part 3: The Effect of Biochemical Disruption (FoxJ1 suppression) on the Developing Planar Cell Polarity of Cilia and Basal Bodies

**Rationale:** We wanted to understand how healthy developing ependymal cells react to the sudden loss of important biomolecules. In this case, we used FoxJ1, a known stabilizer of ependymal cells, to explore the effect of modulation on planar cell polarity.

**Method:** We cultured immature ependymal cells in absence of fluid flow, and in presence of a FoxJ1 enhancer or suppressor drug. We stained cilia and basal bodies, and compared the drug-treated ependymal cells to a healthy control to map any changes in planar cell polarity.

**Summary of results:** Suppressing FoxJ1 led to significant loss in ciliary length on the ependymal surface.
9. FoxJ1 Suppression Leads to Loss of Ependymal Cilia

We cultured ependymal cells with drugs that enhance or suppress FoxJ1, a known transcription factor involved in ependymal stability. Culturing ependyma with a FoxJ1 enhancer led to no visible change in ciliary expression and/or length, based on qualitative analysis (Figure 16). Culturing ependymal cells with a FoxJ1 suppressor led to a significant reduction of ciliary length. Cilia in the FoxJ1 suppressed ependymal cell look shorter and stubbier, with no observed curvature to indicate direction of movement. This may indicate that cilia are being lost after FoxJ1 suppression.

Analysis of gamma tubulin reveals that the IMD treated wholemount has greater variability in the location of gamma tubulin on the cell surface. Distance and distance standard deviation did not vary significantly. Both wholemounts also show evidence for ventral basal body alignment (angular position of basal body was between 0 and 180 degrees). However, the variability of this ventral angular alignment was 119 degrees for an IMD treated tissue, while variability was only 94 degrees for an untreated tissue. Greater angular variability is an indicator that the IMD treated tissue is slightly less ventrally polarized than the untreated tissue; the tissue was more variable. This may be evidence for heightened loss of planar polarity; however, more studies must be conducted to establish a correlation between IMD treatment and increased variability of ventral alignment.
Figure 16. FoxJ1 suppression leads to loss of cilia in ependymal E1 culture.
Green is GFP, a marker of ependymal cell bodies. Gray is acetylated tubulin, a marker of ependymal cilia. FoxJ1 was suppressed using the drug IMD0354, and enhanced using MLN4924. Suppression led to loss of cilia length, with short and stubby cilia, while enhancement produced no change from the untreated control.

10. FoxJ1 Suppression Leads to No Change in Average Basal Body Position, but Led to Higher Angular Variability

<table>
<thead>
<tr>
<th>Untreated</th>
<th>IMD Treated</th>
</tr>
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<tbody>
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<td>N=52</td>
<td>N=48</td>
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<table>
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<th>Distance from the Cell Center</th>
<th>Angular Position</th>
<th>Distance from the Cell Center</th>
<th>Angular Position</th>
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<td>154.0907</td>
<td>Value 5.18975</td>
<td>129.1084</td>
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<tr>
<td>Stdev 1.51521</td>
<td>94.4671</td>
<td>Stdev 1.348898</td>
<td>119.9247</td>
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</tbody>
</table>

Ventral Alignment

Ventral Alignment
Figure 17. FoxJ1 drug treatment suppressor led to no change in basal body distance from the cell center, but led to greater variability in basal body angular position on the cell surface. The average distance from the cell center was not significantly different. Both wholemounts also had a ventral basal body alignment, indicating no significant change. However, the IMD treated wholemount had a much higher deviation of angular estimate. This is shown in the graph above. The standard deviation of the angular mean was 119.92 degrees, meaning that many more basal bodies were skewed dorsally. This finding provides evidence for reduced planar cell polarity after IMD treatment, though more studies must be conducted to establish conclusive evidence.
Discussion

This experiment serves as one of the few in-vitro models of ependymal planar polarity after modulation of healthy tissue. Results show evidence for an age, flow, and FoxJ1 induced change in planar polarity. Absence of CSF flow led to the retraction of basal bodies towards the cell center for p8 mice, and a loss of the normal developing asymmetrical planar cell polarity. This is important because it enhances our understanding of ependymal dysfunction in previously healthy tissue, in a process potentially translatable to human health.

