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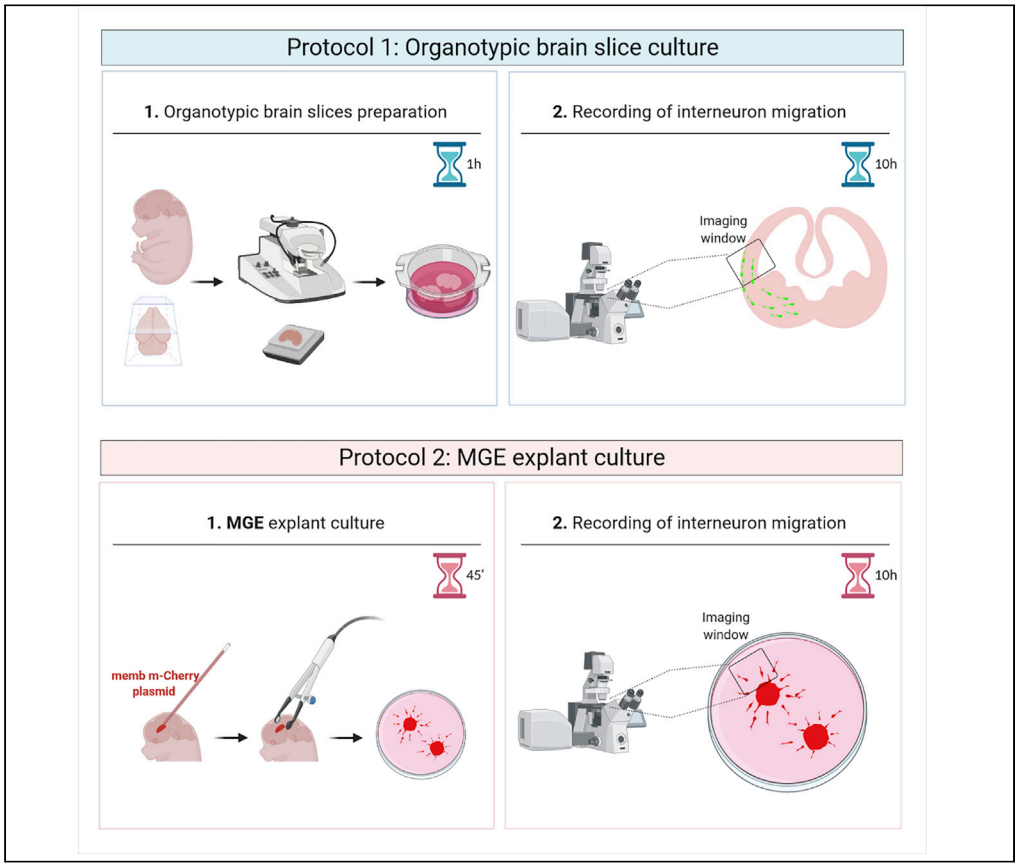
Time lapse recording of cortical interneuron migration in mouse organotypic brain slices and explants



Interneuron migration involves repetitive cycles of pausing and motion that include nucleokinesis. Here, we provide a step-by-step description of how to culture and record the migration of cortical interneurons. We provide two culture models: the first includes organotypic brain slices and the second medial ganglionic eminence (MGE) explants. While organotypic brain slices provide a close-to-physiological context to analyze interneuron migration into cortical streams, MGE explants are appropriate to investigate the fine details of interneuron morphology remodeling during movement.

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Highlights

Descriptive protocols to analyze interneuron migration parameters

Detailed method to generate and maintain mouse organotypic brain slices in culture

Step-by-step procedure for *in vitro* electroporation of DNA plasmids in mouse brain tissues

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Time lapse recording of cortical interneuron migration in mouse organotypic brain slices and explants

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SUMMARY

Interneuron migration involves repetitive cycles of pausing and motion that include nucleokinesis and dynamic branching of the leading process. Here, we provide a step-by-step description of how to culture and record the migration of cortical interneurons. We provide two culture models: the first includes organotypic brain slices and the second medial ganglionic eminence (MGE) explants. While organotypic brain slices provide a close-to-physiological context to analyze interneuron migration into cortical streams, MGE explants are appropriate to investigate the fine details of interneuron morphology remodeling during movement.

