Video Article

The Subventricular Zone En-face: Wholemount Staining and Ependymal Flow

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Abstract

The walls of the lateral ventricles contain the largest germinal region in the adult mammalian brain. The subventricular zone (SVZ) in these walls is an extensively studied model system for understanding the behavior of neural stem cells and the regulation of adult neurogenesis. Traditionally, these studies have relied on classical sectioning techniques for histological analysis. Here we present an alternative approach, the wholemount technique, which provides a comprehensive, en-face view of this germinal region. Compared to sections, wholemounts preserve the complete cytoarchitecture and cellular relationships within the SVZ. This approach has recently revealed that the adult neural stem cells, or type B1 cells, are part of a mixed neuroepithelium with differentiated ependymal cells lining the lateral ventricles. In addition, this approach has been used to study the planar polarization of ependymal cells and the cerebrospinal fluid flow they generate in the ventricle. With recent evidence that adult neural stem cells are a heterogeneous population that is regionally specified, the wholemount approach will likely be an essential tool for understanding the organization and parcellation of this stem cell niche.

Video Link

The video component of this article can be found at http://www.jove.com/video/1938/

Protocol

I. Preparation of Glass Micropipettes Filled with Fluorescent Microbeads for Ependymal Flow Assay (these steps may be skipped if preparing wholemounts for staining purposes only).

1. Secure a Wiretrol 5 ul glass capillary tube onto glass micropipette puller and adjust heater and solenoid settings to pull pipette with a smooth, shallow taper.
2. Attach a source of positive air pressure onto the end of the pulled micropipette and gently lower the tip of the pipette onto a metal grating surface at a 45° angle to create a beveled tip. Positive air pressure helps to clear glass debris from the inside of the pipette. Clean the end of the beveled tip with an ethanol-moistened tissue.
3. Examine the tip of the pipette under a microscope with a micrometer. The tip should have a smooth bevel with an internal opening diameter of ~100 um. Smaller diameters may be used but often result in clogging of the pipette with fluorescent beads.
4. Backfill pipette with mineral oil until half-full, then insert grease-dipped plunger into back of pipette. Advance meniscus of mineral oil to the pipette tip by manually pushing in the plunger.
5. Secure the micropipette and plunger onto a micromanipulator, then screw the micromanipulator pipette holder onto a small stationary arm with adjustable height.
6. Frontload the pipette with the fluorescent microbead solution, consisting of 50% fluorescent microbead stock solution, 45% water, and 5% glycerol. Glycerol is added to increase the density of the solution so that when deposited onto the wholemount the microbeads sink onto the surface.
7. Place the micromanipulator in a safe place where the needle will not be accidentally broken and proceed with wholemount dissection.

II. Wholemount Dissection and Fixation

1. To prepare for wholemount dissection, warm sufficient quantity of L-15 Leibovitz media to 37°C. You will need approximately 10 ml per animal you plan to dissect. Also gather all supplies you will need for dissection and fixation by the stereomicroscope: scissors, toothed large forceps, smooth fine forceps, Sharpoint 22.5° microsurgical stab knife, dissecting dish, paper towel, biohazard bag, and a 24 well plate on ice filled with 4% paraformaldehyde with or without 0.1% Triton X-100. Triton X-100 is used to decrease the surface tension of the PFA solution, which decreases the incidence of shearing the wholemount surface when immersing it in this solution.

2. Pour 5-10 ml of the warmed media into a dissecting dish placed under a fluorescent stereomicroscope. Dissecting dishes are prepared by pouring an elastic polymer, called Sylgard 184, into a 6 cm plastic dish and letting the polymer solution cure for 1 week under vacuum, then thoroughly rinsing dishes in large volumes of water before use. Usually, we let the dishes soak in water in a 1 L beaker for 1 week.

3. The animal is sacrificed by cervical dislocation and the head is cut off.
   Note: If desired, blood may be cleared from the vasculature by perfusing the animal with normal saline prior to dissecting out the brain. This is particularly important if performing chromogenic immunostaining with diaminobenzidine (DAB).

4. A midline incision is made, posterior to anterior, along the scalp to reveal the skull.

5. A series of 4 cuts in the skull are made to open the cranium: one cut spans the two orbits anterior to the olfactory bulbs, the next two cuts are inferior to the cerebellum and separate the cranium from the skull base, and the final cut runs posterior to anterior along the mid-sagittal suture.

6. The cranial flaps are gently retracted and the brain is extracted and placed into the dissecting dish.

7. The remainder of the dissection is performed under the stereomicroscope. First, the olfactory bulbs are dissected away from the brain. If you wish additionally to examine the olfactory bulbs, simply fix them by immersion in 4% PFA overnight and you may subsequently prepare them for sectioning and staining.

