Video Article

In vivo Postnatal Electroporation and Time-lapse Imaging of Neuroblast Migration in Mouse Acute Brain Slices

Martina Sonego*[1], Ya Zhou*[1], Madeleine Julie Oudin[2], Patrick Doherty[1], Giovanna Lalli[1]

[1]Wolfson Centre for Age-Related Diseases, King's College London

[2]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

*These authors contributed equally

Correspondence to: Giovanna Lalli at giovanna.lalli@kcl.ac.uk

URL: http://www.jove.com/video/50905
DOI: doi:10.3791/50905

Keywords: Neuroscience, Issue 81, Time-Lapse Imaging, Cell Migration Assays, Electroporation, neurogenesis, neuroblast migration, neural stem cells, subventricular zone (SVZ), rostral migratory stream (RMS), neonatal mouse pups, electroporation, time-lapse imaging, brain slice culture, cell tracking

Date Published: 11/25/2013

Citation: Sonego, M., Zhou, Y., Oudin, M.J., Doherty, P., Lalli, G. *In vivo* Postnatal Electroporation and Time-lapse Imaging of Neuroblast Migration in Mouse Acute Brain Slices. *J. Vis. Exp.* (81), e50905, doi:10.3791/50905 (2013).

Abstract

The subventricular zone (SVZ) is one of the main neurogenic niches in the postnatal brain. Here, neural progenitors proliferate and give rise to neuroblasts able to move along the rostral migratory stream (RMS) towards the olfactory bulb (OB). This long-distance migration is required for the subsequent maturation of newborn neurons in the OB, but the molecular mechanisms regulating this process are still unclear. Investigating the signaling pathways controlling neuroblast motility may not only help understand a fundamental step in neurogenesis, but also have therapeutic regenerative potential, given the ability of these neuroblasts to target brain sites affected by injury, stroke, or degeneration.

In this manuscript we describe a detailed protocol for *in vivo* postnatal electroporation and subsequent time-lapse imaging of neuroblast migration in the mouse RMS. Postnatal electroporation can efficiently transfect SVZ progenitor cells, which in turn generate neuroblasts migrating along the RMS. Using confocal spinning disk time-lapse microscopy on acute brain slice cultures, neuroblast migration can be monitored in an environment closely resembling the *in vivo* condition. Moreover, neuroblast motility can be tracked and quantitatively analyzed. As an example, we describe how to use *in vivo* postnatal electroporation of a GFP-expressing plasmid to label and visualize neuroblasts migrating along the RMS. Electroporation of shRNA or CRE recombinase-expressing plasmids in conditional knockout mice employing the LoxP system can also be used to target genes of interest. Pharmacological manipulation of acute brain slice cultures can be performed to investigate the role of different signaling molecules in neuroblast migration. By coupling *in vivo* electroporation with time-lapse imaging, we hope to understand the molecular mechanisms controlling neuroblast motility and contribute to the development of novel approaches to promote brain repair.

Video Link

The video component of this article can be found at http://www.jove.com/video/50905/

Introduction

In the mammalian brain, the generation of new neurons (neurogenesis) occurs after birth mainly in two regions, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone in the dentate gyrus of the hippocampus[1]. Considerable evidence gathered in recent years supports a critical role for postnatal neurogenesis in hippocampal and olfactory bulb memory functions[1-3]. Importantly, postnatal neurogenesis also holds therapeutic potential because of its relationship with degenerative neurological disorders, and the ability of neuroblasts to migrate to injured sites in the brain[4-6].

The subventricular zone (SVZ) has recently emerged as a crucial neurogenic niche. SVZ-derived neuroblasts migrate towards the olfactory bulb (OB) via the rostral migratory stream (RMS), making this the longest migration process in the postnatal brain[1,7,8]. The mammalian SVZ/RMS/OB system has become a useful model to study different steps in neurogenesis, such as proliferation, migration and differentiation[1,8]. Many growth factors and extracellular cues regulate SVZ neurogenesis and migration along the RMS, but the intracellular molecular mechanisms are far from being fully understood[1,9]. Proper migration along the RMS is crucial for the subsequent maturation of newborn neurons[10]. Additionally, some studies have shown that SVZ-derived neuroblasts can migrate out of the RMS to brain injury sites[4-6,11-13]. Thus, investigating the signalling mechanisms regulating neuroblast migration is fundamental not only to understand neurogenesis but also for potential therapeutic applications.

Here, we describe a detailed protocol to label SVZ neural progenitors by *in vivo* postnatal electroporation and monitor their migration along the RMS in acute brain slice cultures using time-lapse spinning disk confocal microscopy. Electroporation is widely used in developmental studies from embryonic to adult stages[14-18]. It is a powerful tool to target and manipulate SVZ neural progenitors and represents a cheaper and considerably faster alternative to stereotactic injection of viral vectors or generation of transgenic models[1,15,19,20]. It is a relatively simple procedure that does not need surgery and has high survival rates. Electroporation of shRNA or CRE recombinase-expressing plasmids in mouse

Copyright © 2013 Journal of Visualized Experiments

genetic models employing the LoxP system can be used to target genes of interest or to achieve permanent labeling of SVZ progenitors, thus representing a useful tool for adult neurogenesis studies[21,22].

Imaging RMS neuroblast migration in the intact brain is still challenging due to current technical limitations. However, this process can be monitored using confocal spinning disk time-lapse microscopy of acute brain slices, which provide a suitable system closely resembling the *in vivo* condition also amenable to pharmacological manipulation[23,24]. Coupling *in vivo* postnatal electroporation with time-lapse imaging will facilitate the understanding of the molecular mechanisms controlling neuroblast motility and contribute to the development of novel approaches to promote brain repair.