For complete details on the use and execution of this protocol, please refer to Silva et al. (2018).

BEFORE YOU BEGIN

Timing: 3–5 min

Here, we provide a technical procedure for preparing organotypic brain slices or MGE explants from mouse embryos for live imaging of cortical interneurons expressing fluorescent proteins. This procedure can be adapted to analyze neuronal migration in the brain of other species. A factor to consider is the timing of interneuron generation and migration for each species. For example, organotypic slices from mouse brains are analyzed between embryonic stages (E) E12.5 and E16.5, while MGE explants are usually prepared between E12.5 and E13.5 to image interneuron migration.

Preparation of Culture Medium (for Culture of Brain Slices and Preparation of Cortical Feeder; See step 11 and step 36 in the step-by-step detailed protocol 1 and 2 respectively)

1. Supplement the Neuro-basal Medium with 2% B27 supplement, 1% N2 supplement, 1% penicillin/streptomycin and 1% L-glutamine; protect the solution from light (as vitamin A contained in B27 supplement is light-sensitive) and store at 4°C for up to two weeks.

Reagent	Final concentration	Amount
Neurobasal Medium	-	47.5 mL
B27 supplement	2%	1 mL
N2 supplement	1%	500 µL
penicillin/streptomycin	1%	500 µL
L-glutamine	1%	500 µL

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Low melting agarose	Bio-Rad	1613114
HBSS	Lonza	10-527F
Neurobasal Medium	Gibco, Thermo Fisher Scientific	21103049
B-27 Supplement	Gibco, Thermo Fisher Scientific	17504044
N-2 Supplement	Gibco, Thermo Fisher Scientific	17502048
Penicillin/streptomycin	Gibco, Thermo Fisher Scientific	15140122
L-Glutamine	Lonza	BE17-605E
Isoflurane	Abbott Laboratories	N/A
Rectapur® ethanol 99% denatured	VWR	85830.460
Poly-L-ornithine	Sigma-Aldrich	P4638
Laminin	Sigma-Aldrich	L2020
DPBS	Lonza	17-512F
Fast green	Sigma-Aldrich	F7252
China Ink	Indian Ink	N/A
Critical commercial assays		
NucleoBond Xtra Midi kit for transfection-grade plasmid DNA	Macherey-Nagel	740410.50
Experimental models: organisms/strains		
E13.5 pregnant *RjOrl*:Swiss mice (outbred)	Janvier Labs	N/A
E13.5 pregnant Dlx5/6-Cre-IRES-EGFP *RjOrl*:Swiss mice (outbred)	The Jackson laboratory	(Tg(ml56i-cre,EGFP)1Kc/J
Recombinant DNA		
pCX memb-mCherry	(provided by Dr. Xavier Morin)	N/A
Software and algorithms		
Fiji–ImageJ	(Schindelin et al., 2012)	https://imagej.nih.gov/ij/
MTrackJ–ImageJ	(Meijering et al., 2012)	https://imagescience.org/meijering/software/mtrackj/
Other		
Isoflurane anesthesia station	Harvard Apparatus	34-1041
Routine stereo microscope Leica M80	Leica	N/A
Microwave PHILIPS M742 Sensor Space Cube 50	Philips	N/A
Galaxy® 170 S CO_2 Incubator	Eppendorf	N/A
50-mL Conical bottom tube	Greiner	210261
1.5-mL Reaction tubes	Greiner	616 201
Vertical shaker	Thermo Fisher Scientific	88881002
Vacuum bell	Nalgene	5312-0230
Centrifuge	Eppendorf	5804 R
Razor blade Astra superior platinum	Astra	N/A
Double microspatula	Carl Roth	YL43.1
Hardened Fine Scissors	Fine Science Tools (FST)	14090-11
Dumont #5CO Forceps	Fine Science Tools (FST)	11295-20
ZEISS SteREO Discovery V8 stereomicroscope	Zeiss	N/A
X-Cite series 120Q fluorescence lamp	Excelitas Technologies	N/A
Petri-dish 35 × 10 mm	Thermo Fisher Scientific	153066
Tork® Paper	Tork®	8031900
Perforated spoon	Fine Science Tools (FST)	10370-18
Super Glue-3	Loctite	N/A
Vibratome Leica VT1000S	Leica Microsystems	VT1000S
MatTek glass-bottom 35 mm dishes	MatTek Corporation	P35G-0-20-C
Millicell cell culture insert	Millipore	PICM0RG50

