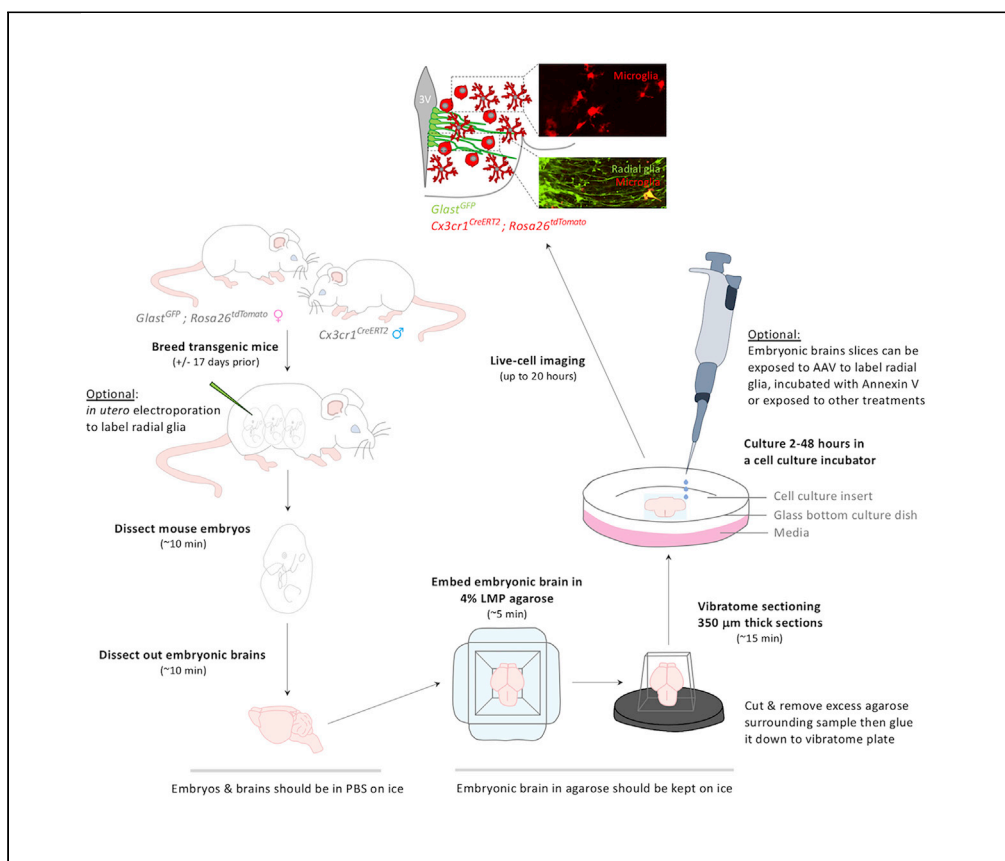


Protocol

Live-cell imaging of microglial interactions with radial glia in transgenic embryonic mouse brains using slice culture



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Highlights

Microglia and radial glia interactions can be captured in embryonic brain slices

Microglial dynamics and interactions with nearby cells can be visualized in real time

Live-cell imaging can be used to study various cell types in the developing brain

Microglial dynamics and interactions with nearby radial glia can be visualized in real time in embryonic mouse brain tissue using time-lapse imaging in slice culture. This live-cell imaging protocol can be used to study the morphology and activities of a number of cell types across a variety of brain regions and developmental time points. The advantage of this brain slice culture model is that it allows for the visualization of cellular interactions and movements in real time, especially across embryogenesis.

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Protocol

Live-cell imaging of microglial interactions with radial glia in transgenic embryonic mouse brains using slice culture

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SUMMARY

Microglial dynamics and interactions with nearby radial glia can be visualized in real time in embryonic mouse brain tissue using time-lapse imaging in slice culture. This live-cell imaging protocol can be used to study the morphology and activities of a number of cell types across a variety of brain regions and developmental time points. The advantage of this brain slice culture model is that it allows for the visualization of cellular interactions and movements in real time, especially across embryogenesis.

For complete details on the use and execution of this protocol, please refer to Rosin et al. (2021).

BEFORE YOU BEGIN

The protocol below describes the specific steps used to visualize microglial interactions with radial glia in the embryonic day 15.5 (E15.5) murine hypothalamus (Rosin et al., 2021). This protocol also has been used to visualize neuronal movements in the embryonic hypothalamus and microglia-radial glia interactions in the embryonic cortex, in addition to microglial dynamics across numerous embryonic time-points from E14.5 onwards. Aspects of the embryonic mouse brain live-cell imaging slice culture protocol are adapted from other organotypic slice culture models described elsewhere (Dailey and Waite, 1999; Daily et al., 2013; Falk et al., 2017; Nelson et al., 2013; Noctor 2011; Pilaz and Silver, 2014; Pilz et al., 2013; Shitamukai et al., 2011; Wiegreffe et al., 2017).

Breeding mice

⌚ Timing: 17 days prior to E15.5 dissection and slice culture experiment

1. Use a *Glast*^{GFP} transgene to label radial glia with GFP:
 - a. Cross a *Glast*^{GFP}; *Rosa26*^{tdTomato} double transgenic mouse to a *Cx3cr1*^{CreERT2} transgenic mouse to label microglia (i.e., *Cx3cr1*-expressing cells) with tdTomato. Plug check daily, as the morning a vaginal plug is detected will represent E0.5.
 - i. Cre recombinase can be induced using 1 mg of 4-Hydroxytamoxifen dissolved in corn oil by warming the corn oil to 37°C and sonicating (store as 100 μL aliquots of corn oil containing 0.5 mg 4-Hydroxytamoxifen at -20°C for up to 6 months). Administer the



4-Hydroxytamoxifen intraperitoneally over two days (e.g., optimized for E11.5 and E12.5 injections if collecting brains between E14.5-E15.5) as two 0.5 mg doses (i.e., 100 μ L volume/dose).