Protocol

This procedure is in accordance with the UK Home Office Regulations (Animal Scientific Procedures Act, 1986). Scientists should follow the guidelines established and approved by their institutional and national animal regulatory organizations.

1. Postnatal Electroporation

1.1. Preparation of Glass Capillaries, DNA Solution and Electroporator

1. Prepare pulled glass capillaries (O.D.: 1.5 mm, I.D.: 0.86 mm) for DNA injection. (Indicative settings for a Sutter P-97 capillary puller are: Heat 283; Pull 50; Velocity 90; Time 50). Make a mark on the capillary corresponding to a volume of approximately 2 µl.
2. Set the voltage of the electroporator to 5 square pulses, 50 msec/pulse at 100 V, with 850 msec intervals (100 V, pulse ON 50 msec, pulse OFF 850 msec, pulse 5).
3. Dilute high-purity (OD 260/280 > 1.80) endotoxin-free plasmid DNA to a final concentration of 1-2 µg/µl with endotoxin free Tris-EDTA buffer or PBS. It is recommended to add 0.1% Fast Green to the DNA solution (the dye should spread in the ventricle when successfully injected).
4. Prepare the 5-7 mm electrodes, the electrode gel and warm up the heating pad.

1.2. Electroporation

1. Remove a postnatal day 2 mouse pup from cage and anesthetize it by isoflurane inhalation (at a flow of ~0.6 L/min).
2. After approximately 1 min, determine the state of anesthetization using the foot pinch response. If no movement occurs, proceed with intraventricular injection.
3. Load capillary needle with 1-2 µl of DNA using an aspirator tube connected to the capillary.
4. Under a cold light source, hold the head of the pup between your thumb and index finger of your less dominant hand. Slightly pull the skin back on the head to help identification of the right injection point.
5. Consider a virtual line between the eye and the craniometric landmark lambda (**Figure 1A**). Insert the capillary needle at about one third of the length of this line from the lambda (about 1 mm from the line midpoint)[15]. Insert the capillary about 2 mm deep, making sure to avoid deep penetration in the brain.
6. Inject plasmid by blowing slowly through mouth (a syringe connected to the capillary can also be used). During this procedure, make sure your fingers are not applying too much pressure on the brain, as this can prevent successful plasmid injection.
7. Stop injection when a minimal amount of DNA solution is left in the capillary. It is advisable to inject less than 1 µl to avoid deleterious increase in intracranial pressure.
8. Coat both electrodes with gel and place them with the positive side on the lateral side of the hemisphere where the DNA was injected (**Figure 1A**). For DNA incorporation into the rostral SVZ, place electrodes slightly rostral to the injection point. Varying electrode position can achieve regional specificity of electroporation in different areas of the SVZ[22,25].
9. Initiate current transfer by pressing the pulse footswitch pedal. When the electroporation is complete, check the voltage on the electroporator display (voltage values should not be below 90 V, since lower voltage values correlate with poor electroporation efficiency).
10. Reanimate the pup under oxygen on heating pad for a few minutes and return it to the cage, placing it away from the mother. Make sure the mother retrieves the pup and reunites it with the rest of the litter. After electroporation, leave pups with their mother for 4-7 days before next step.

2. Preparation of Acute Brain Slice Cultures

2.1. Preparation of Solutions and Tools

1. The following solutions are required (it is also possible to use high-glucose DMEM for dissecting and imaging[17]):

   Dissection Medium (500 ml)

   Gey's Balanced media - 500ml

   45% Glucose - 5ml

   Movie Medium (10 ml)

   45% Glucose - 0.110 ml

   HEPES 1 M - 0.100 ml

Pen/Strep - 0.100 ml

FCS - 0.500 ml

B27 - 0.100 ml

Glutamine - 0.200 ml

Dulbecco Modified Eagle Medium (phenol red-free) - 8.89 ml

2. Warm up movie medium at 37 °C.
3. Place Millicell inserts into a 35 mm glass bottom culture dish containing 1 ml of movie medium and place in a humidified incubator at 37 °C/5% CO_2.
4. Prepare vibratome accessories (screwdriver, chamber, razor blades, glue).
5. Prepare dissection tools: scissors, small spatula, straight forceps.
6. Prepare tools for handling slices: small paintbrush or soft inoculating loop (size: 10 µl), plastic Pasteur pipettes, an ice box and several 6 cm plastic dishes.
7. Precool the dissection medium at 2-4 °C.

2.2. Preparation of Brain Slices

1. Fill a 6 cm dish with precooled dissection medium and place it on ice (to keep freshly cut slices).
2. Following cervical dislocation, use scissors to decapitate the mouse pup. Remove the scalp with a scalpel, cut the skull along the mid-sagittal suture from the OB to the cerebellum and gently remove the cranial flaps using forceps. Make sure the entire brain is exposed and carefully dissect it out using a spatula, taking special care not to damage the tissue. The dissection has to be done carefully but at the same time very quickly (less than a minute if possible). Cervical dislocation is our preferred method because terminal anesthesia with drugs/gas anesthetics can influence the migratory properties of the neuroblasts and the healthy state of the brain slice cultures, which need to be imaged relatively quickly following animal sacrifice.
3. Hemisect the brain with a razor blade (**Figure 2**). Discard uninjected hemisphere or use for other experiments.
4. Place a small piece of tape on the vibratome holder and use the minimal necessary amount of glue to attach the brain hemisphere on top of it (**Figure 2**). This prevents damaging of the holder surface due to repeated glue applications.
5. Let the glue dry for a few seconds.
6. Place holder in the vibratome tray filled with precooled dissection solution. Point the olfactory bulb towards the blade (**Figure 2**).
7. Begin cutting the brain hemisphere using appropriate settings. The following parameters are recommended: slice thickness 300 µm; speed ~3-5; frequency ~9. High frequency and low speed are recommended to prevent damage to the slice.
8. Collect slices using a small paintbrush or a soft inoculating loop. Only keep slices with visible OB (usually 2-3 slices/brain). Typically, the slice containing most of the RMS can be found at ~300 µm from the bottom surface.
9. Check slices under a standard fluorescent microscope for GFP signal (making sure to flip them over to check fluorescence on both sides), and choose the ones showing bright fluorescence along most of the RMS.