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
CELLview™ 4 compartments, glass-bottom 35 × 10 mm dishes	Greiner Bio-One	627871
Easystrainer 40 µm (cell strainer)	Greiner Bio-One	542040
Borosilicate glass capillaries	Harvard Apparatus	30-0016
Micropipette Puller P-97 Flaming/Brown	Sutter Instrument	P-97
Microloader tips	Eppendorf	5242956003
Spring scissors	Aesculap	OC498-R
FemtoJet microinjector	Eppendorf	5252000013
Tweezer platinum electrodes	Sonidel	CUY650P3
Electroporator	BTX	ECM 830
SYLGARD™ 184 Silicone Elastomer Kit	Dow®	N/A
Petri dish 100 × 17 mm	Thermo Fisher Scientific	150350
Micro Knife–Plastic Handle	Fine Science Tools (FST)	10316-14
Nikon A1R confocal microscope	Nikon	N/A
Zeiss LSM 880 confocal microscope	Zeiss	N/A

STEP-BY-STEP METHOD DETAILS

Protocol 1 – recording and analysis of cortical interneuron migration parameters in organotypic brain slices

Organotypic brain slices culture is a well suited protocol to study interneuron migration parameters in a context close to *in-vivo* situation. Here, we used organotypic brain slices at E13.5 from a transgenic mouse model characterized by expression of GFP by interneurons (Dlx5/6-Cre-IRES-EGFP).

Preparation of the embedding solution

Timing: 25 min

1. Prepare a 2%–4% agarose solution for brain embedding by adding 0.5 g to 1 g of low-melting agarose into a 50 mL conical tube containing 25 mL of sterile HBSS.

   Note: a 2% solution is recommended for E12.5 brain, while a 4% solution is well-suited for E13.5 to E16.5 brains.

2. Transfer the tube into a microwave and bring the solution to boil. Keep the tube cap loose until the agarose is completely dissolved.

   Note: prevent excess boiling and overflow while heating the solution; when the solution starts to boil, stop the microwave, close the tube and mix by inverting the tube several times; repeat the heating step until complete dissolution of the agarose. Protect your hands from burns by wearing heat-resistant gloves.

3. Transfer the tube into a 37°C incubator and allow the solution to cool down during approximately 20 min keeping the cap loose to allow air bubbles to escape. It is crucial to cool down the agarose solution to avoid burning the brain meninges during the embedding procedure.

Dissection and embedding of embryonic brains

Timing: 30 min

The embedding of brains in polymerized agarose solution allows mechanical support during slicing, and prevent the expansion of the tissue while maintained in culture.

4. Time-mated females are sacrificed by cervical dislocation followed by decapitation to confirm death.

   Note: Euthanasia methods may vary from one country to another. Please refer to your local ethical regulations. If needed, anaesthesia can be induced before cervical dislocation using an induction chamber filled with a mixture of 96% air and 4% isoflurane.

5. After sacrifice, disinfect the abdomen of the pregnant female with 70% alcoholic solution and open it with fine scissors under a laminar flow hood. Collect the uterus and excise the embryos from the amniotic sac using forceps. Immediately harvest the embryos in sterile ice-cold HBSS; section the head and dissect the embryonic brain. Here, we used E13.5 embryos.

   Note: For embryos between E12.5 and E13.5, brain dissection must be performed carefully in order to preserve the meninges that will maintain the brain structure.

   Note: If a transgenic mice model expressing a fluorescent protein as reporter is used, a fluorescence binocular microscope might be useful to select the transgenic embryos. Here, we used Dlx5/6-Cre-IRES-EGFP transgenic mice.

6. Pour the low-melting agarose solution previously cooled to 37°C in 35 × 10 mm petri-dish. Do not fill completely the dish to prevent the dish cover to touch the agarose.

