Tracking Neuronal Migration in Adult Brain Slices

Karen Bakhshetyan[1] and Armen Saghatelyan[1,2]

[1]Cellular Neurobiology Unit, Institut Universitaire en santé mentale de Québec, Quebec City, Canada
[2]Department of Psychiatry and Neuroscience, Université Laval, Quebec City, Canada

Neuronal migration is one of the fundamental processes underlying the proper assembly and function of neural circuitry. The majority of neuronal precursors are generated far away from their sites of integration and need to migrate substantial distances to reach their final destination. Neuronal migration occurs not only in the embryonic brain but also in a few regions of the adult brain such as the olfactory bulb (OB). The mechanisms orchestrating cell migration in the adult brain are, however, poorly understood, despite their clinical relevance. Here we describe a method for time-lapse imaging of cell migration in acute brain slices. This method, combined with genetic and/or pharmacological manipulations of different molecular pathways, makes it possible to determine the dynamics and molecular mechanisms of cell migration in the adult brain. In addition, time-lapse imaging in acute brain slices makes it possible to monitor cell movement in a microenvironment that closely resembles in vivo conditions and to study neuroblast displacement along with other cellular elements such as astrocytes and blood vessels. © 2015 by John Wiley & Sons, Inc.

Keywords: cell migration • time-lapse imaging • olfactory bulb • rostral migratory stream • blood vessels

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INTRODUCTION

Neuronal precursors born in the adult subventricular zone (SVZ) retain the remarkable capacity to migrate long distances along the rostral migratory stream (RMS) into the olfactory bulb (OB; Alvarez-Buylla and Garcia-Verdugo, 2002; Marin and Rubenstein, 2003). Neuronal precursors first migrate tangentially in chains along the RMS and then, once in the OB, turn and migrate radially and individually out of the RMS into the bulbar layers (Lois and Alvarez-Buylla, 1994; Alvarez-Buylla and Garcia-Verdugo, 2002; Saghatelyan, 2009). A number of molecular cues affect neuronal migration in the adult brain. These include polysialated neural cell adhesion molecule (PSA-NCAM; Cremer et al., 1994; Ono et al., 1994; Hu et al., 1996); members of the Slit (Kaneko et al., 2011), ephrin-B (Conover et al., 2000), and integrin families (Murase and Horwitz, 2002; Belvindrah et al., 2007); astrocyte-derived factor of unknown identity (Mason et al., 2001); the ErbB4 (Anton et al., 2004) and prokineticin 2 (Ng et al., 2005) receptors; neuroblast-derived γ-aminobutyric acid (GABA; Bolteus and Bordey, 2004); as well as various growth factors such as glial cell line-derived neurotrophic factor (GDNF; Paratcha et al., 2006), brain-derived neurotrophic factor (BDNF; Chiaramello et al., 2007; Snapyan et al., 2009), and vascular endothelial growth factor (VEGF; Wittko et al., 2009; Bozoyan et al., 2012). Reelin and tenascin-R, two extracellular matrix molecules,

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also contribute to the radial migration of neuronal precursors (Hack et al., 2002; Saghatelyan et al., 2004; David et al., 2013). In addition to these molecular cues, cellular interactions between neuroblasts, astrocytes, and blood vessels (BVs) are required for faithful migration toward the OB. Adult neuronal precursors use BVs that topographically outline the RMS for their migration (Snapyan et al., 2009; Whitman et al., 2009) and create a migratory path by repelling astrocytic processes (Kaneko et al., 2011).

Understanding the cellular and molecular mechanisms orchestrating neuronal migration in the adult brain may be important for the development of new strategies aimed at the recruitment of endogenous and/or grafted neuronal precursors into damaged/diseased brain areas. Indeed, the possibility of manipulating these mechanisms in order to increase the number and dispersal of endogenous and/or grafted neuronal precursors in diseased areas may open novel avenues for the treatment of devastating neurodegenerative diseases and brain trauma. Interestingly, a massive migration of endogenous neuronal precursors from their generation site (SVZ) to damaged areas of the brain has been observed following stroke and in some neurodegenerative conditions (Arvidsson et al., 2002; Falk and Frisen, 2005). These observations suggest that the injured/diseased brain may re-route the migration of neuronal precursors as a repair/compensation mechanism. If so, this also suggests that intervening in this process might be an effective therapy for treating degenerative diseases and brain injuries.