OR

2. Use a *pCIG2* construct and *in utero* electroporation (IUE) to label radial glia with GFP:
 - a. Cross a *Rosa26^{tdTomato}* transgenic mouse to a *Cx3cr1^{CreERT2}* transgenic mouse and plug check daily. The morning a vaginal plug is detected will represent E0.5.
 - i. Cre recombinase can be induced using 1 mg of 4-Hydroxytamoxifen dissolved in corn oil and administered intraperitoneally over two days (e.g., E11.5 and E12.5) as two 1.5 mg doses (please refer to details above).
 - b. At E14.5 the IUE protocol, described elsewhere (Dixit et al., 2011; Rosin and Kurrasch, 2018), is performed in order to label radial glia with GFP.

OR

3. Use Adeno-associated virus (AAV2-GFP control virus) to label radial glia with GFP:
 - a. Cross a *Rosa26^{tdTomato}* transgenic mouse to a *Cx3cr1^{CreERT2}* transgenic mouse and plug check daily. The morning a vaginal plug is detected will represent E0.5.
 - i. Cre recombinase can be induced using 1 mg of 4-Hydroxytamoxifen dissolved in corn oil and administered intraperitoneally over two days (e.g., E11.5 and E12.5) as two 0.5 mg doses (please refer to details above).
 - b. Follow the AAV2-GFP viral application as described below.

Note: While there are a variety of methods that can be employed to label embryonic radial glia (e.g., transgenic mouse lines, IUE, AAV, etc.), the toolkit available for labeling microglia is much more limited. Accordingly, here we described labeling microglia using the *Cx3cr1^{CreERT2}* transgenic mouse line. While this and other microglial transgenic lines can be employed for this purpose, other tools to label microglia are currently lacking in the field of microglia research.

Slice culture medium preparation and culture dish assembly

⌚ Timing: 1–2 h prior to E15.5 dissection and slice culture experiment

4. Prepare the medium for the slice culture experiment. Culture medium should be filtered and made fresh daily.

Culture medium		
Reagent	Percent composition	Amount
DMEM	56.4%	5.64 mL
F-12	28.2%	2.82 mL
FBS	5%	0.5 mL
Horse serum	5%	0.5 mL
B-27 supplement	2%	0.2 mL
N2 supplement	1%	0.1 mL
GlutaMAX	1%	0.1 mL
Penicillin/Streptomycin	1%	0.1 mL
Fungizone	0.4%	0.04 mL
Total	100%	10 mL

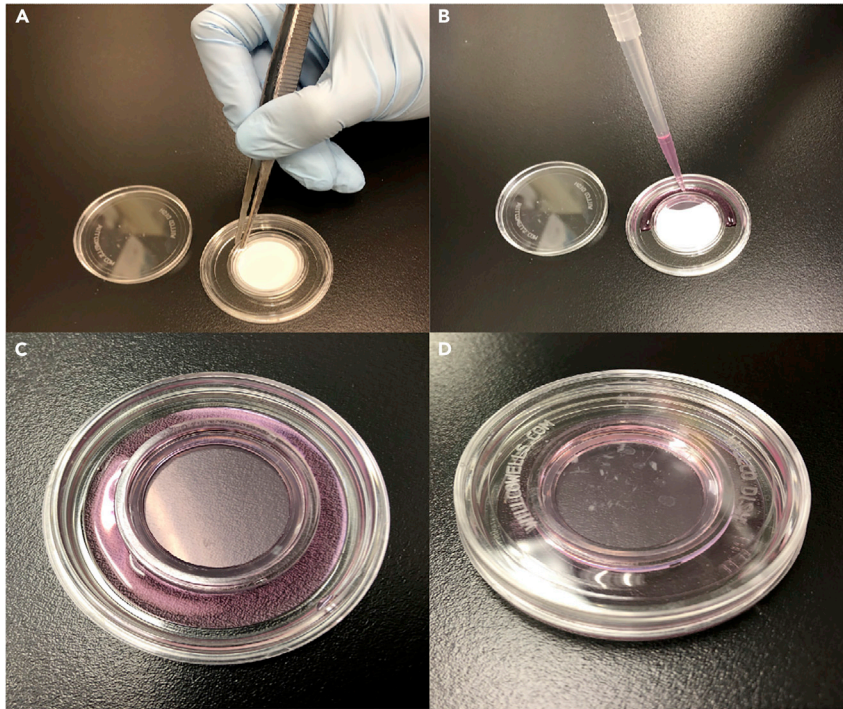


Figure 1. Culture dish setup

Place a 0.4 μm , 30 mm Millicell cell culture insert into a 40 mm glass bottom Petri dish (A), fill the glass bottom Petri dish with culture medium (B and C) and cover with lid (D) in preparation for incubation in a cell culture incubator.

5. Place a 0.4 μm , 30 mm Millicell cell culture insert into a 40 mm glass bottom Petri dish (Figure 1A), fill the Petri dish with 1.5 mL–2.0 mL of culture medium (Figures 1B and 1C), and place the covered Petri dish (Figure 1D) in a cell culture incubator with ambient oxygen and 5% CO_2 at 37°C to warm for at least 1 h prior to the start of the E15.5 dissection and slice culture experiment.