2.3. Culturing Brain Slices

1. In a cell culture hood, cut away the most caudal third of the brain slice and remove any glue traces using fine straight tweezers or a microdissection scalpel.
2. Delicately aspirate the slice using a plastic Pasteur pipette (cut the tip of the pipette to create a bigger opening, this will avoid damaging the slice) and place it on the center of a prewarmed Millicell insert.
3. Make sure the side with the brightest fluorescent signal is placed in contact with the Millicell insert (for imaging with an inverted microscope).
4. Remove excess dissection solution on top of the insert with a pipette.
5. Leave slice cultures to settle in a 37 °C/5% CO_2 incubator for at least for 1 hr before imaging.

3. Time-lapse Imaging of Neuroblast Migration

1. At least 2 hr before imaging, turn on the Perkin Elmer UltraViewVoX confocal spinning disk system, inverted Nikon Ti-E microscope, Hamamatsu C10600-10B (ORCA-R2) cooled digital CCD camera, and heating system (Solent Scientific) set at the constant temperature of 37 °C.
2. Open the Volocity software Acquisition module and click on "VIz" button and select the laser(s) for imaging.
3. Within 2 hr of brain slice preparation, place the glass bottom dish containing the brain slice in the imaging chamber on the microscope stage.
4. Use a Nikon CFI Super Plan Fluor ELWD 20X/0.45 objective under the appropriate fluorescent light to select and focus the area of the slice that will be imaged (*e.g.* the first descending part of the RMS just after the injection site, or the elbow region of the RMS, or just after the elbow before neuroblasts enter the OB).
5. Set up the time-lapse imaging with the following actions:
    1. On the microscope, click on the L100 button to allow laser scanning of the sample.
    2. In Volocity, open the UltraVIEW Laser Changer by selecting the appropriate laser (*e.g.* 488 nm laser for GFP).
    3. Set the exposure time (usually between 100-500 msec) and the laser intensity (usually 20-30%) depending on the intensity of the fluorescent signal.
    4. Adjust the image digital gain to improve cell visualization.
    5. Select the z-stack interval to image *inside* the brain slice (usually over a 100-120 µm interval). Choose an interval with a suitable number of isolated cells to avoid possible overlaps as much as possible (this will facilitate the subsequent tracking analysis).
    6. Select the spacing between each z-stack image (usually 2-4 µm).
    7. Choose the time interval between each z-stack capture (*e.g.* 3 min) and the total imaging duration (*e.g.* 3 hr).
    8. Click on the "save" icon to save the changes to the imaging parameters.

Copyright © 2013 Journal of Visualized Experiments

9. Press the recording button to start imaging.

6. Alternate imaging of control and experimental samples (for example vehicle/drug treatment or different electroporated plasmids) throughout the same day.

4. Analyzing Neuroblast Migration

1. In the Volocity Quantitation module, open the desired library created after completing a time-lapse experiment. Select "Extended Focus" from the top left box (**Figure 4A**, step 1).
2. Click on the "Measurements" tab to display the Measurement window (**Figure 4A**, step 2).
3. A list of tasks is visible in the bottom left of the screen. Drag "Track" (present under the "Miscellaneous" heading at the bottom of the list) on the space above. This will prompt the opening of a new window called "Track" at the top left section of the screen (**Figure 4A**, step 3).
4. Select "Points" from the "Input" tab in the Track window (**Figure 4A**, step 4).
5. Click on the Point Tool (**Figure 4A**, step 5).
6. Start tracking the migrating neuroblast by clicking with the mouse on the central area of the cell body and keep tracking the cell movement until the last time point of the time-lapse is reached (for example point number 61 for a 3 hr-long movie).
7. To obtain data choose "Make Measurement Item" from the Measurements Menu (**Figure 4B**, steps 6-7). A window will appear at the center of the screen.
8. In this window, select "A new measurement item called:" and type a name (**Figure 4C**, step 8). Remember to select the "All timepoints" option before pressing OK (**Figure 4C**, step 9). A measurement item file will appear under the time-lapse file and will contain parameters for quantitative analysis (*e.g.* migration distance, velocity, displacement, and displacement rate).
9. Double-click on the measurement item file to open it as a window (**Figure 4D**, step 10).
10. To visualize the single tracks of analyzed cells, choose "Tracked Point" from the "Display" options (**Figure 4D**, step 11).
11. Right click on the Measurement Item file and export it as a text file, which can then be imported in programs like Excel to analyze migration parameters.
12. To measure movements between consecutive frames and pauses made by each cell during the filming period, in the Measurement Item file select from the "Display" options "Point" or "Populations" and click on ID (every track has an unique ID). Export the resulting file by proceeding as explained in step 4.10.