In this unit we will describe a method for monitoring and studying the migration of adult neuronal precursors in acute brain slices in a microenvironment that closely resembles in vivo conditions. We will describe stereotaxic injections of viral particles to infect neuronal precursors in vivo, the labeling of BVs, the preparation of acute adult brain slices, time-lapse video-imaging of cell migration, and analysis. While in this unit we describe neuronal migration along BVs in the adult RMS, we have successfully applied the same procedure for monitoring neuronal migration along BVs in the ischemic striatum (Grade et al., 2013) and along astrocytes in the developing and adult RMS (Bozoyan et al., 2012). A video showing some of the procedures in this unit can be viewed at *http://www.jove.com/video/4061/* (Khlghatyan and Saghatelyan, 2012).

NOTE: All protocols using live animals must be reviewed and approved by Institutional Animal Care and Use Committees (IACUC) and must comply with applicable government legislation and regulations on the humane care and use of laboratory animals.

BASIC PROTOCOL

TIME-LAPSE VIDEO-IMAGING OF NEURONAL MIGRATION IN ADULT ACUTE BRAIN SLICES

This protocol describes the basic steps for visualizing and studying neuronal migration in acute adult brain slices. The use of acute slices makes it possible to assess cell migration in a microenvironment that closely resembles in vivo conditions and in brain regions that are difficult to access by in vivo imaging. While neuronal migration can also be visualized using differential interference contrast (DIC) optics (Snapyan et al., 2009), we recommend using time-lapse video-imaging of fluorescently labeled neuronal precursors, which makes it possible to reliably monitor the tangential and radial migration of individual cells and to follow the dynamics of leading processes of neuroblasts (Snapyan et al., 2009; Bozoyan et al., 2012; Khlghatyan and Saghatelyan, 2012; David et al., 2013; Grade et al., 2013). In the Support Protocol, we describe the procedure to stereotaxically inject viral particles into the SVZ in order to infect neuronal precursors.

Materials

Adult C57Bl/6 mice (Charles River)

Acute brain slices prepared from adult mice infected with viral particles 3 to 7 days (for monitoring tangential migration in the RMS) or 7 to 12 days (for monitoring radial migration in the OB and the RMS of the OB; see Support Protocol)
Artificial cerebro-spinal fluid (ACSF; see recipe)
Cutting solution (see recipe)
Dextran Texas Red (Molecular Probes)
4% agar block (prepared using agar powder [Sigma-Aldrich]; see recipe)
95% O_2/5% CO_2

Surgical instruments for extracting the brain (e.g., scissors, scalpel, forceps)
Vibratome (Microm HM 650 V; Thermo Scientific)
Fluorescence wide-field upright microscope, with motorized Z-drive (BX61WI; Olympus)
482/35 nm and 536/40 nm excitation and emission filters for imaging GFP-labeled cells
14-bit cooled CCD camera with 1392 × 1040 imaging pixels (CoolSnap HQ2; Photometrics)
40× water immersion objective lens with a 0.8 numerical aperture or higher (Olympus)
Illumination system, equipped with a 175 W xenon lamp (30 to 100 msec excitation time per z plane, Lambda DG-4; Sutter Instruments)
Imaging chamber, mounted on a microscope stage (PH1 Series 20; Harvard Apparatus)
Automatic heating system (to maintain temperature in the imaging chamber at ~32°C; Harvard Apparatus, cat. no. TC-344B)
Multidimensional time-lapse data acquisition software (MetaMorph, Molecular Devices)
Software for Z-stack image acquisition, every 15 sec for 1 hr (usually 5 to 10 z-planes at 3-μm intervals; MetaMorph, Molecular Devices)
Custom-made ACSF delivery system for continuous perfusion of slices with oxygenated ACSF at a 1 to 2 ml/min flow rate
Slice fixation mesh (nylon with 0.12-mm diameter and ~1-mm^2 holes)

NOTE: For standard animal techniques including stereotaxic surgery, anesthesia, and analgesia, see *APPENDIX 4A* and *APPENDIX 4B*.

NOTE: The imaging and acquisition systems described in this unit are used in the authors' laboratory and serve only as examples of the equipment required to perform time-lapse imaging in acute slices. Other commercial and custom-made systems can be used. However, we strongly recommend using imaging systems that can acquire time-lapse images at least every 15 sec. This is important to ensure the unambiguous identification of the migratory and stationary phases of neuronal precursors, to better understand the dynamics of cell migration.

Preparation of acute slices and imaging

1. Inject 200 μl dextran Texas Red (10 mg/ml) into the tail vein and wait 15 to 30 min before decapitating the mouse. Alternatively, inject 200 μl dextran Texas Red into the left ventricle of the heart of a deeply anesthetized mouse and wait 2 to 3 min before decapitating the animal.

2. Anesthetize mouse in which neuronal precursors are labeled with green fluorescent protein (GFP)-encoding viral vectors (see Support Protocol) and BVs are labeled with dextran Texas Red.

3. Decapitate mouse and rapidly immerse head in ice-cold cutting solution. Remove scalp using scissors and make an incision in the skull over the cerebellum.