△ CRITICAL: The final volume of culture medium in the Petri dish will vary from 1.5 mL–2.0 mL depending on the embryonic stage used and the number of sections placed on the cell culture insert (i.e., maximum of three). When using this culture model, be sure to not use more than 2.0 mL of medium in the glass bottom Petri dish because it will cause the cell culture insert to lift up and move during imaging.

Confocal microscope setup

⌚ Timing: 1 h prior to starting live-cell imaging

6. Turn on the confocal microscope (e.g., Zeiss LSM 700 inverted confocal microscope).
 - a. Set the incubation temperature to 37°C to warm up the live-cell imaging chamber.
7. Open the gas tank (e.g., 5% CO_2 /40% O_2 balanced with nitrogen (Falk et al., 2017)) and turn the bubbler on so that the imaging chamber can equilibrate (i.e., appropriate gas composition and humidity).

Vibratome setup

⌚ Timing: 10–30 min prior to E15.5 dissection and slice culture experiment



Figure 2. Vibratome setup

The base surrounding the slicing chamber should be filled with ice and the slicing chamber should be filled with ice-cold PBS.

8. Turn on the vibratome (e.g., Leica VT1200 S vibratome), attach the components for fresh tissue (i.e., it is best to have a separate reservoir and blade holder for ‘fixed’ and ‘fresh’ tissue) and perform any alignment protocols that are necessary.
 - a. Following vibratome alignment, fill the base surrounding the slicing chamber with ice and pour ice-cold phosphate-buffered saline (PBS) into the slicing chamber (Figure 2).
 - b. In preparation for slicing, set the vibratome to slice the embryonic brain tissue at a thickness of 300–350 μm with a vibratome sectioning speed of 0.3–0.5 mm/s and an amplitude of 1.00 mm.

△ CRITICAL: The thickness of the brain tissue section is an important consideration. By sectioning embryonic brain slices between 300–350 μm , there is enough depth to image in the middle of the slice while also avoiding cells directly adjacent to the cut tissue. This is important for microglial studies since microglia located near where the tissue was sliced become more reactive and amoeboid in response to the tissue insult. In some experiments, sectioning the embryonic brain thicker than 350 μm might be advantageous for providing a larger imaging area but this thickness can also be a challenge for detecting weaker fluorescent signals and sometimes imaging quality. It is also important to note that the oxygen levels may fluctuate depending on the thickness of the slices and the age of the sample preparation (Jiang et al., 2013). Therefore, the optimal thickness is best decided on a case-by-case basis.

Low-melting point (LMP) agarose preparation

⌚ Timing: 5–10 min prior to E15.5 dissection and slice culture experiment

9. Prepare 4% LMP agarose using PBS (total volume of 4% LMP will depend on how many embryonic brain samples being prepared for the experiment).

Note: Preparing 50 mL of 4% LMP agarose in PBS is usually a good starting point for two embryonic brains. Do not microwave the agarose solution until the E15.5 dissection is finished.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Adeno-associated virus (AAV2-GFP) control virus	Cedarlane	Cat#AAV-302
Chemicals, peptides, and recombinant proteins		
Annexin-V conjugated to Alexa Fluor 647	Life Technologies	Cat#1964400
Annexin V	ImmunoTools	Cat#31490010

(Continued on next page)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
4-Hydroxytamoxifen	Sigma	Cat#H7904
UltraPure LMP (low melting point) agarose	Invitrogen	Cat#16520050
DMEM	Gibco, Thermo Fisher Scientific	Cat#11965-092
F-12	Gibco, Thermo Fisher Scientific	Cat#11765-054
Fetal bovine serum (FBS)	Gibco, Thermo Fisher Scientific	Cat#A3840001
Horse serum	Gibco, Thermo Fisher Scientific	Cat#26050-070
B-27 Supplement	Gibco, Thermo Fisher Scientific	Cat#17504-044
N2 Supplement	Gibco, Thermo Fisher Scientific	Cat#17502-048
GlutaMAX	Gibco, Thermo Fisher Scientific	Cat#35050-061
Penicillin and Streptomycin	Gibco, Thermo Fisher Scientific	Cat#15140-148
Fungizone	Gibco, Thermo Fisher Scientific	Cat#15290-018
HBSS	Gibco, Thermo Fisher Scientific	Cat#14025-092
PBS	Gibco, Thermo Fisher Scientific	Cat#10010-023
Hoechst 33342	Life Technologies	Cat#H3570
Experimental models: organisms/strains		
<i>Glast</i> ^{GFP} mice (<i>Glast1-EMTB-GFP</i>)	Dr. Eva Anton, University of North Carolina	N/A (gifted)
*transgenic mouse genetic background: C57BL/6J		
<i>Cx3cr1</i> ^{CreERT2} mice (B6.129P2(Cg)- <i>Cx3cr1</i> ^{tm2.1(cre/ERT2)Litt/WganJ})	The Jackson Laboratory	JAX: 021160
*transgenic mouse genetic background: C57BL/6J		
<i>Rosa26</i> ^{tdTomato} mice (B6.Cg-Gt(ROSA) <i>26Sox2</i> ^{tm14(CAG-tdTomato)Hze/J})	The Jackson Laboratory	JAX: 007914
*transgenic mouse genetic background: C57BL/6J		
Recombinant DNA		
Plasmid: <i>pCIG2</i>	Hand et al., 2005	N/A (gifted)
Other		
0.4 μm, 30 mm Millicell cell culture insert	Millipore	Cat#PICMORG50
40 mm Glass bottom petri dish	Ted Pella Inc.	Cat#14026-20
5% CO ₂ /40% O ₂ balanced with nitrogen gas tank	Praxair	N/A (custom order)
Peel-A-Way disposable plastic tissue embedding molds	Polysciences Inc.	Cat#18985-1
Zeiss LSM 700 inverted confocal microscope	Zeiss	N/A (custom order)
Leica VT1200 S fully automated vibrating blade microtome	Leica	N/A (custom order)

STEP-BY-STEP METHOD DETAILS

This section lists the major steps involved in the embryonic mouse brain slice culture protocol and provides step-by-step details and timing.