Representative Results

Labeling of SVZ-derived migratory neuroblasts can be observed along the RMS, usually 4-8 days after a successful electroporation (**Figure 1B**). Longer time points can also be chosen, but less cells will be found in the RMS since most of them will have entered the OB. Neuroblasts start acquiring typical morphology and features of mature granule cells in the OB around 2-3 weeks after electroporation (not shown). After culturing for ~1 hr, brain slices from electroporated mouse pups can be reliably imaged for up to 3-4 hr. As previously reported[23,26], neuroblasts can display complex migration dynamics (**Figure 3** and **Video 1**), which can be quantitatively analyzed using cell tracking (**Figure 4**).

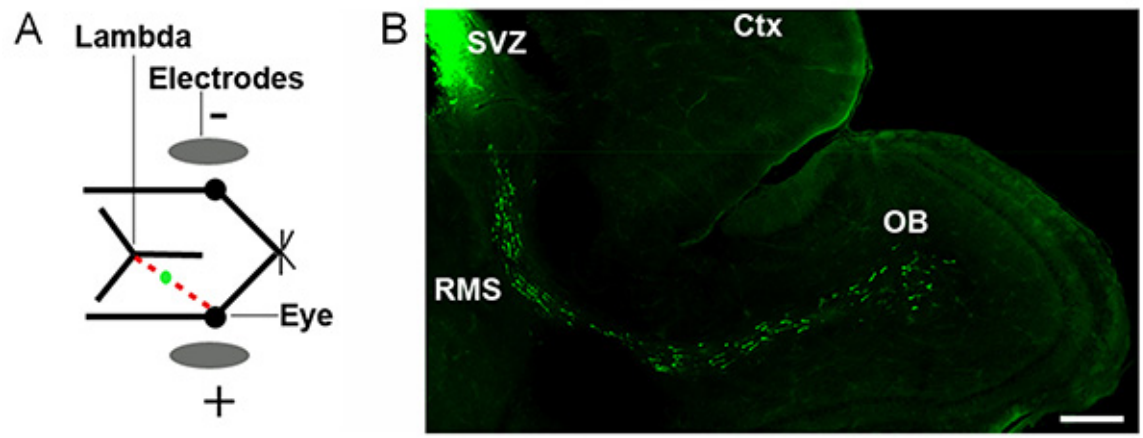

Figure 1. Postnatal *in vivo* electroporation. (A) Schematic drawing of *in vivo* electroporation of a postnatal day 2 mouse pup. A dotted line (red) connecting the eye to the craniometrical landmark lambda serves as a positional marker for capillary insertion. The injection point is indicated as a green dot. Grey oval shapes indicate electrode position as described in Boutin *et al.*[15] **(B)** Sagittal mouse forebrain slice immunostained for GFP 5 days after electroporation of a GFP-expressing plasmid. SVZ-derived migrating neuroblasts are visible along the RMS and some of them have started to reach the OB. Ctx: cortex; SVZ: subventricular zone; RMS: rostral migratory stream; OB: olfactory bulb. Bar, 400 µm. Click here to view larger image.

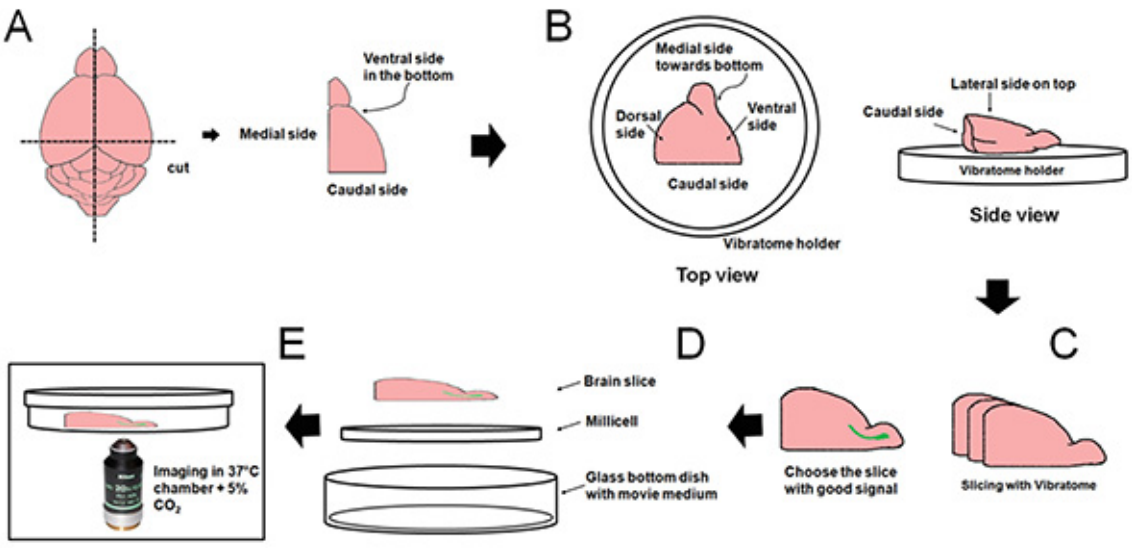

Figure 2. Schematic steps in the preparation of acute brain slice cultures for imaging. (A) Caudal and intrahemispheric cuts (dotted lines) are performed on a freshly dissected brain. **(B)** The electroporated brain hemisphere is placed onto a vibratome holder. **(C)** Sagittal slices are obtained with a vibratome and observed under a standard fluorescent microscope. **(D)** Slices with the best GFP signal are cultured for at least 1 hr on a Millicell insert inside a glass-bottom dish, and **(E)** subsequently placed in an environmental chamber for imaging by an inverted confocal spinning disk system. Click here to view larger image.