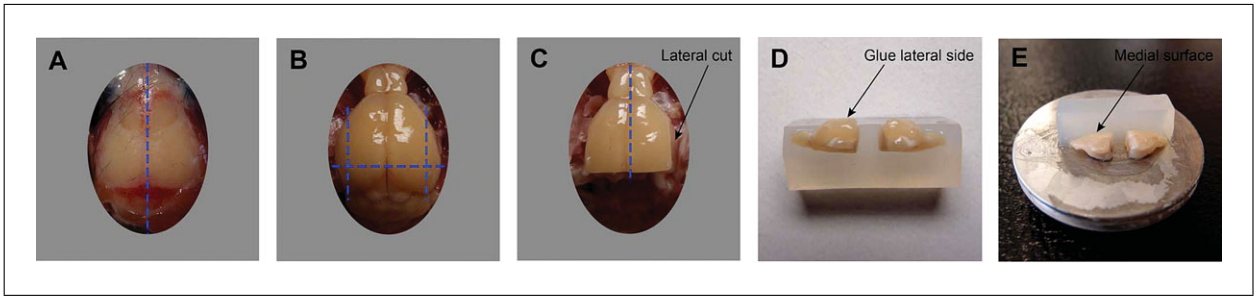

Figure 3.28.1 Preparation of sagital acute brain slices. (**A**) Dorsal view of the mouse head with removed scalp. Dotted line represents the line of incision for removal of cranial bones. (**B**) Dorsal view of the brain with removed cranial bones. (**C**) Dorsal view of the brain with removed caudal and lateral parts. (**D**) Separated hemispheres placed on agar block. (**E**) The agar block with hemispheres glued on vibratome platform.

4. Using forceps, gently remove cranial bones to expose the lateral and dorsal sides of the brain (Fig. 3.28.1A,B). Irrigate the surface of the brain with ice-cold cutting solution.

5. To prepare sagital sections, cut out the caudal part of the brain using a scalpel without removing the brain from the skull. Then cut out sagitally ~2 mm of brain tissue from the most lateral part of each hemisphere (dotted lines in Fig. 3.28.1B) and cut the brain sagitally in the midline to separate the two hemispheres from each other (Fig. 3.28.1C).

6. Gently remove both hemispheres from the skull using a spatula and place in ice-cold cutting solution.

7. Place the two hemispheres side by side on a 4% agar block with the dorsal or ventral sides touching the agar (Fig. 3.28.1D). Then glue the agar block with both hemispheres to the platform of a vibratome with the laterally cut sides facing the platform, keeping the medial side face up (Fig. 3.28.1E).

8. Place platform in the chamber of the vibratome and fill platform with cutting solution. Bubble 95% O_2/5% CO_2 through the solution to keep it oxygenated throughout the slice preparation procedure.

9. Prepare 250-μm thick slices using the vibratome.

   Cut slices at low speed and with high-frequency vibration to obtain high-quality slices.

10. Gently remove slices from the cutting solution and place them in an incubation chamber filled with oxygenated ACSF maintained at 32°C in a water bath.

    This procedure should be performed as quickly as possible to ensure high-quality slices.

    For monitoring radial migration of neuroblasts in the OB we use horizontal sections (Fig. 3.28.2C). To prepare horizontal sections, follow the same procedure as described above, but do not perform sagital cut at the most lateral parts of each hemisphere, separate two hemispheres, place them on the agar block with medial part touching the agar and glue the agar block with hemispheres to the platform of a vibratome with the ventral side facing to the platform, and keeping the dorsal side face up.

11. Start perfusion of the imaging chamber mounted on a fluorescent upright microscope (BX61WI; Olympus) with ACSF (1 to 2 ml/min).

    Make sure the temperature in the imaging chamber remains stable at ~32°C.

12. Carefully transfer the slice into the imaging chamber of the microscope. To avoid slice drift during imaging, gently cover the slice with a nylon mesh stretched on a silver frame.