Mouse embryo dissection and brain extraction

⌚ Timing: 15–20 min

The goal of this step is to dissect embryos from a pregnant dam and remove several intact embryonic brains in a timely manner.

1. Anaesthetize the pregnant dam using isoflurane and immediately euthanize the animal using cervical dislocation.
 - a. Make an incision along the abdomen of the pregnant dam and remove the E15.5 embryos from both uterine horns.
 - b. Place the embryos in ice-cold PBS in a Petri dish sitting on ice.
2. One at a time, place a single embryo into a second Petri dish filled with ice-cold PBS under a dissecting microscope on the benchtop.
 - a. Cut off the embryo head and discard the body.

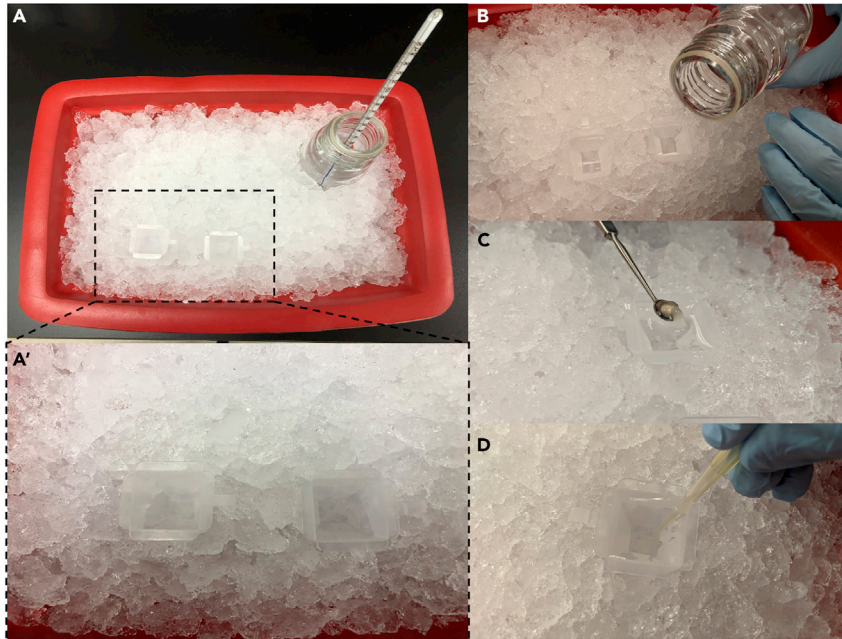


Figure 3. Embedding embryonic brains in LMP agarose

The bottle of 4% LMP agarose is placed on ice with a thermometer in the agarose to measure the temperature (A), and the Peel-A-Way disposable plastic tissue embedding molds are placed individually on ice (A'). Once at the appropriate temperature, the 4% LMP agarose can be poured into the plastic molds (B) and the embryonic brains can be transferred to (C) and appropriately positioned within (D) the agarose.

- b. Carefully harvest the E15.5 brain from the head using forceps and a dissecting spoon.
- c. Place the E15.5 brain in a Petri dish filled with ice-cold PBS and place back on ice.

Sample preparation and vibratome slicing

⌚ Timing: 15–20 min

The goal of this step is to mount the embryonic brain in agarose for stability and section the brain into thick sections in preparation for culturing and live-cell imaging.

3. Microwave the 4% LMP agarose until the agarose powder has dissolved and the solution is a clear homogenous liquid.
 - a. Place the bottle of 4% LMP agarose on ice with a thermometer in the agarose to measure the temperature (Figure 3A).
 - b. Place the plastic tissue embedding molds on ice (i.e., molds used to set the embryonic brains into the agarose; Figure 3A').
 - c. Pour the 4% LMP agarose into the plastic molds once the temperature is between 45°C to 55°C (Figure 3B).
4. Place the E15.5 mouse brain into the 4% LMP agarose within the plastic molds once the temperature is at 40°C (Figure 3C).
 - a. Use a pipette tip to quickly orient (e.g., the ventral aspect of the embryonic brain should face the bottom of the plastic mold if coronal sections are preferred) the brain in the 4% LMP agarose before it solidifies (Figure 3D).

⚠ **CRITICAL:** When the embryonic brain is transferred from the PBS to the 4% LMP agarose using a dissecting spoon, make sure that excess PBS is not transferred into the agarose