Part 1: Age Effects

Summary of Results:

1. We observed very few basal bodies in a p3 wholemount. This indicates that most ependymal cells have either not differentiated from radial glial precursors, or they have not yet developed basal bodies on their surface.
2. Cells with basal bodies in p3 are clustered and likely originate from the same precursor cell.
3. Some ependyma differentiate and form basal bodies faster than others. At p3, some cells have become ependyma with full basal body expression, while others are still undifferentiated radial glial precursors. All stages of development are present on the surface.
4. There is evidence for development of asymmetry occurring to move basal bodies further from the cell center. The furthest basal bodies in p11 have migrated significantly further from the cell center than p3 or p8.
5. P11 animals had greater ventral alignment of basal bodies in one common region of the cell. There is evidence for increasing development of translational planar polarity occurring to move basal bodies to one position in the cell.

6. P11 had greatest tissue-level variability. Variability showed evidence for flux, whereby dorsally aligned basal bodies were migrating ventrally at p11. There are cell-specific differences in basal body development and alignment, with some basal bodies migrating faster to their final position.

7. Another feature of ependymal planar cell polarity is the gradual clustering of cilia tips and alignment of beating in a common direction. At p3 we see few ependymal cilia. At p8 we see disorganized cilia beating in random directions, at p11 we see very organized ciliary beating with clustered ciliary tips. The clustering is observed to curve with the tips pointing ventrally.

8. At p11, ependymal planar polarity is immature.

9. Culturing immature healthy ependyma (p8) outside of the normal flow environment reverses developments in planar polarity. Basal bodies retract significantly towards the cell center and cilia appear more disorganized.

10. Suppressing an ependymal cell stabilizer like FoxJ1 led to loss of cilia.

11. FoxJ1 suppressor led to a greater disorganization of ependymal basal body angular position on the cell surface.

12. These results indicate that Immature healthy ependymal cells are sensitive to environmental changes.
In the p3 animal, very few basal bodies were observed on the wholemount (N=29). These basal bodies were always observed in clusters. We know at p3 that most ependymal cells have not fully matured from their radial glial precursors (24). Some radial glial cells have differentiated into ependymal cells, but most cilia and basal bodies have not fully formed on the surface. By p8, all cells in our region seem to show basal body expression. Our observations were consistent with the development outlined by Spassky et. al. (24), which shows that ependyma differentiate and form cilia during the first week of post-natal development. We observed that ependymal cells with cilia and basal bodies were sparsely populated on the ventricular surface: we only observed 29 basal bodies, as opposed to n>46 in our p8 and p11 animals.

There are clusters of fast-maturing ependymal cells that already exhibit basal bodies at p3. These basal bodies are polarized with other ependymal cells in their same cluster. However, these are not polarized in their final ventral position which we observe at p11. Ependymal cells that are clustered together are likely derived from the same progenitor(s), and exhibit a similar or identical morphology. This may falsely lead to the conclusion of planar cell polarity that actually results from a small sample size all taken from genetically/morphologically identical ependyma at p3, because these are the only cells expressing basal bodies for our analysis.

The relatively small cell diameter may have also confused our results, because there is less surface area on which basal bodies can possibly be located. Other factors include smaller cell diameter at p3, and small sample size (29 cells).

Another confounding factor comes from basal body development. Basal body formation occurs underneath the surface in a conserved part of the cell, and
subsequently migrates upwards towards the surface, followed by migration to final position on the surface (30). What we may be witnessing on the p3 wholemount are newly-formed basal bodies that have recently migrated upwards to the cell surface from a conserved region of formation, thus they are initially expressed in a similar surface location, but are not yet in their final location.

**Basal Bodies Have Greater Asymmetry at p11 than p8, evidenced by basal bodies which are further from the cell center. Asymmetry is evidence that planar polarity is developing at p11.**

The furthest basal body in the p3 mouse was 7.7 microns from the cell center. The furthest basal body in the p8 mouse was 9.8 microns from the cell center. In the p11 mouse the furthest basal body was 11 microns from the cell center (Figure 5). The furthest basal body in the p11 wholemount was much further than that of the p3 and p8. This indicates that basal bodies are migrating significantly further away from the center. Asymmetrical migration of basal bodies away from the cell center, which we see at p11, is a feature of planar cell polarity.

**Ventral alignment increased with age. p11 animals have a greater ventral alignment of basal bodies, while p8 ependyma show high variability in regional alignment. Ventral alignment is evidence for developing planar polarity at p11.**

Ventral alignment increased with age between p8 and p11. At p8, basal bodies could be found spanning an entire 360 degrees of locations within the cell. 58% of basal bodies were dorsal, and 41% ventral. However, it was much easier to see the ventral skew of basal bodies in the p11 wholemount. 83% of basal bodies in the p11 animal were in the ventral-most region of the cell (Figure 5). Thus, we see basal bodies which
were once aligned across the span of 360 degrees, were now aligning in a common ventral region of the cell.