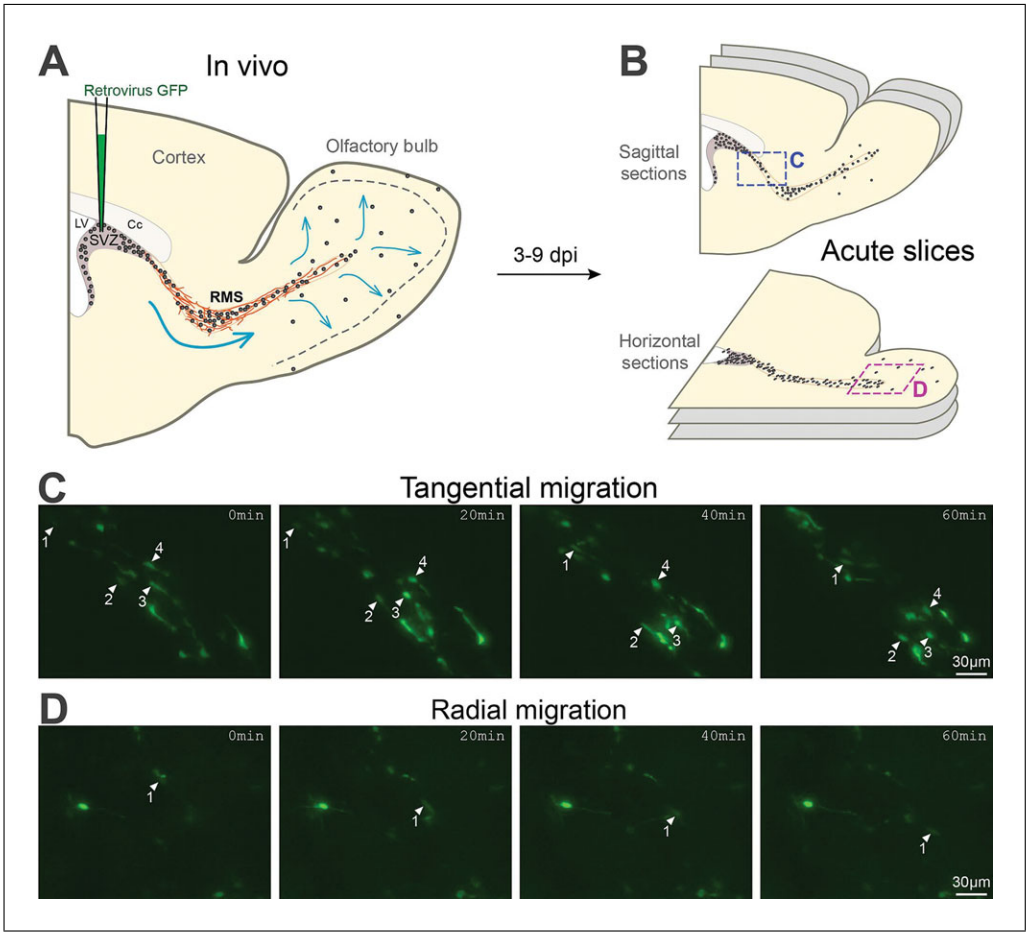

Figure 3.28.2 Tracking tangential and radial migration in adult brain slices. (**A**) Schematic diagram of adult-born cell labeling by stereotaxic injection of GFP-encoding viral particles into the SVZ. The adult forebrain slices are prepared 3-7 days or 7-12 days after the injection of the viral vectors for monitoring tangential and radial migration, respectively. (**B**) Tangential migration is monitored in the RMS with slices cut sagitally, while radial migration is recorded in the horizontal sections of OB and the RMS of the OB. (**C**) Time-lapse wide-field imaging of GFP-labeled neuronal precursors in acute slices of the adult mouse forebrain. GFP-expressing retrovirus was injected into the SVZ 4 days before time-lapse imaging in the RMS. The arrows and numbers indicate the soma of the different migratory cells. Time is indicated in minutes in the upper right corner of each photograph. (**D**) Time-lapse imaging of GFP-labeled neuroblasts in acute OB slices. GFP-expressing retrovirus was injected into the SVZ 8 days before time-lapse imaging. The arrows and numbers indicate the soma of different migratory cells. Time is indicated in minutes in the upper right corner of each photograph.

13. Engage a 10× objective to find the region of interest and make sure the nylon mesh does not obstruct the field of view. Slightly reposition the mesh if it obstructs the field of view and gently press down on the silver frame on which the nylon mesh is stretched.

14. Engage a 40× objective and select the region for imaging.

15. Set the time-lapse acquisition parameters in MetaMorph (exposure time, the number and distance of z-planes, time-lapse intervals, and total imaging duration).

    The acquired time-lapse data is automatically saved by MetaMorph and the z-stack of each time point is stored as a multipage TIFF file.

16. To avoid changes in the focal plane during imaging, continuously monitor the amount and temperature of the ACSF in the imaging chamber and make sure the perfusion of the ACSF remains constant.

We recommend occasionally verifying the data acquired during the imaging by selecting Review Multidimensional Data in Metamorph. If there are significant drifts or changes in focus that could affect the analysis, stop, discard the data, resolve the issue, and restart the time-lapse recording.

17. Perform imaging for at least 1 hr, or more, to ensure that both the migratory and stationary phases of cell migration are recorded.

    Avoid imaging cells that are too close to the surface of the slice; image at a depth of 20 to 100 μm.

    For reliable cell tracking, we recommend short acquisition intervals (15 to 30 sec).