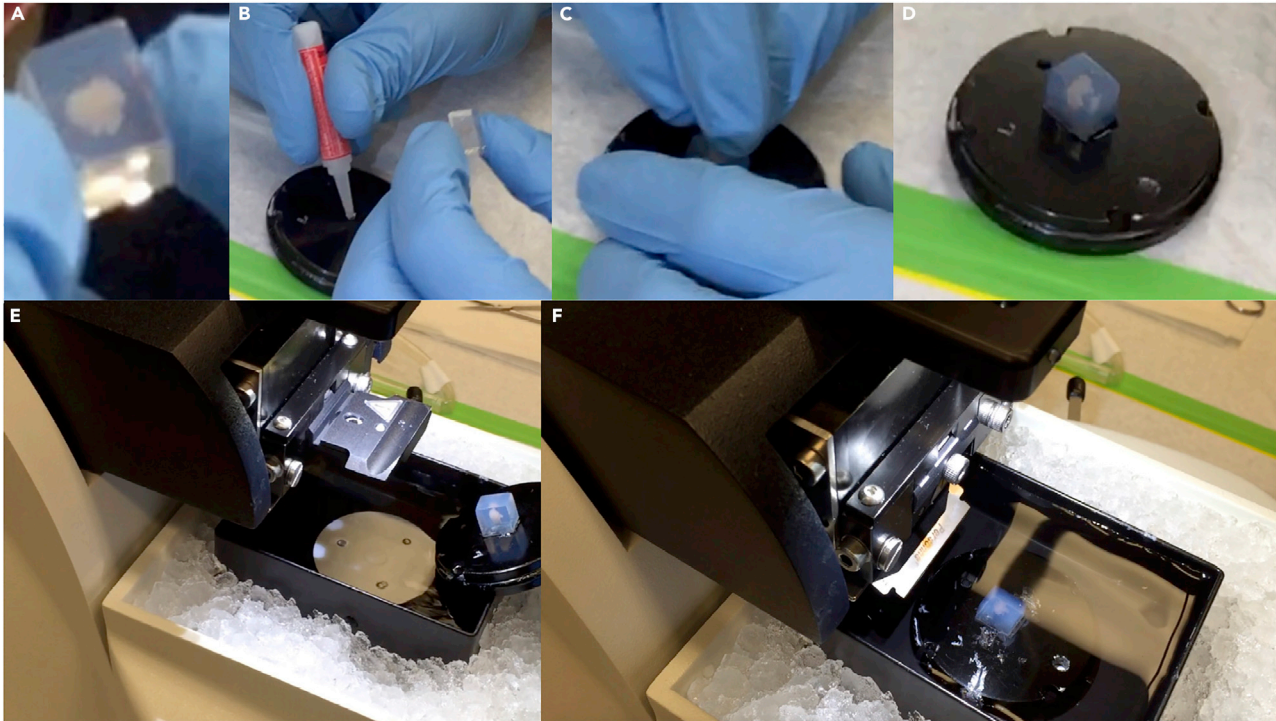


Figure 4. Sample preparation and vibratome Sectioning

Krazy glue is used to adhere the agarose embedded sample onto the metal vibratome sectioning plate (A–D), which is then placed into the vibratome tray containing ice-cold PBS (E and F).

alongside the brain. Once the embryonic brain is placed within the agarose, use the pipette tip to swirl the agarose around the brain to dilute any PBS that was adhering to the brain. Also, remove or pop any nearby bubbles. These steps are important to achieve the solid seal between the brain and the agarose that prevents the brain from detaching from the surrounding agarose during vibratome sectioning.

- b. Once the agarose has solidified, carefully tear apart the plastic mold and place the agarose embedded sample on a Petri dish on ice.
 - c. Cut the excess agarose surrounding the embryonic brain using a razor blade (Figure 4A).
 - d. Use a super glue such as Krazy Glue® to adhere the agarose-embedded brain onto the metal vibratome plate used for sectioning (Figures 4B–4D).
5. Attach the metal vibratome plate with the glued sample to the vibratome such that the sample is submerged in the vibratome reservoir containing ice-cold PBS and surrounded by ice (Figures 4E and 4F).
 - a. Use the vibratome to trim and section through excess agarose surrounding your sample and brain regions that are not of interest (e.g., section through a significant proportion of the embryonic cortex to reach the embryonic hypothalamus).
 6. Once you reach your brain region of interest, begin collecting the 300–350 μm vibratome slices using a brush and metal spatula (Figures 5A and 5B).
 - a. Place the 300–350 μm vibratome slices on the cell culture insert within the glass bottom Petri dishes containing the medium (Figures 5B and 5C). [Troubleshooting 1](#)
 - b. Use a pipette to remove any PBS that was transferred from the vibratome sectioning chamber to the cell culture insert (Figure 5D).
 - c. Add 20–40 μL slice culture medium to the top of each embryonic brain section (Figure 5E).

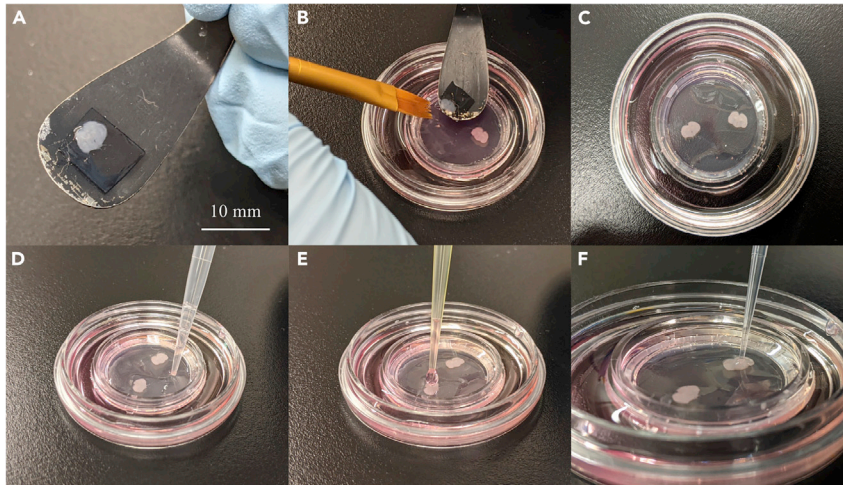


Figure 5. Placement, treatment and culturing of embryonic brain slices

Sliced embryonic brain sections are transferred from the vibratome sectioning chamber to a cell culture dish using a brush and spatula (A and B), where up to three embryonic brain sections can be placed on the cell culture insert (C). Excess PBS that was transferred from the vibratome chamber is aspirated using a pipette (D) and slice culture medium is added to the top of each embryonic brain slice (E). AAV viral transduction can be achieved using a pipette and localized application (F). Scale bar represents 10 mm (A).