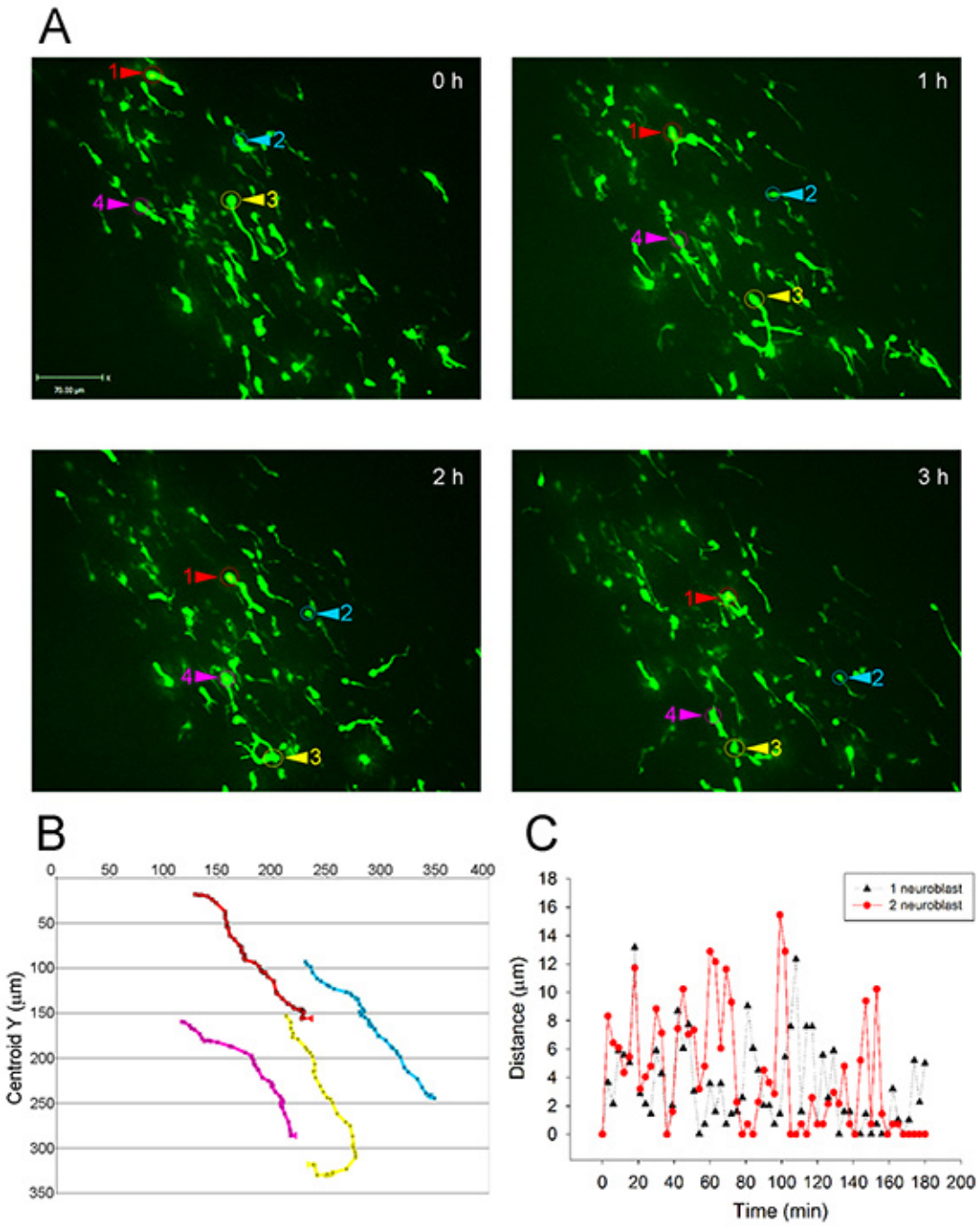

Figure 3. Time-lapse imaging of migrating neuroblasts. (A) Spinning disk time-lapse images of neuroblasts taken from a mouse sagittal brain slice 5 days after electroporation of a GFP-expressing plasmid. Images are 1 hr apart. Each panel is a z-stack projection of 28 consecutive images 4 µm apart. Arrowheads indicate 4 representative neuroblasts migrating along the RMS towards the olfactory bulb (located out of the picture at the bottom right corner). **(B)** Representative migratory paths obtained from time-lapse imaging of the 4 neuroblasts highlighted in **(A). (C)** Graph showing the distance migrated with time by 2 representative neuroblasts. Cells display a typical saltatory motile behavior. Bar, 70 µm. Click here to view larger image.