    To study particular molecular factors that might play a role in neuroblast migration, the ACSF can be supplemented with pharmacological agents, for example agonists/antagonists, or growth factors/blockers. Alternatively the expression of particular genes of interest can be affected using viral vectors (for example, gain-of-function or loss-of-function approaches using overexpression constructs, siRNA, miRNA, or the injection of Cre viruses into animals where the gene of interest is flanked by two loxp sites).

Analysis of cell migration

Use Imaris software (Bitplane) for the three-dimensional (3D) analysis of migrating cells.

18. Load the acquired time-lapse movie by dragging and dropping the first time point TIFF file onto the Imaris program icon.

19. When the movie is loaded in Imaris, adjust the brightness in the Display adjustment window so that the cells are clearly distinguished from the background. Set the voxel size based on objective calibration data and define the time-lapse acquisition interval in the Image Properties and Set Equidistant Time Points windows, respectively.

20. To initiate cell tracking, run the Spots function in the Surpass menu and then follow the wizard-style steps. In Source Channel, set the spot diameter and then define the threshold for cell detection. Adjust the threshold if not all the migrating cells were tracked. Next, set the maximal distance between the spot positions of consecutive time points as well as the maximal time gap in cell tracking.

    After creating the tracks, it is possible to make corrections to tracks whenever necessary. For example, different tracks that are actually the same cell but were not recognized as such by Imaris can be connected together. The parts of tracks that on careful examination belong to different cells but were misinterpreted as one cell can also be disconnected.

21. Delete all irrelevant tracks such as those that were tracked for too short a time. Also delete the tracks that remained stationary throughout the imaging period because these cells may have been damaged during the cutting procedure or may be non-migratory cells.

22. Once all the corrections have been made, export the cell tracking data to an Excel file by clicking on Export All Statistics to File icon.

    The exported file contains per time point information on the number of tracks and cell speed and displacement, as well as per cell information on displacement length, track duration, and track length.

23. Load the data from Imaris into a custom-made Excel template to extract the final migration data and to identify the migratory and stationary phases of each cell.

    The stationary phases are defined by a speed threshold of 0.03 μm/sec. This corresponds to the means (± SD) of cell displacement per second for all cells multiplied by 1.5.

The final cell tracking statistics data include the following parameters: total migration distance per cell, mean speed per cell, percentage of migratory phases per cell, and percentage of migrating cells per time point.

STEREOTAXIC INJECTION OF VIRAL VECTORS INTO THE SVZ OF THE ADULT MOUSE

SUPPORT PROTOCOL

While neuroblast migration in acute brain slices can be also monitored using DIC optics (Snapyan et al., 2009), we recommend imaging fluorescently labeled neuronal precursors (Snapyan et al., 2009; Bozoyan et al., 2012; Khlghatyan and Saghatelyan, 2012; David et al., 2013; Grade et al., 2013). This makes it possible to reliably track cell migration during prolonged periods, which is quite difficult using DIC optics. In addition, cells can be tracked deeper in the tissue, and the dynamics of the leading process of neuroblasts can be visualized (Grade et al., 2013). In this Support Protocol, we describe the basic steps for labeling neuroblasts with GFP-encoding viral vectors. This procedure can also be used to overexpress or knock-out and/or knock-down the protein of interest and study its role in neuronal precursor migration. We use adeno-associated virus (AAV), retroviruses, and/or lentiviruses to label neuronal precursors in the SVZ using the following coordinates: anterio-posterior (AP) 0.70; medio-lateral (ML) 1.20, and dorso-ventral (DV) 1.90.

Materials

Adult C57Bl/6 mice (Charles River)
Proviodine (Rougier) or 70% ethanol (Sigma-Aldrich)
Paraffin oil (Sigma-Aldrich)
Solution containing viral particles (obtained from Vector Core Facilities, Institut Universitaire en santé mentale de Québec or University of North Carolina)
Ketamine (Bioniche)/xylazine (Bimeda) or isoflurane (PPC)
Lidocaine (AstraZeneca)
Anafen (Merial)

Micropipet puller
Stereotaxic injection apparatus equipped with a digital stereotaxic coordinate read-out system (WPI)
Mouse adaptor mounted on a stereotaxic apparatus (WPI)
Sterile surgical instruments (e.g., scissors, scalpels, forceps)
Microdrill system
Heating pad
Nanoliter injector with nanoliter injector controller (WPI)
Binocular microscope

NOTE: For standard animal techniques including stereotaxic surgery, anesthesia, and analgesia, see *APPENDIX 4A* and *APPENDIX 4B*.

1. Sterilize all instruments before starting the surgery using a bead sterilizer or an autoclave.
2. Pull very thin-tipped (1- to 2-μm) micropipets using a micropipet puller.