8. Divide the brain along the interhemispheric fissure.

9. A coronally-oriented cut is then made at the posterior-most aspect of the interhemispheric fissure, allowing the caudal hippocampus to be visualized in cross-section.

10. The hippocampus, which forms the medial wall of the lateral ventricle at this position, must then be released from the overlying cortex, which forms the dorsal-lateral wall of the ventricle. First, the knife is inserted into the small ventricular space between the cortex and hippocampus dorsally, and a cut is made in the cortex where it reflects ventrally, away from the midline, to join the hippocampus. After this cut is made, the cortex can be slowly peeled away from the hippocampus to reveal the lateral ventricle moving from dorsal to ventral. This maneuver is expedited by cutting off a wedge of cortex at the corner where the hippocampus was released. After reaching the ventral-most extent of the lateral ventricle at this position, you may either visualize or feel where the cortex again wraps around, this time reflecting back medially, to join the hippocampus. Another cut must be made in this position to completely release the hippocampus or medial wall of the lateral ventricle from the cortex or lateral wall of the lateral ventricle.

11. It will then be easy to pull the hippocampus away from the cortex, medially and anteriorly, to open the lateral ventricle widely.

12. Continue to gently pull the hippocampus anteriorly using small strokes of the forceps and knife to retract the medial and lateral walls apart.

13. Once the resistance to this retraction begins to increase, additional cuts are needed. First, to increase your exposure to the lateral ventricle and in particular, the lateral wall and SVZ, dissect away the cortex. The cortex is cleanly dissected away by visualizing the interface between the corpus callosum and the VZ/SVZ. Simply cut along this interface staying on the callosal side to avoid damaging the SVZ.

14. In order to continue retracting the medial wall away from the lateral wall, two more cuts are needed: one cut dorsally where the lateral wall, medial wall, and cortex all converge, and one cut ventrally where the lateral wall, medial wall, and thalamus converge. With these cuts made, further gentle retraction on the medial wall allows the anterior-most extent of the lateral ventricle to be opened.

15. Proper lighting is essential throughout the procedure and especially in the next step where the medial and lateral walls are separated anteriorly. At this anterior position in the lateral ventricle, the medial wall reflects back and is continuous with the lateral wall. Adjust the lighting such that shadows cast between the two walls reveal this reflection point, which appears like a valley between the two walls. Cut exactly in this valley to separate these two walls.

16. Finally, completely expose the lateral wall by removing any overhanging cortex dorsally and the thalamus ventrally.

17. If preparing wholemounts for immunostaining, carefully transfer the wholemount, ventricle side up, from the dissection dish into a 24-well plate filled with 4% PFA with or without 0.1% Triton-X100 for an overnight fixation at 4°C. For fixation-sensitive antigens, wholemounts may be fixed for shorter periods of time. Then proceed to section 4 on immunostaining wholemounts.

18. If preparing wholemounts for ependymal flow analysis, transfer the wholemount to a clean dissection dish filled with fresh, 37°C Leibovitz medium and proceed to the next section.

III. Ependymal Flow Analysis using Fluorescent Microbeads

1. Immobilize the wholemount on a clean dissecting dish using 2 insect pins, one in the thalamus and one in anterior-dorsal corner of the wholemount.

2. Place the base of the stationary arm holding the micromanipulator next to the stereomicroscope. Make sure the height of the adjustable arm is maximally elevated to avoid breaking the needle against the dissection dish.

3. Carefully dip the tip of the needle in media in an ependorf tube to clean off microbeads that are present on the exterior tip of the needle. If these are not cleaned away, they may subsequently be deposited on the wholemout surface inadvertently during needle positioning and reduce the overall quality of the movie.

4. Position the needle tip over the dorsal surface of the lateral wall and lower the arm to bring the needle tip into the medium. The needle should be lowered until it is just above the lateral wall surface.

5. With the needle in position, adjust the zoom and focus on the stereomicroscope to cover a desired field. If you will be making a recording of the ependymal flow, begin acquiring images at this time.

6. Eject ~5 nl of the microbead solution onto the surface of the wholemount. Once the initial bolus of beads has been cleared off the surface by ependymal flow, additional rounds of bead ejection can be performed.

IV. Immunostaining Wholemounts

1. Wholemounts dissected for immunostaining are immersion-fixed overnight in 4% PFA with or without 0.1% Triton-X100 at 4°C. The use of Triton-X100 is preferred for antigens that tolerate this treatment, but can be left out in cases where staining quality is diminished by detergent.