At p8, there is low distance variability, but high angular variability. At p11, however, there was high distance variability and low angular variability (ventral alignment). There was no statistically significant difference in the average basal body distance from the cell center, between p8, and p11, but large difference in the standard deviations of these estimates.

For p8: Low distance variability means that p8 basal bodies were more consistently clustered around 6 microns from the cell center. High angular variability means that regional location on the surface was non-specific and could not be determined. For p11: High distance variability means that some basal bodies were very far from the cell center, others were very close to the cell center; the average of these distances evened-out to a similar 6 microns, but the individual cells were variable. Low angular variability means most cells were ventrally aligned.

Between p3 and p11 days post-natally, there was no statistically significant difference in the average distance of basal bodies from the cell center (p3=5.8, p8=6.8, p11=5.7, Figure 5). In fact, the average was smaller in p11 animals, though this was not statistically significant. This was contrary to our expectations that basal bodies would be further out from the cell center at p11 than they were at p3 and p8. The high distance variability can explain this result: averaging the distance for basal bodies significantly further and significantly closer creates an average which appears similar, but in fact is matched with greater variability.
High distance variability is explained by the fact that p11 had the furthest and closest basal bodies. We propose that high distance variability is caused by flux, as explained in the next section. At p8, the closest basal body was 3.8 microns. At p11 the closest basal body was 2.4 microns. P8 had distance values which clustered closer to ~6 microns, but these basal bodies had no ventral specificity; they were found scattered randomly in different cellular regions. P11 has both the closest and furthest basal bodies from the cell center; the average of these values was also ~6 microns, but was met with a significantly higher standard deviation. This result may explain why the average was similar, and the variability high. Reasons for variability might be the existence of fast-maturing and slow-maturing ependymal cell types, and the beginning stages of flux as basal bodies migrate towards their final positions.

**At p11, basal bodies might be in flux towards their final ventral position.**

With variability, we might be observing dorsal basal bodies moving towards the ventral region of the cell, while already-ventral basal bodies are migrating outwards to their final position. This is what Figure 10 and 11 would suggest. The basal bodies which appear closer to the center might have been the dorsal p8 observations being pushed towards the center, and back out towards the ventral region. We predict that this push is caused by hydrodynamic forces (ie flow of cerebrospinal fluid pushing cilia towards the ventral edge of the cell) and/or cellular signals. At this time, we see increasing variability because basal bodies are still in flux to their final position on the cell surface. We would expect, as the slow-migrating ependymal cell basal bodies find their positions, and “catch up” to the faster-migrating cells, that variability would eventually decrease.
Clustered fast-maturing ependyma at p3, and high variability at p11, imply that certain ependymal cells achieve differentiation, basal body formation, and surface migration faster than others. There may be multiple cell types on the ependymal surface distinguished as fast-maturing and slow-maturing.

Cells coming from different progenitors may exhibit slightly different morphologies that affects the speed at which the basal bodies migrate to their final positions. Variability indicates that some cells show basal body migration and their mature polarity faster than others.

Our evidence shows that differentiation of ependyma, formation and migration of basal bodies, occur earlier in some cells than others. Are the basal bodies developing faster at p3, also the basal bodies reaching their final position faster at p11? What are the features that distinguish cells that develop faster, or slower? These questions may be explored in future studies by genetic and morphological similarities or differences between fast and slow developing ependymal cells.

Cilia Align Intracellularly and Intercellularly. At p8, cilia are disorganized. At p11, cilia are observed to cluster and are curved in a common ventral direction.

We see from qualitative analysis of Figure 12 that planar cell polarity is marked by ciliary development (p3), followed by the appearance of disorganized cilia that beat in random directions. The p8 image shows cilia floating without any coordination of their directionality. The p11 image, in contrast, shows that the tips of cilia are tightly clustered; these clustered tips seem to be pointing in a common direction which might be the direction of flow.
As previously stated, this result should not be taken as strong conclusive evidence without the use of live-imaging studies. Rather, this result is a preliminary analysis of ciliary planar polarity; this remains consistent with that observed in basal bodies. We do observe the preliminary development of intracellular ciliary clustering, and intercellular ciliary curvature towards a common ventral direction. Strong conclusions about the coordinated direction of movement cannot be established from fixated cilia, because ciliary beating cannot be observed once the tissue is fixed in paraformaldehyde. We can only observe the position and location that cilia were in at the time they were placed in paraformaldehyde. Future researchers should conduct high-speed live imaging studies to watch cilia beating physically. This will allow researchers to track the direction of beating in freely-moving, unfixed cilia, to establish stronger conclusive evidence for coordinated movement.