   We use glass capillaries suitable for the nanoliter injector.
3. Backfill pipets with paraffin oil until half full and insert plunger of a nanoliter injector.
4. Using a nanoliter injector controller, force paraffin oil downwards with the plunger until a small drop extrudes from the tip. Lower pipet tip into a small drop (0.5

to 1 μl) of solution containing the viral particles (Vector Core Facilities, Institut Universitaire en santé mentale de Québec or University of North Carolina).

5. Backfill pipet using the withdraw function of the nanoliter injector.
6. Anesthetize 22- to 24-g adult C57Bl/6 mice using an intraperitoneal injection of ketamine/xylazine (10 mg/1 mg per 10 g of body weight) or by isoflurane inhalation.
7. Shave the head of the mouse and thoroughly clean the surgical site using wet gauze.
8. Inject lidocaine under the skin at the surgical site and administer an analgesic intraperitoneally (Anafen, 10 mg/kg).
9. Place mouse in the stereotaxic frame on the heating pad and fix head in place using ear and tooth bars.
10. Disinfect surgical site with gauze soaked with Proviodine or 70% ethanol.
11. Cut the skin at the surgical site and expose the underlying skull.
12. Set the anterio-posterior (AP) and medio-lateral (ML) stereotaxic coordinates, respectively, using the bregma and midline as the zero coordinates.
13. Drill a small hole in the skull over each hemisphere at the desired coordinates.

    Avoid damaging the underlying brain tissue.
14. Carefully remove the dura using an angled needle or forceps.
15. Set the zero for the dorso-ventral (DV) stereotaxic coordinates on the surface of the brain.
16. Slowly insert glass micropipet tip into the brain using the desired coordinates and slowly inject a small amount of the solution (for example, we inject 100 to 500 nl at 5 nl/sec) containing the viral particles.
17. Wait 1 to 2 min and then slowly withdraw the glass micropipet.
18. Suture the skin over the surgical site. Wait until the animal recovers, and return it to its cage.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2A; for suppliers, see SUPPLIERS APPENDIX.

Agar blocks, 4% (w/v)

Thoroughly dilute agar powder (Sigma-Aldrich) in distilled water to generate a 4% w/v mixture then heat to boiling. Fill a petri dish with hot agar solution and let it cool to room temperature. Store at 4°C for 5 to 7 days.

Artificial cerebro-spinal fluid (ACSF)

125 mM NaCl
3 mM KCl
1.3 mM $MgCl_2 \cdot 6H_2O$
2 mM $CaCl_2 \cdot 2H_2O$
26 mM $NaHCO_3$
1.25 mM NaH_2PO_4
20 mM glucose
Maintain at pH 7.3 to 7.4, and continually oxygenate by bubbling 95% O_2/5% CO_2 through the solution. Maintain temperature at 32°C during slice incubation.
Prepare fresh on the day of experiment

Cutting solution

210.3 mM sucrose
3 mM KCl
3 mM $MgCl_2 \cdot 6H_2O$
0.5 mM $CaCl_2 \cdot 2H_2O$
26 mM $NaHCO_3$
1.25 mM NaH_2PO_4
20 mM glucose
Chill until ice cold using liquid nitrogen. Maintain at pH 7.3 to 7.4, and continually oxygenate by bubbling 95% O_2/5% CO_2 through the solution.
Prepare fresh on the day of experiment

COMMENTARY

Background Information

Neuronal migration is one of the fundamental processes underlying proper brain development. In the adult brain, neuronal migration has largely ceased and the SVZ-OB pathway is probably the only region where massive neuronal migration still occurs under normal conditions. It should be noted, however, that neuronal migration in the adult brain can be induced by brain damage and/or neurodegenerative diseases (Arvidsson et al., 2002; Lindvall et al., 2004; Ohab et al., 2006; Grade et al., 2013). Interestingly, induced neuronal migration toward damaged/diseased brain areas shares at least some features of constitutive neuronal migration in the SVZ-OB pathway. For example, neuronal precursors use BVs as a physical scaffold and a source of molecular cues in both the RMS (Snapyan et al., 2009; Whitman et al., 2009) and the ischemic striatum (Ohab et al., 2006; Grade et al., 2013). Astrocytes, which play an important role in neuroblast migration in the RMS (Lois and Alvarez-Buylla, 1994; Snapyan et al., 2009; Kaneko et al., 2011), are also involved in neuronal migration in ischemic brain regions (Imitola et al., 2004; Yan et al., 2007). Studying the cellular and molecular mechanisms of neuronal migration in the SVZ-OB pathway is thus not only important from a fundamental point of view but may also have clinical relevance. It is conceivable that a better understanding of the mechanisms controlling neuronal migration in the undamaged adult brain may be of use in the development of new strategies to re-route neuronal precursors from their natural migratory pathway and/or to increase the dispersal of grafted neuronal progenitors in diseased/damaged brain areas (Lindvall et al., 2004; Falk and Frisen, 2005; Massouh and Saghatelyan, 2010). Since ischemic damage and neurodegeneration mostly occurs in the adult/aged brain, studying the mechanisms of neuronal migration in the adult SVZ-OB where massive neuronal migration is observed (Lois and Alvarez-Buylla, 1994; Marin and Rubenstein, 2003) may be particularly important for the development of new cell replacement strategies.