2. The following morning, PFA is aspirated from the 24-well plate and the wholemounts are washed 3 times for 5 minutes each in 0.1 M PBS with or without 0.1% Triton-X100. As before, the use of Triton-X100 is preferred, but not required, for all washes in this protocol. Throughout this protocol, exchanging solutions over the wholemount requires careful aspiration of the solution from the side of the well. Then, the well is refilled with solution using a transfer pipette angled such that the solution washes over the side of the well, not directly onto the wholemount. Take care to keep the ventricle side of the wholemount facing up at all times. Vigorous pipetting of solutions will often flip the wholemount. We prefer to exchange solutions over 1 wholemount at a time to prevent tissue from drying.

3. After washing off the PFA, wholemounts are incubated for 1 hour at room temperature in blocking solution, containing 10% normal goat or donkey serum in 0.1 M PBS with or without Triton-X100. If using Triton-X100 for your staining, you may choose to use either 2% or 0.5% Triton-X100 in the blocking solution. We use 2% Triton-X100 when staining for antigens that require deeper antibody penetration into the tissue, such as those antigens located in the SVZ. However, when staining for antigens located closer to the surface of the lateral wall, such as antigens found in the apical surface of ependymal cells, we use 0.5% Triton-X100. In addition, Triton-X100 can be left out for cell-surface or other antigens that are removed or altered by detergent.

4. Next, remove the blocking solution and add primary antibodies diluted in the same blocking solution and incubate for 24 or 48 hours at 4°C. Choice of the incubation period depends on the antigen, similar to choice of 0.5% or 2% Triton. For antigens located on the surface of the wholemount, 24 hour incubation suffices. However, for antigens located deeper, such as in the SVZ, 48 hour incubation periods provide better results.
   For example, to study the apical surface and basal bodies of cells lining the lateral ventricle wall, stain with antibodies to β-catenin, to label the cell membrane, and γ-tubulin, to label basal bodies. Dilute mouse anti-β-catenin antibodies (1:500) and rabbit anti-γ-tubulin antibodies (1:1000) in 0.1 M PBS containing 10% normal goat serum and 0.5% Triton-X100. Incubate at 4°C for 24 hours.
   To stain the adult neural stem cells, or type B1 cells, stain the lateral wall with GFAP antibody. Dilute mouse anti-GFAP antibodies (1:500) in 0.1 M PBS containing 10% normal goat serum and 2% Triton-X100. Incubate at 4°C for 48 hours.

5. Primary antibodies are washed off initially by 2 quick rinses in PBS with or without 0.1% Triton-X100. Then do 3 additional washes for 20 minutes each at room temperature.

6. Dilute secondary antibodies in the same blocking solution used for primary antibodies and add to wholemounts to incubate for the same length of time as for primary antibodies at 4°C.
   For example, for staining for β-catenin and γ-tubulin: dilute Alexa Fluor 488 goat anti-mouse antibodies (1:400, recognizes mouse anti-β-catenin) and Alexa Fluor 594 goat anti-rabbit antibodies (1:400, recognizes rabbit anti-γ-tubulin) in 0.1 M PBS containing 10% normal goat serum and 0.5% Triton-X100. Incubate at 4°C for 24 hours.
   For GFAP immunostaining: dilute Alexa Fluor 488 goat anti-mouse antibodies (1:400, recognizes mouse anti-GFAP) in 0.1 M PBS containing 10% normal goat serum and 2% Triton-X100. Incubate at 4°C for 48 hours.

7. Secondary antibodies are washed off the wholemount using the same washes performed for primary antibodies.

8. If desired, nuclear counter-staining can be performed at this point by incubating in DAPI diluted in PBS for 30 minutes at room temperature and then washing one time in PBS.

V. Mounting Immunostained Wholemounts onto Slides for Confocal Microscopy

1. For high-resolution confocal imaging, following immunostaining the wholemounts needed to be sub-dissected to preserve only the lateral wall of the lateral ventricle as a sliver of tissue 200-300 um thick. Separating the lateral wall from the underlying striatum allows it to be mounted onto a slide and covered with a coverslip in a flat manner.

2. Return to the stereomicroscope with immunostained wholemounts and the following tools and equipment: smooth fine forceps, Sharpoint 22.5° microsurgical stab knife, dissecting dish, microscope slides and coverslips, 0.1 M PBS, and Aquamount mounting medium.

3. Transfer the wholemount from the 24-well plate to the dissecting dish containing 0.1 M PBS being careful to keep the ventricle side up.

4. First, completely remove the dorsal cortex of the wholemount from posterior to anterior by cutting precisely along the line where the corpus callosum meets the lateral wall. This is recognized as the interface between the callosal white matter and the pink-appearing SVZ.

5. Then make a long horizontally-oriented cut across the ventral aspect of the wholemount. This cut surface will provide a platform onto which you can stabilize the wholemount during the next step in the dissection.