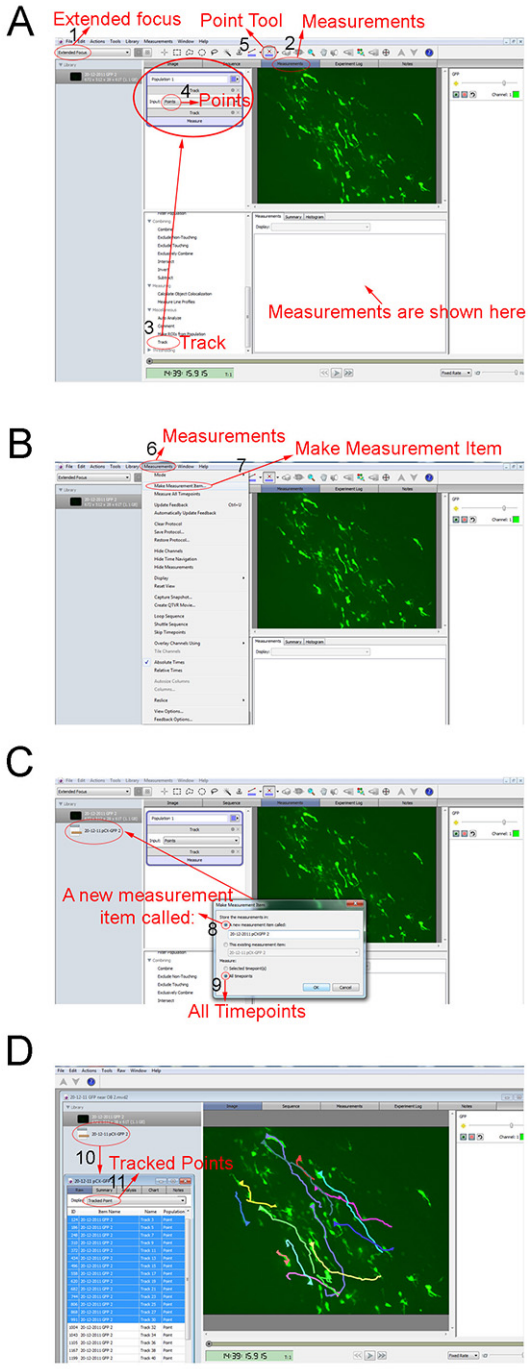

Figure 4. Tracking analysis of migrating neuroblasts. Sequential steps used to track migrating neuroblasts using Volocity software. Please see text for detailed description. Click here to view larger image.

Video 1: Time-lapse imaging of migrating neuroblasts. The movie shows a section of the mouse RMS with GFP-labeled migrating neuroblasts obtained 5 days after electroporation of a GFP-expressing plasmid. The OB is located out of view towards the bottom right corner. Confocal z-stacks were captured on a spinning disk confocal with a 20X objective every 3 min for 3 hr over a 120 µm interval. Playing speed: 10 frames/sec.

Discussion

Efficient migration of neural progenitors along the RMS ensures their subsequent maturation into functional neurons[10]. Prominent streams of neural progenitors directed towards the OB are visible in human infancy and are likely to play an important role in early postnatal human brain development[27]. Moreover, these cells are able to target sites of the brain affected by injury and neurodegeneration[4,28]. Being able to monitor in

real time the effect of gene manipulation on neuroblast dynamics becomes essential to fully understand how neuroblast movement is guided and regulated.

Here we have described a protocol to monitor SVZ-derived neuroblast migration by coupling *in vivo* postnatal electroporation with time-lapse confocal spinning disk microscopy of acute brain slice cultures. Specifically, we have shown how migrating neural progenitors can be labeled by electroporating in the SVZ a plasmid encoding GFP. Depending on the purpose of the study, several types of plasmids can be used (*e.g.* allowing expression of other fluorescent proteins or co-expression of wild type/mutant proteins of interest, CRE recombinase, or shRNA together with fluorescent proteins). We strongly recommend using plasmids containing the chicken beta actin CAG promoter[29] for *in vivo* expression. Imaging of brain slices obtained from electroporated animals can also be used to monitor the radial migration of neural progenitors in the OB at longer time points (7-10 days) after electroporation[30].

After an initial period of practice, *in vivo* postnatal electroporation becomes a very reliable method, allowing robust neuroblast labeling for both time-lapse imaging and immunofluorescence analysis. Moreover, this technique offers a fundamental advantage over transgenic mice expressing fluorescent proteins under neuroblast-specific promoters, where the majority of neuroblasts are labeled. Indeed, electroporation allows sparse labeling of neuroblasts, thus allowing detailed analysis of their morphology and migration dynamics. Compared to the stereotactic delivery of viral vectors[31], this technique is cheaper, faster and is very well tolerated by mouse pups. The main drawback consists in the fact that it is limited to early postnatal stages. Indeed, we strongly recommend using postnatal day 2 mouse pups, since we observed a substantial decrease in labeling efficiency at later times, when viral delivery methods become more suitable[31]. However, strategies like electroporation of CRE-expressing plasmids in appropriate mouse genetic models may be used to study neurogenesis in adult stages[22].

Two-photon, standard confocal, spinning disk confocal, and wide-field fluorescence microscopy can all be used to visualize neuroblast migration[17,23,24]. Spinning disk confocal microscopy is a cheaper alternative to two-photon microscopy. It allows 3D imaging at higher speed through multiple z planes, limiting photobleaching compared to standard confocal microscopy and offering a higher resolution than wide-field fluorescence imaging[31-33]. The majority of migrating neuroblasts have a clearly visible soma and a highly dynamic leading process tipped with a growth cone.