There are several approaches for studying neuroblast migration. These include two-dimensional (2D) cultures of SVZ progenitor cells (Lois et al., 1996), 3D cultures of SVZ explants on Matrigel (Lois et al., 1996; Saghatelyan et al., 2004; Courtes et al., 2011), organotypic cultures (Murase and Horwitz, 2002; Mejia-Gervacio et al., 2012a,b), and acute brain slices (Bolteus and Bordey, 2004; Platel et al., 2008; Snapyan et al., 2009; Comte et al., 2011; Bozoyan et al., 2012; David et al., 2013; Grade et al., 2013). Each of these methods has advantages and limitations. Time-lapse imaging of adult acute slices allows the monitoring of neuroblast migration in a microenvironment that closely resembles in vivo conditions. In addition, complex cellular and molecular interactions with other cellular elements such as astrocytes and BVs can be studied (Bolteus and Bordey, 2004; Platel et al., 2008; Snapyan et al., 2009; Comte et al., 2011; Bozoyan et al., 2012). The main drawback of this method is that cutting-induced damage may affect neuronal migration.

If the role of a particular molecular pathway in neuroblasts needs to be studied without interference from astrocytes and/or endothelial cells, 2D and 3D SVZ cultures may be used (Saghatelyan et al., 2004; Courtes et al., 2011). In addition, co-cultures of neuroblasts and astrocytes (Garcia-Marques et al., 2010; Bozoyan et al., 2012) or endothelial cells (Snapyan et al., 2009) may be used to study the role of these cells in neuronal migration. It should be noted, however, that neuroblasts migrating out of SVZ explants are in

a microenvironment that is completely different from in vivo conditions, which may affect the dynamics and mechanisms of cell migration. Organotypic cultures have also been used to study neuronal migration, especially in the developing RMS (Murase and Horwitz, 2002; Mejia-Gervacio et al., 2012a,b). This method provides the advantage of studying neuroblast migration over long periods of time (up to several days). It should be kept in mind, however, that cellular organization (Law et al., 1999; Peretto et al., 2005; Bozoyan et al., 2012) and neuronal migration (Bozoyan et al., 2012; David et al., 2013) are quite different in the developing and the adult RMS. Moreover, long-term culturing may also alter the migratory properties of the cells. Regardless of the method used to study neuroblast migration, we recommend using short intervals (15 to 30 sec) between consecutive acquisitions in order to reliably identify the migratory and stationary phases and to quantify the speed of migration solely during migratory phases. Moreover, an assessment of the periodicity of the migratory and stationary phases as well as the duration of the migratory phases provide additional information on the dynamics of cell movement and the impact of particular treatments on cell migration (Bortone and Polleux, 2009; Snapyan et al., 2009).

Critical Parameters and Troubleshooting

Preparation of acute brain slices

The quality of the acute brain slices is particularly important for the successful implementation of time-lapse video imaging of cell migration. It is thus important to carefully inspect the quality of the slices using DIC optics before starting the time-lapse video imaging. Acute slices from the adult mouse brain (2 to 3 months old) are particularly challenging to prepare. A number of modifications in the ionic composition of the cutting solution can improve cell viability and decrease cellular swelling and damage. For example, increasing Mg^{2+} and decreasing Ca^{2+} concentrations to dampen synaptic transmission as well as replacing Na^{+} by sucrose to dampen neuronal excitability (see cutting solution recipe) during the cutting procedure improve the quality of the slices. It is also important to prepare the slices as quickly as possible. The brain should be rapidly extracted and immersed in ice-cold cutting solution. The vibratome chamber should also be filled with oxygenated ice-cold solution, and the entire slicing procedure should be performed when the cutting solution is still ice cold. The solution should be oxygenated throughout the slice preparation, and the brain should be cut at low speed and high frequency vibration to obtain high-quality slices.

If BVs do not need to be labeled, we recommend that the mouse be transcardially perfused with ice-cold cutting solution before removing the brain. In our hands, this approach markedly improves the quality of the slices.