6. With the dorsal surface of the wholemount facing up, you will be able to visualize the thickness of the SVZ from anterior to posterior along the lateral wall. Note that this view was made possible by the intial removal of the cortex allowing the underlying striatum and SVZ to be seen. The SVZ is identified as the thin band of tissue extending from the ventricular surface to the striatum. The SVZ has a homogeneous pink appearance while the striatum is infiltrated by cords of white matter. Note that the SVZ is thicker anteriorly and becomes progressively thinner posteriorly.

7. Once you have identified the interface of the SVZ and striatum, carefully begin cutting at this interface at the anterior-most aspect of the lateral wall, advancing the knife from dorsal to ventral. To do this accurately, stabilize the wholemount with your forceps used as two pins. You can also use your forceps to slightly turn the wholemount to visualize the blade advancing from dorsal to ventral across the lateral wall. The key to this dissection is for the resulting sliver of tissue to be very flat. This means that as you slice off the SVZ from anterior to posterior across the lateral wall, the orientation of the cuts you make must remain parallel to the ventricular surface at all times.

8. Remember that more posteriorly, the SVZ becomes thinner. Rather than thinning out your dissection to cut off only the SVZ at this point, it is important that as you advance posteriorly, the thickness of the tissue being dissected remains the same. This will ensure that the sliver of tissue you will subsequently mount is flat.

9. After completely separating the lateral wall from the underlying striatum, carefully remove all other surrounding tissues from this sliver that are not part of the ventricular wall.

10. Then pick up this sliver from below using your forceps and position it in the center of a microscope slide. Apply a few drops of aquamount directly onto the wholemount and gently place a coverslip centered over this, trying not to introduce bubbles onto the surface of the tissue. The weight of the slide will ensure that the aquamount disperses evenly and will produce a refined flattening of the lateral wall surface. The

amount of aquamount used and the size of the coverslip depend on the age of the tissue being dissected. For embryonic and early postnatal tissues, we prefer 1 drop of aquamount and a 22" x 30" coverslip. Heavier coverslips may distort the tissue and more aquamount will interfere with imaging quality because the confocal lasers will be less able to penetrate a thin layer of aquamount residing between the coverslip and the tissue surface. For later postnatal and adult tissues, we use 4 drops of aquamount and a 24" x 60" coverslip.

11. The slides are then stored flat in a slide book at 4°C for 1-2 days before imaging to allow the coverslips to settle.

Representative Results

Wholemount approaches have provided several key insights into the germinal activity of the adult SVZ. The network of chains of migrating young neurons in the SVZ was first observed after wholemounts of the lateral wall of the lateral ventricle were immunostained with antibodies to polysialylated neural cell adhesion molecule (PSA-NCAM) [1]. These chains of migrating neuroblasts can also be seen after immunostaining wholemounts with doublecortin antibodies (**Figure 1**). Remarkably, the network of chains has a stereotyped pattern, with two general streams of cells, one running dorsally over and one running ventrally around the adhesion point. Wholemounts of the SVZ also provide a comprehensive view of the proliferative activity of progenitors in this region, as seen with Ki67 staining in **Figure 2**. Interestingly, two recent studies suggest a close interaction between dividing SVZ cells and the local vasculature [2,3] (**Figure 2**).

When examined under high power confocal microscopy, the en-face view provided by wholemounts allows a unique perspective of the apical surface of cells lining the ventricular system. This en-face perspective has recently revealed that SVZ type B1 cells, the adult neural stem cells, are part of a mixed neuroepithelium with non-dividing differentiated ependymal cells [4]. The apical surface of type B1 cells contacts the lateral ventricle and is surrounded by large apical surfaces of ependymal cells in a pinwheel configuration (**Figure 3**, arrows indicate B1 apical surfaces). Furthermore, close examination of the apical surface of ependymal cells has revealed that the translational position and rotational orientation of their basal bodies are indicators of their planar polarity [5]. Ependymal cell basal bodies are clustered in a patch on the apical surface. This patch is displaced from the center of the apical surface in the downstream" direction with respect to CSF flow (translational polarity); within this patch, each basal body is rotated about its long axis such that the basal foot, an accessory of the basal body, points in the direction of flow (rotational polarity). Neighboring ependymal cells have their basal bodies oriented in the same direction. Importantly, videomicrographs of the ependymal flow assay can be used to directly compare the flow in a specific region of the lateral wall to the orientation of ependymal cell basal bodies in that region (**Figure 4**).