Slice culture, viral transduction, and treatments

⌚ Timing: 2–48 h

The goal of this step is to culture the embryonic brain slice in a cell culture incubator for 2 h or longer, in order to allow the slice to rest and normalize following the trauma experienced during the slicing procedure. At this stage during the protocol, embryonic brain slices can be exposed to virus, treated with Annexin V or other compounds, or stained with Hoechst, for example.

- Place the embryonic brain slices atop the culture insert within the glass bottom Petri dish into a cell culture incubator with ambient oxygen and 5% CO₂ at 37°C for at least 1–2 h prior to imaging.

Note: Embryonic brain slices can remain in the incubator for up to 48 h prior to imaging; however, the slice culture medium should be changed daily.

Optional:

AAV Viral Transduction

In order to label radial glia and/or other cells with GFP (as per [Rosin et al., 2021](#)), 1–2 μL Adeno-associated virus (e.g., AAV2-GFP control virus) can be diluted in 5 μL slice culture medium and applied to the surface of the *Cx3cr1^{CreERT2}; Rosa26^{tdTomato}* vibratome-sectioned embryonic brain tissue in the region of interest (e.g., hypothalamus, [Figure 5F](#)), and placed in a cell culture incubator with ambient oxygen and 5% CO₂ at 37°C for 24–48 h before imaging.

Annexin V Treatment

Vibratome sectioned embryonic brain slices atop cell culture inserts within glass bottom Petri dishes can be treated with Annexin V to block externalized phosphatidylserine ([Brelstaff et al., 2018](#); [Rosin](#)



Figure 6. Tissue separation from agarose

Embryonic brain slices can separate from the surrounding agarose during vibratome sectioning and/or during the transfer from the vibratome sectioning chamber to the culture dish.

et al., 2021) after removing the slice culture medium. By blocking externalized phosphatidylserine, interactions between microglia and radial glia that result in radial glia breakage and phagocytosis by microglia, can be reduced (Rosin et al., 2021). Annexin V can also be used to detect apoptotic cells. Once the slice culture medium has been aspirated, a solution containing 7.5 μL Annexin V conjugated to Alexa Fluor 647 diluted in 1,132 μL HBSS and 375 μL 10 mM CaCl_2 can be placed in the glass bottom Petri dish and incubated at room temperature ($\sim 20^\circ\text{C}$ – 22°C) for 15 min in the dark. Following the incubation, the glass bottom Petri dish should be washed with slice culture medium twice, whereby the final solution added to the glass bottom Petri dish is 1.5 mL slice culture medium containing 5.4 μL Annexin V (100 $\mu\text{g}/100 \mu\text{L}$). The vibratome sectioned embryonic brain slices can then be placed in a cell culture incubator with ambient oxygen and 5% CO_2 at 37°C for 2–48 h before imaging.

Hoechst staining

Vibratome sectioned embryonic brain slices can be stained with Hoechst in order to visualize the nuclei of individual cells in the sliced brain during imaging, if visualizing the movement and interactions of all cells in the slice is of interest. Once the slice culture medium has been aspirated, 1.5 mL of a solution containing 1 μL Hoechst diluted in 7.5 mL of PBS can be applied to the glass bottom Petri dish, with at least 50 μL of the solution added to the brain slice itself, and incubated for 10–15 min in the dark. Following the incubation, the glass bottom Petri dish should be washed with PBS three times. Fresh slice culture medium can be added to the glass bottom Petri dish and the vibratome sectioned embryonic brain slices can then be placed in a cell culture incubator with ambient oxygen and 5% CO_2 at 37°C for 2–48 h before imaging.

Live-cell imaging and analysis

⌚ Timing: Up to 20 h

The goal of this step is to visualize microglial interactions with radial glia. Microglial dynamics and/or their interactions with other cell types can also be visualized in embryonic brain slices using this protocol by modifying the transgenic mouse lines and labeling tools employed.

8. For live-cell imaging, glass bottom Petri dishes containing the embryonic brain slices atop cell culture inserts can be placed in a 37°C heated and humidified imaging chamber and exposed to 5% $\text{CO}_2/40\% \text{O}_2$ balanced with nitrogen (Praxair) via a gas bubbling system (Figures 7A–7C).
9. Live-cell imaging can be achieved using a Zeiss LSM 700 inverted confocal microscope equipped with a 20 \times long working distance objective (air / 0.4 numerical aperture).
 - a. Z-stacks should be taken from the center (e.g., 30–100 μm) of the 300–350 μm slice using a pinhole of 1–2 AU, depending on the microscope being used and the fluorescent signal observed, with a Z-step size of 3–7 μm , again depending on the total Z-plane size.
 - b. Each Z-stack requires between 5–15 min to acquire, depending on the microscope being used and the parameters listed above; therefore, the slices should be given 5 min to rest between Z-stack acquisitions.
 - c. Laser power should be maintained around 2% to prevent photobleaching and toxicity.



Figure 7. Confocal microscope setup

The glass bottom Petri dish containing the vibratome sectioned embryonic brain samples is placed in the confocal microscope incubation chamber (A) and the glass imaging chamber lid is placed on top of the chamber bottom (B) before imaging on the confocal microscope (C) can begin.