Stable conditions during the time-lapse video imaging

An irregular flow of ACSF, unstable temperature, and slice drift may all lead to changes in the focal plane and unreliable imaging of cell migration. Make sure that all these parameters are stable before transferring the slice into the imaging chamber and starting the time-lapse video imaging. ACSF perfusion can be pump driven or gravity fed. In both cases, irregular flows of ACSF may be resolved by adjusting the rates of inflow and outflow. We use a gravity-fed perfusion system and an ultraquiet imaging chamber (RC-27D; Warner Instruments) that eliminates vibrations and provides a more constant flow of ACSF.

Temperature fluctuations during imaging is another critical parameter that must be controlled. In fact, one of the most effective ways of blocking cell migration is to perform imaging at room temperature. We usually image cell migration at about 32°C. In our hands, increasing the temperature above this value leads to unstable imaging of cell migration. This is mainly due to the formation of numerous small bubbles at the interface between the heated ACSF and the air in the open-bath chamber. The temperature should not fluctuate more than ± 1°C to ensure stable imaging. Preheated ACSF can be used to avoid overheating the imaging chamber.

Slice drift is another parameter that may affect the time-lapse video imaging of cell migration. This can be controlled using slice anchors. We use custom slice anchors made from nylon mesh tightly stretched over and glued to a silver frame. We usually prepare new slice anchors every month or as soon as we notice significant slice drift.

Selecting the region for imaging

It is important to carefully inspect the slice and select the appropriate region for imaging. The RMS in the field of view should be continuous and uninterrupted. Do not image cells in the region where the RMS was cut or is spanned with axonal bundles. Avoid imaging cells that are too close to the slice

surface. Rather, image cells at a depth of 20 to 100 μm. Perform the imaging for at least 1 hr, or more, so that both the migratory and stationary phases of cell migration are recorded.

Anticipated Results

Time-lapse video imaging of cell migration in acute adult brain slices improves our understanding of the dynamics and cellular and molecular mechanisms of cell movement in a microenvironment that closely resembles in vivo conditions. Typical real-time video images of neuroblasts migrating tangentially and radially are shown in Figure 3.28.2. Neuronal precursors labeled by the stereotaxic injection of GFP-encoding retroviruses into the SVZ are shown in Figure 3.28.2A, B. Images of tangential migration in the RMS in acute slices prepared 5 days after the viral vector injections are shown in Figure 3.28.2C. Images of radial migration in the OB in acute slices prepared 9 days after the injection of the viral vector into the SVZ are shown in Figure 3.28.2D. Regions of interest containing GFP-labeled neuroblasts were selected based on the parameters described in the Critical Parameters and Troubleshooting section. Time-lapse video imaging of cell migration was performed for 1 hr with 15 sec intervals between consecutive acquisitions. The exposure time was 30 msec, and seven z-planes with 3-μm intervals were used to acquire time-lapse movies. Time-lapse imaging snapshots containing several migrating cells in the RMS (numbered arrows; each number reflects a different cell) are shown in Figure 3.28.2C. Migration in the RMS was relatively rapid and ranged from 120 to 180 μm/hr. The mean distance that neuroblasts migrate during 1 hr is ~60 μm, and cells spend ~40% in the migratory phase.

When neuroblasts arrive in the OB, they change their mode of migration from tangential to radial (Fig. 3.28.2D). Several modes of migration can be studied in the OB. Tangential migration in the RMS of the OB can be studied to determine whether particular molecular cues play a role in stopping tangential migration (David et al., 2013). Radial migration in the RMS of the OB and the granule cell layer (GCL) can also be studied. For example, time-lapse imaging of neuroblasts in the OB showed that extracellular matrix glycoprotein does not affect tangential migration in the RMS of the OB but sustained radial migration in the RMS of the OB and the GCL (David et al., 2013). The distance covered by tangentially and radially migrating neuroblasts in the OB and the RMS of the OB is shorter than that of tangentially migrating cells in the RMS (see Video 3.28.1). This is mainly due to lower speed of migration (David et al., 2013). Radially migrating neuroblasts in the OB migrated at about 80 to 100 μm/hr, had roughly 40% of migratory phases, and propagated 20 to 40 μm during 1 hr of imaging, considering stationary phases as well.

Time Considerations

It takes an average of 30 to 40 min to perform the stereotaxic injections of the viral vectors into the SVZ. The survival time after injection can vary and depends on the type of migration to be studied. If tangential migration of neuroblasts is to be studied, the preparation of the acute slices and time-lapse imaging may be performed 3 to 7 days after the viral vector injection. If radial migration of neuroblasts in the OB and the RMS of the OB is to be studied, the time-lapse imaging of acute slices may be performed 7 to 12 days after the injection of the viral vector into the SVZ. The acute slices should be prepared as quickly as possible and can be kept for up to 6 to 8 hr.

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