In addition to providing a panoramic perspective of the largest germinal region in the adult brain, with higher power imaging, wholemounts allow a more complete and detailed analysis of individual cellular morphologies in the SVZ. High power confocal imaging of GFAP immunostaining on wholemounts has revealed that type B1 cells, in addition to their short ventricle-contacting apical process, have a long basal process in contact with blood vessels (**Figure 5**) [4]. This cytoarchitecture had not been appreciated previously in coronal sections because the basal process runs mostly parallel to the ventricular wall. Serial sectioning therefore cuts individual cells into small fragments, making it nearly impossible to reconstruct a cell s complete morphology, or to understand its relationship to other cell types in the SVZ. The wholemount approach has several advantages over classical sectioning techniques, both in providing panoramic views with low power microscopy and a complete perspective of individual cells with high power microscopy. This technique will continue to be an important complement to future studies of this adult brain germinal zone.
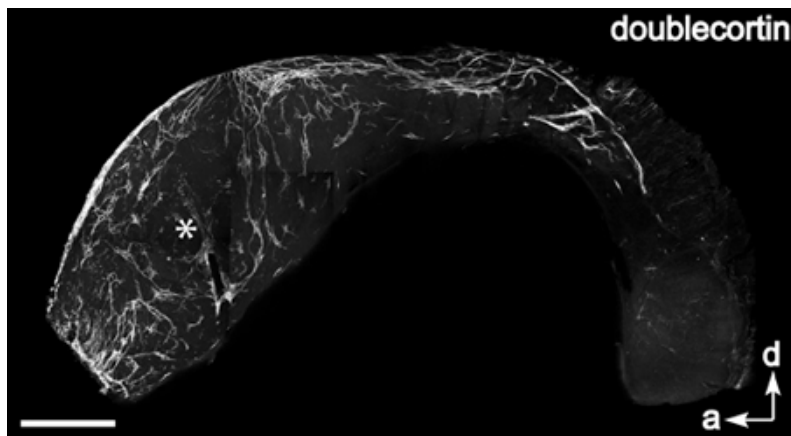

Figure 1. Network of migratory neuronal chains in the SVZ. Tiled confocal images reconstruct a lateral wall wholemount that was stained with antibodies to doublecortin, which labels migrating neuroblasts throughout the SVZ. There are two general streams of migration, one running dorsally over and one running ventrally around the adhesion point, indicated by the asterisk (*). Arrows indicate anterior (a) and dorsal (d) directions. Scale bar = 1 mm.
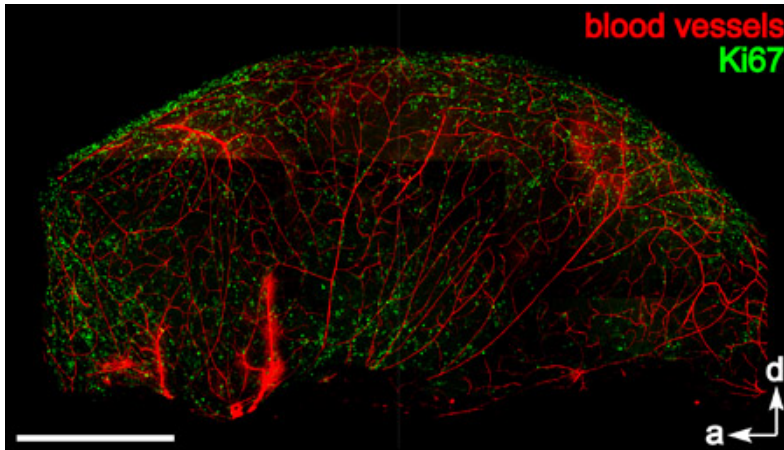
Figure 2. Relationship between the vasculature and dividing cells in the SVZ. This lateral wall wholemount was immunostained with antibodies for Ki67, to label dividing cells in green, and antibodies against mouse immunoglobulins, to label the vasculature in red. Because this wholemount was not perfused with saline prior to staining, the endogenous mouse IgG molecules remain within blood vessels and are stained by secondary anti-mouse antibodies. Recent work suggests that dividing SVZ precursors (green) are located in close proximity to blood vessels (red) {Shen, 2008 #6523}{Tavazoie, 2008 #6522}. Arrows indicate anterior (a) and dorsal (d) directions. Scale bar = 1 mm.
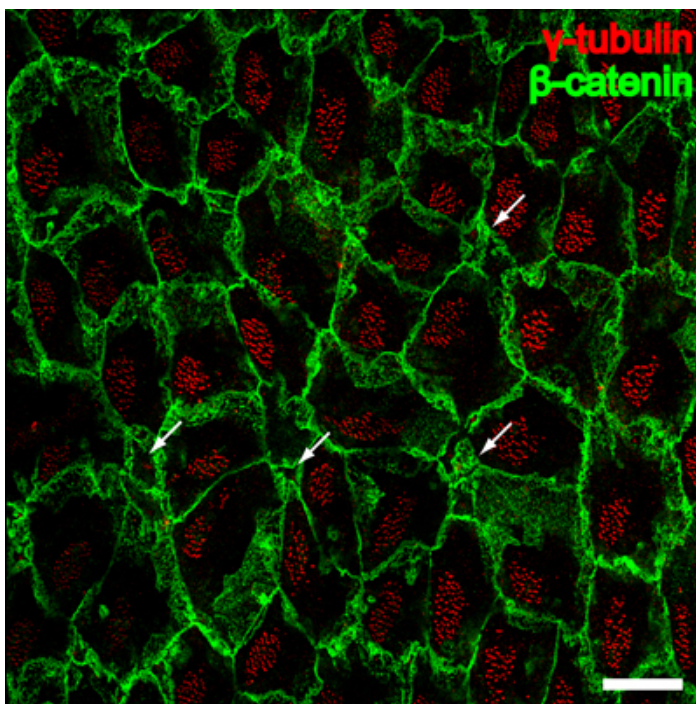