As described by others[17], having a perfusion chamber would allow to directly assess the effects of control and drug treatments on the same brain slice. While we consider this an important advantage, the protocol described here shows that it is not absolutely necessary for cell viability to perfuse slices with oxygenated artificial cerebrospinal fluid (aCSF) or high glucose DMEM[17,33]. Moreover, using an inverted microscope allows easier objective manipulation compared to upright microscopes equipped with water immersion objectives. We found that using a 20X long-distance objective is an optimal compromise, enabling tracking of a good number of cells (usually 25-40 per slice) and producing good resolution images (**Video 1**). Automatic tracking is also possible, however it is not always reliable. For this reason we recommend visual and, if necessary, manual verification of automatic tracking data. Higher magnification imaging of single neuroblasts can be performed using a Nikon Fluor DIC M/N2 40X/0.80W objective. The freely available ImageJ plugin MTrackJ can also be used to measure basic track statistics[33], however some raw data acquisition files may be incompatible with this software and may need to be converted to suitable formats for analysis, which in certain cases can be time-consuming. The combination of image acquisition and analysis modules provided by the Volocity software allows an immediate transition from image capturing/processing to quantitative analysis of migratory parameters (speed, displacement, *etc.*), which can be readily visualized in a variety of graphs (*e.g.* showing migratory patterns/distance/directionality/speed/persistence/time spent immobile, *etc.*).

Neuroblasts display a saltatory movement, alternating migratory to stationary phases[34,35] (**Figure 3C**). In light of the fact that stationary phases can be between 4-10 min[32], we believe that capturing images every 3 min represents a good compromise to follow migrating neuroblasts with minimum illumination and photodamage. We rarely see cells stopping for more than 6 min, and find that, over a 3-hr period, they generally spend an average of 30 min immobile. According to previous reports, neuroblasts have an average speed of 60-100 μm/hr and an average meandering index (actual displacement over the migrated distance), slightly above 0.6[23,36], which is in agreement with our observations. Typically, about 60% of cells show a "migratory" behavior (*e.g.* following almost linear migration paths, with a meandering index between 0.6-1) and 20-25% are "exploratory" (with a meandering index between 0-0.4), while the remaining ~20% can be classified as "intermediate" (alternating migratory and exploratory phases, with a meandering index between 0.4-0.6).

We recognize that there are still limitations to this technique, since we cannot film neuroblasts for more than 3-4 hr without observing substantial alterations in their motility. Filming duration could be increased by lowering imaging frequency (however this will affect the accuracy of the migration analysis) as well as by adopting a perfusion chamber to optimize environmental conditions for imaging. However, the protocol described here including a 3-4 hr imaging period represents a suitable compromise enabling the collection of sufficient data for quantitative analysis of migration. Indeed, so far we have managed to detect the effects produced on migration by protein overexpression/downregulation[37] or by pharmacological manipulation after comparison with appropriate controls (Sonego and Zhou, unpublished results).

In conclusion, the combination of *in vivo* postnatal electroporation with spinning disk imaging of brain slice cultures represents a powerful tool to monitor the dynamics of neuroblast migration in a system closely resembling the *in vivo* environment, offering the possibility to investigate in depth the molecular mechanisms controlling neuroblast motility.

Disclosures

The authors have nothing to disclose.

Acknowledgements

M.S. and Y.Z. are supported by KCL and KCL-China PhD studentships. M.O. was funded by a Biotechnology and Biological Sciences Research Council PhD studentship. We thank Masaru Okabe and Jun-ichi Miyazaki for the pCX-EGFP plasmid and Alain Chedotal and Athena Ypsilanti for valuable advice on electroporation.