**Mature Ependymal Planar Cell Polarity arises after P11. At p11, ependymal planar cell polarity is still developing.**

In conclusion for part 1, the features of planar cell polarity include appearance and organization of basal bodies and cilia. Basal bodies appear postnatally, first in random orientations, then gradually migrate to their final position in the ventral region of the cell. Vangl2 aligns ventrally from its initial uncoordinated position. Cilia also appear postnatally and beat in random directions until they gradually align and cluster to beat in a common ventral direction. We speculate that flow orients ependymal cilia and their basal bodies. Figure 14 depicts the proposed age alignment of cilia and basal bodies across 3 stages of mouse brain development.
Figure 14. The proposed development of ciliary and basal body Planar Cell Polarity from ages 3 to 11 days postnatally, including differentiation, cilia and basal body formation, and transition from disorganized to organized cilia and basal bodies. Black is Vangl2 marker, white dot indicates basal bodies, and red hairs are cilia on the ependymal surface.
Planar cell polarity is described in the literature as a final stage of ependymal maturation. Our observations are consistent with the literature: ependymal planar polarity is maturing, but still undergoing development at p11. At p8, we observed ependymal cells with basal bodies positioned far from the center however they lacked tissue wide angular polarity. This may suggest that these cells have basal bodies that are still in migratory flux to identify a singular positional orientation. By p11, basal bodies have acquired angular polarity to a common ventral orientation on the ependymal surface, but the translational migration away from the cell center showed great variability. At p11, some ependymal cells with angular basal body polarity lack distance from the cell center, while others showed both angular polarity and distance from the center. In mature mice, we’d expect this variability to decrease as slow-maturing cells eventually “catch up” to fast-maturing cells. In future studies, these changes could best be observed by live imaging.

**Part 2**

Culturing p8 ependymal cells in absence of CSF flow significantly reverses development of planar cell polarity. This includes migration of basal bodies towards the cell center, loss of cilia and their coordinated beating. P8 tissues are very sensitive to environmental changes.

Ependymal cells were cultured in-vitro to model the absence of CSF flow. Culturing ependymal cells in-vitro for 48 hours led to a statistically significant retraction of basal bodies towards the center of the cell. Comparison of basal body distances from the cell center on uncultured and 48 hour cultured ependymal cells reveals that culturing
ependyma 48 hours in absence of CSF flow significantly reduced the distance of basal bodies from the cell center (3.60 ± 1.39) compared to the acute p8 non-transgenic mouse (6.78 ± 1.20). A decrease in basal body distance from the cell center was also observed for 24-hour cultured ependymal cells (4.12 ± 1.49), but this was not statistically significant enough to establish correlation.

This result may indicate that ependymal cells in absence of the normal CSF flow show retraction of their basal bodies towards the cell center. However, the similarity in standard deviation indicates that there is still tissue-level alignment of basal bodies. Most or all cells are retracting basal bodies towards the cell center. The tissue’s normal asymmetrical planar polarity, required for propulsion of CSF flow, was lost.

We modulated the ependymal flow environment during a critical period of mouse brain development. It is important to note at this age (p8), planar polarity has not fully developed. This is evidenced by planar polarity differences in the age experiment. During this critical period of maturation, ependyma and other cells may be especially prone to physical modulations such as these.

Figure 15 shows a visual analysis of our result that culturing cells leads to a significant retraction of basal bodies towards the cell center. This is an asymmetrical loss of basal body position, an important feature of planar polarity. We might speculate that, if flow is directing basal bodies away from the cell center as we found in Part 1, that removing flow would have an opposite effect of directing basal bodies towards the cell center, as we see here.

In future studies, it will be important to understand whether certain cells are protected against modulation. We might speculate that older mice, with more mature
planar polarity, might show a less significant reduction of planar polarity. There is also a potential that fast-maturing ependymal cells, identified from Part 1, have greater protection from modulation.

**Flow Removal Induces Loss of Asymmetrical Planar Polarity**

![Diagram of Flow Removal Induces Loss of Asymmetrical Planar Polarity]

**Figure 15.** The proposed loss of planar cell polarity after 24 and 48 hours of ex-vivo culturing. Absence of fluid flow leads to movement of basal bodies towards the cell center, and gradual disorientation of ciliary beating.