Figure 3. The apical surface of ventricle-contacting cells on the lateral wall. High power confocal image of a wholemount immunostained for β-catenin, to label cell membranes in green, and γ-tubulin, to label basal bodies in red, reveals the planar organization of these epithelial cells. Type B1 cells, the adult neural stem cells, have a small apical surface with a single basal body, indicated by arrows. The apical surface of these cells is surrounded by the large apical surface of ependymal cells in a pinwheel configuration. Ependymal cells have planar polarity indicated by the position of their multiple basal bodies on the apical surface. Neighboring ependymal cells have their basal body clusters located on the same side of the apical surface (downward and leftward in this region), corresponding to the direction of CSF flow {Mirzadeh, 2010 #6573}. Scale bar = 10 μm.
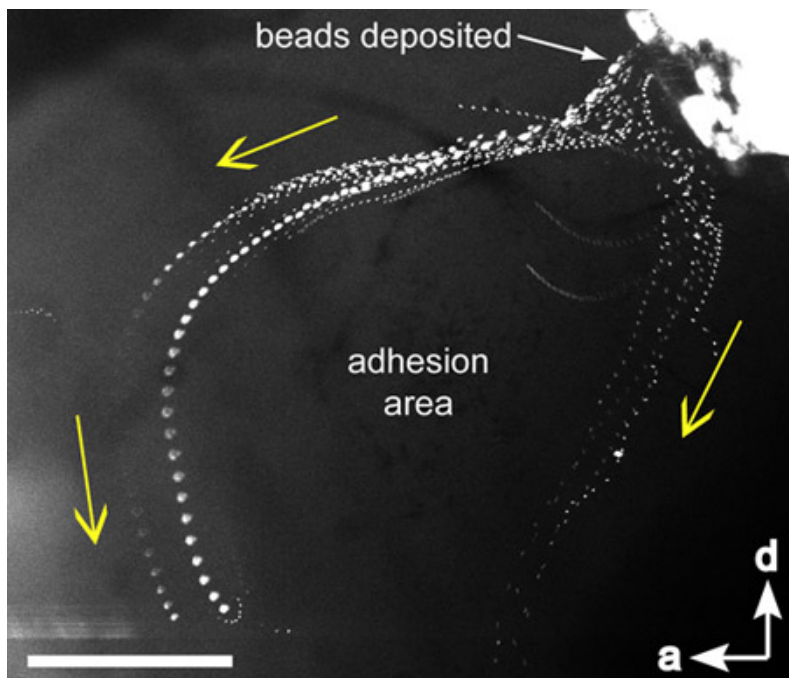
Figure 4. The ependymal flow assay. Composite image created by merging 100 sequential frames from a movie taken during the ependymal flow assay. Fluorescent microbeads deposited dorsal and posterior to the adhesion area were propelled by ependymal cilia in two oriented streams, one over and one under the adhesion, towards the foramen of Monro. This oriented flow reveals the functional planar polarity of ependymal cells. Each flow line depicts the position of a single bead at consecutive points in time. Scale bar = 0.5 mm.
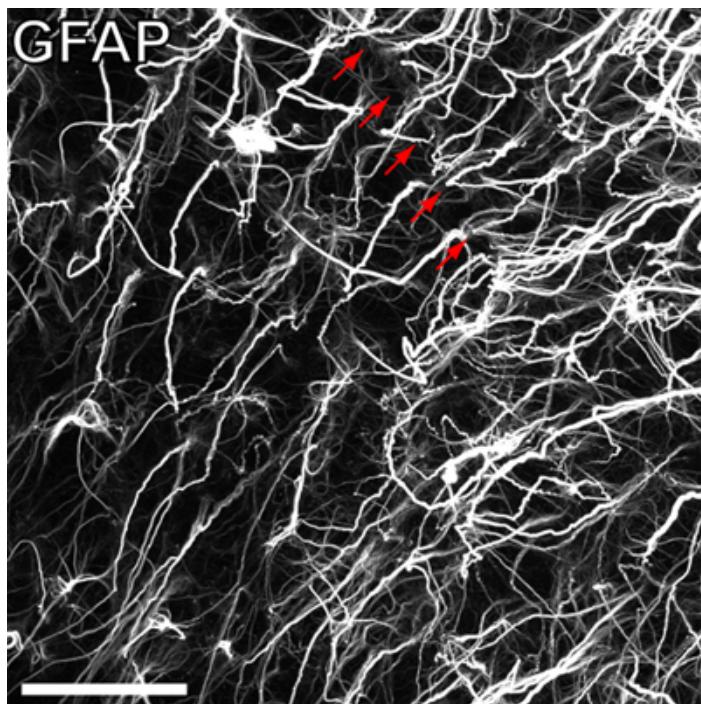