- d. Imaging can be set to continue for any length of time, up to 20 h, depending on the conditions and parameters listed above.
10. If the live-cell imaging experiment is performed on a Zeiss microscope, the resulting live-cell imaging videos can be analyzed using ZENBlack software. [Troubleshooting 2](#)
 - a. If the time-lapse live-cell imaging video is acquired on a Zeiss microscope, ZENBlack software can be used to convert the resulting .czi file to a maximum intensity projection to merge the Z-stacks to generate a video that can be viewed and from which stills can be obtained ([Rosin et al., 2021](#)).
 - b. In the ZENBlack software program, the 3D function can be used on the original .czi video acquired to generate 3D renderings of microglial-radial glia interactions ([Rosin et al., 2021](#)).
 - c. Microglia numbers and microglial-radial glia interactions were quantified manually using the original .czi file acquired in ZENBlack using the Ortho function, which allowed for visualization of microglia wrapping their processes around radial glial projections ([Rosin et al., 2021](#)).

△ CRITICAL: Given the slice culture setup and distance between the glass bottom Petri dish and the embryonic brain slice atop the cell culture insert ([Figure 5C](#)), it is important to image the tissue on an inverted microscope that is equipped with a long working distance objective. Imaging with an objective that is not equipped to account for this additional distance will result in poor imaging quality and suboptimal resolution of the cells of interest. It is possible that these issues could be bypassed by imaging the embryonic brain slices using two-photon microscopy.

Note: While every effort should be used to maintain the laser power around 2% to prevent slice photobleaching and toxicity, fluorescence from the *Glast^{GFP}* transgene often appears fairly weak in embryonic brain slices, therefore, the laser power was set between 6%–8% during these experiments ([Rosin et al., 2021](#)), which did not show any obvious signs of impacting slice health. Again, it is possible that this issue could be bypassed by imaging the embryonic brain slices using two-photon microscopy.