References

1. Ming, G. L. & Song, H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron.* **70**, 687-702, doi:10.1016/j.neuron.2011.05.001 (2011).
2. Deng, W., Aimone, J. B. & Gage, F. H. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **11**, 339-350, doi:10.1038/nrn2822 (2010).
3. Lazarini, F. & Lledo, P. M. Is adult neurogenesis essential for olfaction? *Trends Neurosci.* **34**, 20-30, doi:10.1016/j.tins.2010.09.006 (2011).
4. Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. & Lindvall, O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat. Med.* **8**, 963-970, doi:10.1038/nm747 (2002).
5. Emsley, J. G. & Hagg, T. alpha6beta1 integrin directs migration of neuronal precursors in adult mouse forebrain. *Exp. Neurol.* **183**, 273-285 (2003).
6. Sundholm-Peters, N. L., Yang, H. K., Goings, G. E., Walker, A. S. & Szele, F. G. Subventricular zone neuroblasts emigrate toward cortical lesions. *J. Neuropathol. Exp. Neurol.* **64**, 1089-1100 (2005).
7. Doetsch, F. & Alvarez-Buylla, A. Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 14895-14900 (1996).
8. Ming, G. L. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223-250, doi:10.1146/annurev.neuro.28.051804.101459 (2005).
9. Pathania, M., Yan, L. D. & Bordey, A. A symphony of signals conducts early and late stages of adult neurogenesis. *Neuropharmacology.* **58**, 865-876, doi:10.1016/j.neuropharm.2010.01.010 (2010).
10. Belvindrah, R., Nissant, A. & Lledo, P. M. Abnormal neuronal migration changes the fate of developing neurons in the postnatal olfactory bulb. *J. Neurosci.* **31**, 7551-7562, doi:10.1523/JNEUROSCI.6716-10.2011 (2011).
11. Gotts, J. E. & Chesselet, M. F. Mechanisms of subventricular zone expansion after focal cortical ischemic injury. *J. Comp. Neurol.* **488**, 201-214, doi:10.1002/cne.20609 (2005).
12. Goings, G. E., Sahni, V. & Szele, F. G. Migration patterns of subventricular zone cells in adult mice change after cerebral cortex injury. *Brain Res.* **996**, 213-226 (2004).
13. Romanko, M. J. *et al.* Roles of the mammalian subventricular zone in cell replacement after brain injury. *Prog. Neurobiol.* **74**, 77-99, doi:10.1016/j.pneurobio.2004.07.001 (2004).
14. Saito, T. *In vivo* electroporation in the embryonic mouse central nervous system. *Nat. Protoc.* **1**, 1552-1558, doi:10.1038/nprot.2006.276 (2006).
15. Boutin, C., Diestel, S., Desoeuvre, A., Tiveron, M. C. & Cremer, H. Efficient *in vivo* electroporation of the postnatal rodent forebrain. *PloS One.* **3**, e1883, doi:10.1371/journal.pone.0001883 (2008).
16. Barnabe-Heider, F. *et al.* Genetic manipulation of adult mouse neurogenic niches by *in vivo* electroporation. *Nat. Methods* **5**, 189-196, doi:10.1038/nmeth.1174 (2008).
17. Platel, J. C. *et al.* NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. *Neuron.* **65**, 859-872, doi:10.1016/j.neuron.2010.03.009 (2010).
18. Pathania, M. *et al.* miR-132 enhances dendritic morphogenesis, spine density, synaptic integration, and survival of newborn olfactory bulb neurons. *PloS One.* **7**, e38174, doi:10.1371/journal.pone.0038174 (2012).
19. dal Maschio, M. *et al.* High-performance and site-directed in utero electroporation by a triple-electrode probe. *Nat. Commun.* **3**, 960, doi:10.1038/ncomms1961 (2012).
20. Oudin, M. J. *et al.* Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain. *J. Neurosci.* **31**, 4000-4011, doi:10.1523/JNEUROSCI.5483-10.2011 (2011).
21. Lacar, B., Young, S. Z., Platel, J. C. & Bordey, A. Imaging and recording subventricular zone progenitor cells in live tissue of postnatal mice. *Front. Neurosci.* **4**, doi:10.3389/fnins.2010.00043 (2010).
22. Feliciano, D. M., Lafourcade, C. A. & Bordey, A. Neonatal subventricular zone electroporation. *J. Vis. Exp.* **72**, e50197, doi:10.3791/50197 (2013).
23. Nam, S. C. *et al.* Dynamic features of postnatal subventricular zone cell motility: a two-photon time-lapse study. *J. Comp. Neurol.* **505**, 190-208, doi:10.1002/cne.21473 (2007).
24. James, R., Kim, Y., Hockberger, P. E. & Szele, F. G. Subventricular zone cell migration: lessons from quantitative two-photon microscopy. *Front. Neurosci.* **5**, 30, doi:10.3389/fnins.2011.00030 (2011).
25. Fernandez, M. E., Croce, S., Boutin, C., Cremer, H. & Raineteau, O. Targeted electroporation of defined lateral ventricular walls: a novel and rapid method to study fate specification during postnatal forebrain neurogenesis. *Neural Dev.* **6**, 13, doi:10.1186/1749-8104-6-13 (2011).
26. Saha, B., Ypsilanti, A. R., Boutin, C., Cremer, H. & Chedotal, A. Plexin-b2 regulates the proliferation and migration of neuroblasts in the postnatal and adult subventricular zone. *J. Neurosci.* **32**, 16892-16905, doi:10.1523/JNEUROSCI.0344-12.2012 (2012).
27. Sanai, N. *et al.* Corridors of migrating neurons in the human brain and their decline during infancy. *Nature.* **478**, 382-386, doi:10.1038/nature10487 (2011).
28. Tattersfield, A. S. *et al.* Neurogenesis in the striatum of the quinolinic acid lesion model of Huntington's disease. *Neuroscience.* **127**, 319-332, doi:10.1016/j.neuroscience.2004.04.061 (2004).
29. Niwa, H., Yamamura, K. & Miyazaki, J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene.* **108**, 193-199 (1991).
30. Lledo, P. M., Alonso, M. & Grubb, M. S. Adult neurogenesis and functional plasticity in neuronal circuits. *Nat. Rev. Neurosci.* **7**, 179-193, doi:10.1038/nrn1867 (2006).
31. Khlghatyan, J. & Saghatelyan, A. Time-lapse imaging of neuroblast migration in acute slices of the adult mouse forebrain. *J. Vis. Exp.* **67**, e4061, doi:10.3791/4061 (2012).
32. Snapyan, M. *et al.* Vasculature guides migrating neuronal precursors in the adult mammalian forebrain via brain-derived neurotrophic factor signaling. *J. Neurosci.* **29**, 4172-4188, doi:10.1523/JNEUROSCI.4956-08.2009 (2009).
33. Platel, J. C., Heintz, T., Young, S., Gordon, V. & Bordey, A. Tonic activation of GLUK5 kainate receptors decreases neuroblast migration in whole-mounts of the subventricular zone. *J. Physiol.* **586**, 3783-3793, doi:10.1113/jphysiol.2008.155879 (2008).

Copyright © 2013 Journal of Visualized Experiments

34. Schaar, B. T. & McConnell, S. K. Cytoskeletal coordination during neuronal migration. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13652-13657, doi:10.1073/pnas.0506008102 (2005).
35. Valiente, M. & Marin, O. Neuronal migration mechanisms in development and disease. *Curr.Opin. Neurobiol.* **20**, 68-78, doi:10.1016/j.conb.2009.12.003 (2010).
36. Comte, I. *et al.* Galectin-3 maintains cell motility from the subventricular zone to the olfactory bulb. *J. Cell Sci.* **124**, 2438-2447, doi:10.1242/jcs.079954 (2011).
37. Sonego M., *et al.* Fascin regulates the migration of subventricular zone-derived neuroblasts in the postnatal brain. *J. Neurosci.* **33**, 12171-12185, doi:10.1523/JNEUROSCI.0653-13.2013 (2013).

Copyright © 2013 Journal of Visualized Experiments