Figure 5. GFAP+ type B1 cells have a long basal fiber with end-feet on blood vessels. Maximum projection of a high power confocal stack taken from a lateral wall wholemount immunostained with GFAP antibodies to label SVZ astrocytes. This staining labels adult neural stem cells, or type B1 cells, which have an apical ending on the ventricular surface, and as shown here, a long GFAP+ basal fiber that ends on blood vessels (arrows). Blood vessels are stained here because the secondary antibody used to visualize mouse anti-GFAP antibodies recognize endogenous mouse IgG within the vasculature. Scale bar = 50 μm.

Discussion

Most studies of neurogenesis in ventricular and subventricular zones have relied on classical sectioning techniques to examine the microanatomy and cellular relationships in these regions. Here we describe an alternative technique, first used to analyze the network of

migratory chains of neuroblasts generated in the SVZ [1], then used to study regeneration of the SVZ progenitor population following anti-mitotic treatment [6], and most recently used to study the precise apical and basal cell-cell interactions of adult SVZ neural stem cells [2,3,4]. Interestingly, this technique has revealed that the neural stem cells, or type B1 cells, of the adult SVZ are part of a mixed neuroepithelium with differentiated non-dividing ependymal cells. En-face imaging using wholemounts has shown that this mixed neuroepithelium has pinwheel architecture consisting of the apical endings of type B1 cells surrounded by large apical surfaces of ependymal cells [4]. This en-face analysis has clarified our understanding of the lineage of neural stem cells in embryonic and adult brains as consisting of cells with apical endings at the ventricle surface and basal processes contacting a vascular niche. These findings would have been nearly impossible using classical sectioning techniques. Wholemounts also facilitate the identification of neural stem cells via their ventricle-contacting apical process. As more specific markers for these stem cells are found, wholemounts will be an integral part of identifying and analyzing neural stem cell behavior.

Wholemounts of the lateral ventricle walls also provide the ideal perspective for studying the planar polarity of ependymal cells. Ependymal cells are multiciliated cells lining the ventricles that function to propel CSF in a coordinated manner. With the wholemount technique, the entire ependymal epithelium is exposed en-face and can be stained and studied comprehensively from its anterior to posterior and dorsal to ventral boundaries. Furthermore, ependymal flow assays performed on acutely dissected, live wholemounts robustly demonstrate the planar polarized flow generated by ependymal cilia. Recent work using wholemount approaches has uncovered cellular determinants of this ependymal planar polarity [5]. Interestingly, wholemount studies have also suggested that ependymal-generated CSF flow establishes gradients of chemorepellents that guide the migration of young neurons in the SVZ [7]. Wholemount approaches that initially identified the network of migratory neuronal chains are therefore continuing to provide insights into mechanisms regulating chain migration.

Analysis of the VZ and SVZ by wholemount imaging adds a new approach for both future studies and a way to clarify our understanding of existing studies. For example, a recent study suggested that neural stem cells in the adult SVZ were CD133+/CD24- cells in contact with the ventricle [8]. Based on their immunostaining in sections, these authors claimed that these cells were a subpopulation of multiciliated ependymal cells. However, in our study using the wholemount approach, which gives a more comprehensive view of the entire ependymal epithelium, we found that all ependymal cells express CD24 and the only ventricle-contacting cells that were CD133+/CD24- were a subset of the type B1 cells [4]. Furthermore, the wholemount technique promises to be useful in future studies examining the recently described mosaic organization of neural stem cells in the adult brain [9]. Several studies have shown that neural stem cells in the adult brain are not a homogeneous population, but are regionally specified and normally produce only specific subtypes of olfactory bulb interneurons. These studies have proposed that different subpopulations of neural stem cells may be distinguished either by the expression of specific transcription factors [10,11,12,13,14] and/or by their regional localization along the dorsal-ventral and anterior-posterior extents of the lateral wall [9,15,16]. As more molecular markers of the regionally specified subpopulations of adult neural stem cells are identified, wholemount imaging should provide a comprehensive view of the parcelation of these different progenitor domains along the ventricular wall.