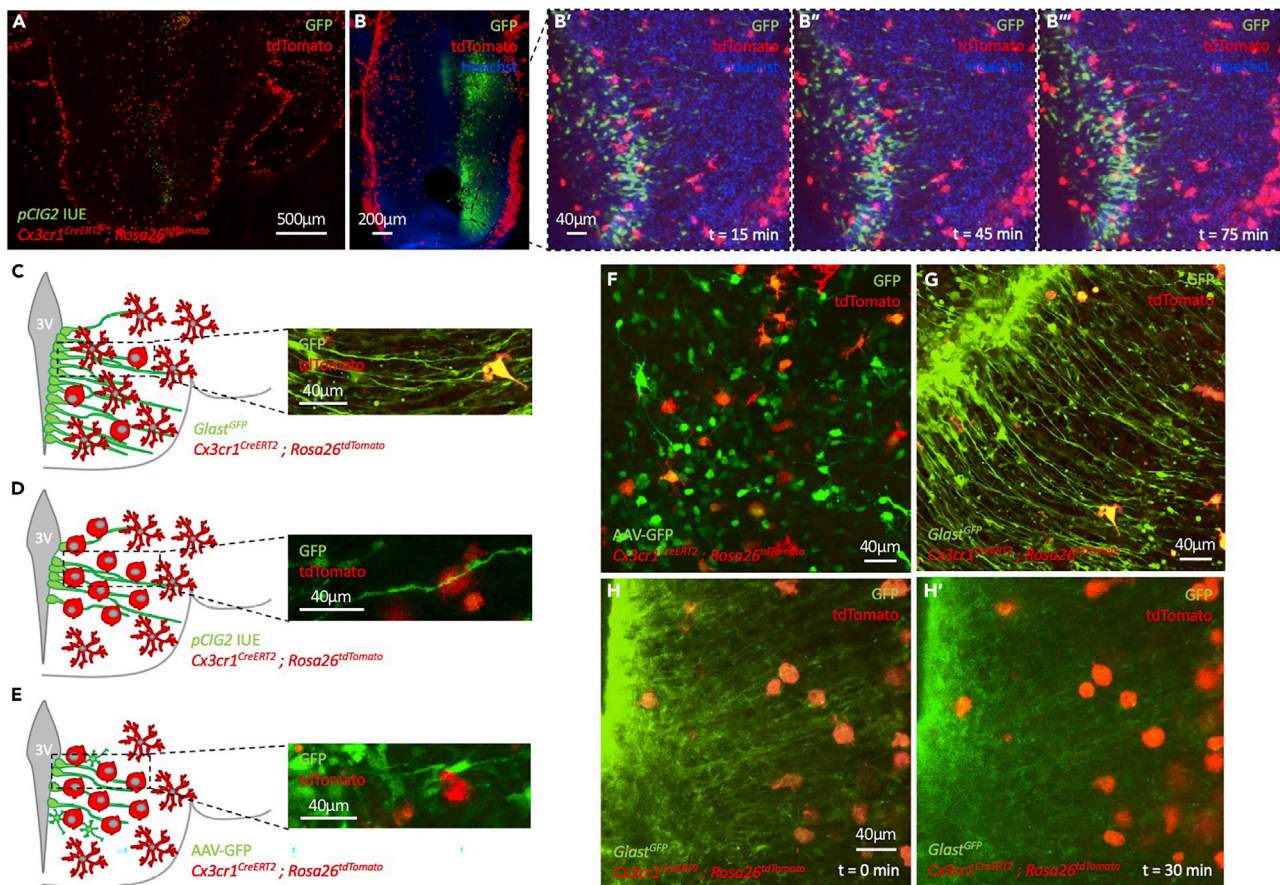


Figure 8. Expected live-cell imaging outcomes

Visualizing the embryonic brain slice under a confocal microscope should show clear fluorescent signal for microglia (e.g., tdTomato from $Cx3cr1^{CreERT2}; Rosa26^{tdTomato}$) and radial glia (e.g., GFP from $pCIG2$ IUE) (A). Hoechst can be applied to the embryonic brain slices (B) during the culturing procedure to stain and visualize independent cells across time during confocal imaging (B'–B'''). Cartoon schematic diagrams and fluorescent images outline a variety of tools that can be used to label radial glia alongside microglia, such as using a transgene (e.g., GFP from $Glast^{GFP}$) (C), IUE (e.g., GFP from $pCIG2$ IUE) (D), or viral transduction (e.g., GFP from AAV2-GFP) (E). Regardless of the approach used to label radial glia, there should be clear bright fluorescent signal visible from both microglia and radial glia (F and G), which can appear dim or blurred if the health of the slice is compromised (H–H'). Scale bars represent 500 μm (A), 200 μm (B) or 40 μm (B'–H').

EXPECTED OUTCOMES

Anticipated outcomes of the embryonic brain slice culture protocol include visualizing cellular dynamics for microglia and radial glia in the embryonic brain (Figure 8A) up to 20 h following the start of imaging (Rosin et al., 2021). Hoechst can be used to stain the nuclei of all cells in the brain region of interest (Figure 8B), allowing microglial-radial glia dynamics to be studied in the context of surrounding cellular dynamics across time (Figures 8B'–8B''') regardless of the transgenes or labeling tools employed to visualize each population of cells (Figures 8C–8E). As mentioned above, this protocol can also be used to visualize neuronal movement in the embryonic brain, in addition to microglia-radial glia interactions in regions outside of the hypothalamus, such as the embryonic cortex. Visualizing other cell types or different brain regions requires adjustment of the transgenic animal model and/or labeling tools used (e.g., IUE, AAV, Annexin V, etc.). Moreover, this protocol can be employed across numerous embryonic time-points, although we have only employed it from E14.5 onwards with similar outcomes.

LIMITATIONS

The largest hurdle associated with live-cell imaging in the embryonic brain using slice culture is maintaining specimen health and normal cellular functions across the imaging experiment. As discussed

in more detail in the [troubleshooting](#) section below, despite careful execution of all aspects of this live-cell imaging protocol, in rare instances the embryonic brain slice will start to die and be visibly unhealthy during live-cell imaging for no apparent cause. This is an uncommon phenomenon that does not occur in the majority of embryonic brain slice culture preparations. Furthermore, it is important to point out that the embryonic brain is being removed from its native environment and is experiencing damage from the vibratome sectioning. Therefore, while this technique allows for the visualization of cellular dynamics in real-time in the brain during embryogenesis, the influences of an *in vivo* versus *ex vivo* environment might reveal differences in findings if ever compared to *in vivo* live-cell imaging, a technique that has been employed to image the mouse placenta ([Zenclussen et al., 2012](#)) but has yet to be mastered for imaging cells in the embryonic hypothalamus.

TROUBLESHOOTING

Problem 1

Sliced embryonic brain tissue can sometimes detach from the surrounding agarose, either during vibratome sectioning or during the transfer from the vibratome sectioning chamber to the culture dish ([Figure 6](#); step 6).

Potential solution

The embryonic brain tissue could be detaching from the surrounding agarose as a result of problems during the embedding process. When transferring the embryonic brain from the vibratome chamber containing PBS to the 4% LMP agarose using a dissecting spoon, make sure that excess PBS is not transferred into the agarose alongside the brain. Excess PBS can be soaked up by lightly dabbing the brain and spoon with a tissue. Once the embryonic brain is in the agarose, a pipette tip can be used to swirl the agarose around the brain to dilute out any excess PBS that was transferred alongside the brain. Swirling the agarose surrounding the embryonic brain can also be used to remove or pop any nearby bubbles, which can also cause issues and tissue separation during sectioning. The embryonic brain tissue could also be detaching from the surrounding agarose during vibratome sectioning because the vibratome sectioning speed is too high. The vibratome sectioning speed should be kept between 0.3–0.5 mm/s when sectioning through the embryonic brain region of interest to help prevent the tissue from separating from the surrounding agarose and folding onto itself.

Problem 2

Sliced embryonic brain tissue can sometimes die or become unhealthy during culturing, which can be visualized as weak, blurred, or unfocused fluorescent signal and/or minimal movement of the cell types being imaged, such as microglia (compare [Figures 8F and 8G](#) to [Figures 8H and 8H'](#); step 9).

Potential solution

The embryonic brain tissue could be dead or dying because either: (1) the brain was placed in the agarose when it was too hot; (2) the embryonic brain tissue was not kept on ice during the appropriate steps; (3) because any number of steps during the processing took too long. The embryonic brain tissue can also start to die during the imaging portion of the protocol, as a result of phototoxicity, acidified medium, tissue drying and/or temperature fluctuations. Accordingly, at the start of each imaging session, fresh medium should be applied to both the glass bottom Petri dish and the top of the slice ([Figures 1B and 5E](#)) to prevent medium acidification and sample drying, and the microscope temperature probe should be placed in the sample chamber to provide an accurate reading of the sample temperature. It should be noted that in rare instances, even when everything in the protocol is performed correctly, sometimes a brain slice will start to die and be visibly unhealthy during the live-cell imaging experiment, with no apparent cause.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Deborah M. Kurrasch (kurrasch@ucalgary.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any unique datasets or code.

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AUTHOR CONTRIBUTIONS

J.M.R. prepared the manuscript. J.M.R., F.M., and D.M.K. edited the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

D.M.K. is the co-founder of Path Therapeutics, focused on the development of drugs for rare pediatric epilepsies.

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