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**Part 3**

*FoxJ1 drug suppression via treatment with IMD0354 leads to a significant loss of cilia on the ependymal surface.*

These results show that suppressing a biomolecule important for ependymal stability leads to a significant loss of cilia, and a slight loss in basal body planar polarity. Qualitative analysis of the suppressor-treated ependymal cells reveals extremely short or absent cilia. Quantifying the angular position of basal bodies shows IMD treated ependyma maintain ventral alignment, but have a larger variability compared to the untreated condition.
The ependymal cells treated with a FoxJ1 stabilizer, however, showed no significant difference in their ciliary expression from untreated. This result may show that FOXJ1 enhancers, such as MLN4924, stabilize ependymal cells and maintain features of planar cell polarity in developing cells.

Putting it Together

Taken together, these results indicate that immature ependymal cells are sensitive to physical and environmental modulation, namely loss of CSF and FOXJ1 expression. We first show evidence that p11 ependyma are immature, but developing signs of planar polarity through basal body asymmetry, basal body ventral localization, basal body colocalization with a planar cell polarity protein (vangl2), and observed coordinated clustering and alignment of cilia. Modulations to immature ependymal cells led to a loss of planar cell polarity, marked by reduced cilia expression and retraction of basal bodies towards the cell center.

These results are important because they provide in-vitro models of healthy tissue modulation. Oftentimes, CSF flow is lost after stroke and brain injury; thus, it is important to understand the effect of these modulations on ependymal cells, as we would see after these dysfunctions.

Understanding cilia and basal body alignment is important for the proper functioning of a variety of tissues that extend beyond the brain. Ciliary alignment is an important feature in tissues as diverse as the respiratory system, where cilia mediate flow of air and mucus (32), and the reproductive system, where cilia propel ova out of the fallopian tubes (33). Loss of cilia in the eye can lead to blindness. Dysfunctional cilia are involved in the development of polycystic kidney disease, affecting 12.5 million
people worldwide (34). Similar mechanisms of planar cell polarity are involved to align cells in these tissues. Thus, a ciliopathy, or a disease marked by dysfunctional cilia, can be devastating to the proper functioning of nearly all body systems (35).

**Future Directions:** Continue age-point analysis across the span of mouse development to track planar polarity.

Comparison was conducted only between p3, p8, and p11, when mice are still considered in their infancy. Thus, we are tracking development of planar polarity across immature ependyma. Change from disorganized to organized is well documented between an immature and mature ependymal cell, but few comparisons track the process of migration in very young mice.

In the mature mouse, all basal bodies eventually migrate to a similar tissue-and-region specific asymmetrical position far from the cell center. If we continue our age analysis in older mice, we would expect to see basal bodies migrating even further away from the cell center, with greater distance variability immediately after p11, and reduced distance variability at maturity. This would happen as slower cells “catch up” to faster migrating basal bodies. We would also expect to see continued reduction of angular variability, as basal bodies migrate to a similar region of the cell. Ultimately, in mature mice, we would expect a higher mean distance from the cell center, conserved angular position, and low angular and distance variability. These speculations must be explored through future experiments.

For the p8 time-point, when the tissue is still developing, culturing healthy tissue in absence of its normal environment leads to a reversal in the normal planar cell
polarity. Future studies can enhance these findings by mapping effects in older mice, to get a fuller understanding of planar polarity across development.

As indicated by our age analysis, we expect that the effect of culturing may differ by age. I would expect that a mature mouse with a fully developed tissue polarity would show more stability and resistance to environmental modulation. It may take longer, or be impossible, to lose planar polarity in fully developed tissue. This would indicate that mature cells are more resilient to change.

Another important question for future research is whether normal developing polarity can be reestablished when the tissue is removed from flow, then reintroduced? Such a study would require use of an in-vitro biomechanical machine which can propel fluid at the direction and the speed of CSF in the brain. Future researchers must also establish a mechanism to culture ependymal cells for 96 hours or more, without the effects of tissue deterioration.
References


Acknowledgements

Special thanks is extended to the mentors that made this research possible in the lab of Chay Kuo, MD, PhD. Research for Part 1 was mentored directly by Dr. Brent Asrican, PhD and Dr. Patri Paez-Gonzalez, PhD. Research for Part 2 was mentored directly by Dr. Khadar Abdi, PhD. Revision for this document was provided by Dr. Pelin Volkan, PhD in addition to the aforementioned laboratory mentors. Funding was provided by the Undergraduate Research Support Office and the Howard Hughes Summer Research Fellowship. The invaluable support of Dr. Ronald Grunwald, PhD was essential for these opportunities.