The wholemount dissection and imaging techniques presented here may also be used to analyze the ventricular walls in the embryo. The dissection of the embryonic lateral wall is performed, step-by-step, in the same manner. There are only slight differences in the level of difficulty; the embryonic ventricles are relatively larger making the dissection easier, but the tissue is softer making manipulation more difficult. In particular, a similar exposure of the lateral ventricle can be used in embryos to dissect the cortical wall of the ventricle to study cortical neurogenesis. Recent evidence suggests that asymmetric centrosome inheritance maintains radial glia at the ventricular surface during cortical neurogenesis [17]. En-face imaging of radial glial apical surfaces may provide insights into how centrosomes within these dividing cells are asymmetrically inherited.

As with most techniques, especially those involving precise dexterity, mastery requires practice. There are, however, a few elements in the dissection that are key to better results: 1) lighting adjusting the illumination of the sample to create shadows provides invaluable contrast during the dissection of tissue that is otherwise relatively homogeneous, 2) using the forceps like two insect pins the forceps in this technique are never used to pinch together or pick up tissue, but are used as maneuverable pins that can be continually readjusted to stabilize the tissue while cutting, 3) a balance of gentle retraction and cutting the knife should not only be used to cut but also to provide gentle retraction to separate the medial and lateral walls, remembering that the majority of this dissection is actually performed through gentle retraction with only intermittent cutting.

Acknowledgements

References

1. Doetsch, F. & Alvarez-Buylla, A. Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc. Natl. Acad. Sci. USA* **93**, 14895-14900 (1996).
2. Shen, Q. *et al.* Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* **3**, 289-300, doi:S1934-5909(08)00397-4 [pii] 10.1016/j.stem.2008.07.026 (2008).
3. Tavazoie, M. *et al.* A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* **3**, 279-288, doi:S1934-5909(08)00396-2 [pii] 10.1016/j.stem.2008.07.025 (2008).
4. Mirzadeh, Z., Merkle, F. T., Soriano-Navarro, M., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* **3**, 265-278 (2008).
5. Mirzadeh, Z., Han, Y. G., Soriano-Navarro, M., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Cilia organize ependymal planar polarity. *J Neurosci* **30**, 2600-2610, doi:30/7/2600 [pii] 10.1523/JNEUROSCI.3744-09.2010 (2010).
6. Doetsch, F., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Regeneration of a germinal layer in the adult mammalian brain. *Proc. Natl. Acad. Sci. USA* **96**, 11619-11624 (1999).

7. Sawamoto, K. *et al.* New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* **311**, 629-632 (2006).

8. Coskun, V. *et al.* CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain. *Proc. Natl. Acad. Sci. USA* **105**, 1026-1031 (2008).

9. Merkle, F. T., Mirzadeh, Z. & Alvarez-Buylla, A. Mosaic organization of neural stem cells in the adult brain. *Science* **317**, 381-384, doi:1144914 [pii] 10.1126/science.1144914 (2007).

10. Young, K. M., Fogarty, M., Kessaris, N. & Richardson, W. D. Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. *J Neurosci* **27**, 8286-8296, doi:27/31/8286 [pii] 10.1523/ JNEUROSCI.0476-07.2007 (2007).

11. Waclaw, R. R. *et al.* The zinc finger transcription factor Sp8 regulates the generation and diversity of olfactory bulb interneurons. *Neuron* **49**, 503-516 (2006).

12. Kohwi, M., Osumi, N., Rubenstein, J. L. & Alvarez-Buylla, A. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. *J Neurosci* **25**, 6997-7003 (2005).

13. Kohwi, M. *et al.* A subpopulation of olfactory bulb GABAergic interneurons is derived from Emx1- and Dlx5/6-expressing progenitors. *J Neurosci* **27**, 6878-6891 (2007).

14. Hack, M. A. *et al.* Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci* (2005).

15. Ventura, R. E. & Goldman, J. E. Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. *J Neurosci* **27**, 4297-4302 (2007).

16. Kelsch, W., Mosley, C. P., Lin, C. W. & Lois, C. Distinct mammalian precursors are committed to generate neurons with defined dendritic projection patterns. *PLoS Biol* **5**, e300 (2007).

17. Wang, X. *et al.* Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. *Nature* **461**, 947-955, doi:nature08435 [pii] 10.1038/nature08435 (2009).