   Note: Do not embed the brains in an agarose solution containing air bubbles; discard the top part of the solution containing air bubbles if necessary, before pouring the solution in the petri-dish.

7. Quickly transfer the brains into the agarose solution using a perforated spoon; remove the excess of HBSS from the spoon using Tork® paper.
8. Carefully rotate the brains with forceps until the olfactory bulbs face the bottom of the petri-dish, and the hindbrain locating at the top.

   Note: This orientation is optimal to perform coronal slices at the vibratome by starting the slicing from the olfactory bulbs; for sagittal or horizontal slicing, it might be easier to orient the brains differently during embedding.

   ⚠ CRITICAL: Step 6 to 8 must be completed quickly; agarose solution undergoes polymerization in few minutes at a temperature under 37°C and in few seconds at 4°C. Rotation of the brains in a rigid polymerizing agarose solution could damage the tissue because of the pressure.

9. Place the petri-dish on ice ensuring that brains hold a good orientation; repeatedly rectify brain position with the help of forceps until the agarose solution is solid enough to maintain the brains.

   ⚠ CRITICAL: A good orientation of brains within the agarose block at this step is key to allow proper slicing with the vibratome. The parallel positioning of the sectioning plane and the bottom of the petri-dish allows you to immediately glue the top side of the agarose block, without additional adjustments. However, if the brain is not perfectly oriented after agarose polymerization, adjust progressively the orientation by cutting the bottom of the agarose block with a razor blade.

10. Let the agarose block solidify on ice for 20 min.

Slicing and culture of brain slices

Timing: 30 min

Organotypic brain slices are obtained with a vibratome and maintained on a millicell membrane placed in a MatTek® glass-bottom dish filled with culture medium. This method is recommended to maintain the viability of organotypic slices for one to three days.

11. Prepare the MatTek® dish under the cell culture hood by adding 1.6 mL of culture medium. Install the millicell with sterile forceps. Place it progressively, starting with an angle of 30° to prevent the formation of air bubbles underneath. Transfer the dish into an incubator set at 37°C and 5% CO_2.
12. Fill the vibratome tray with ice-cold HBSS and surround the tray with ice. Set the following parameters: section thickness: 300 μm; speed: 3; frequency: 3.

Note: These parameters are adjusted for a vibratome Leica VT100S and might need optimization according to the type of vibratome.

13. Cut the agarose block with a truncated pyramid shape using a razor blade, with the hindbrain towards the major base (at the bottom) and the olfactory bulbs facing the top of the pyramid (Figures 1A and 1B). This shape allows a good stability of the agarose block and prevents its break.
14. Glue the agarose block on its major base on the vibratome support making sure that the olfactory bulbs face upward. With this orientation, the vibratome will perform slicing from rostral (olfactory bulbs) to caudal (hindbrain) regions.
15. Place the support with the brain in the vibratome tray. The ventral part of the brain should face the blade of the vibratome (Figure 1C), as starting to slice from the cortex might tear it apart. By positioning the brain this way, the razor blade will perform a forward movement starting from the ventral part of the brain and finishing by the dorsal part (cortex) for each slice.

Note: It is possible to cut several brains at the same time. However, make sure that the brains placed on the vibratome support fit with the size of the razor blade (otherwise the brains would not be cut properly).

16. Proceed to the slicing until obtaining medial to caudal brain slices.

Note: Only comparable slices from different brains should be used for the analysisin order to minimize the analysis bias due to regional differences in interneuron migration streams.

17. Collect brain slices with a spatula without detaching the tissue from its surrounding agarose. Transfer the slices on the millicell insert (Figure 1D) and place the dish in the incubator at 37°C with 5% CO_2.

⚠ CRITICAL: Keep adding more ice around the tray during the whole procedure if needed. Keeping a low temperature reduces cell death and prevents the separation of the tissue from the agarose.

Note: Up to five slices can be collected on a millicell, depending on the width of the agarose block. Do not overlap the edges of the slices as it will compromise the proper live-imaging acquisition (e.g., expansion of the tissue; blurred images).

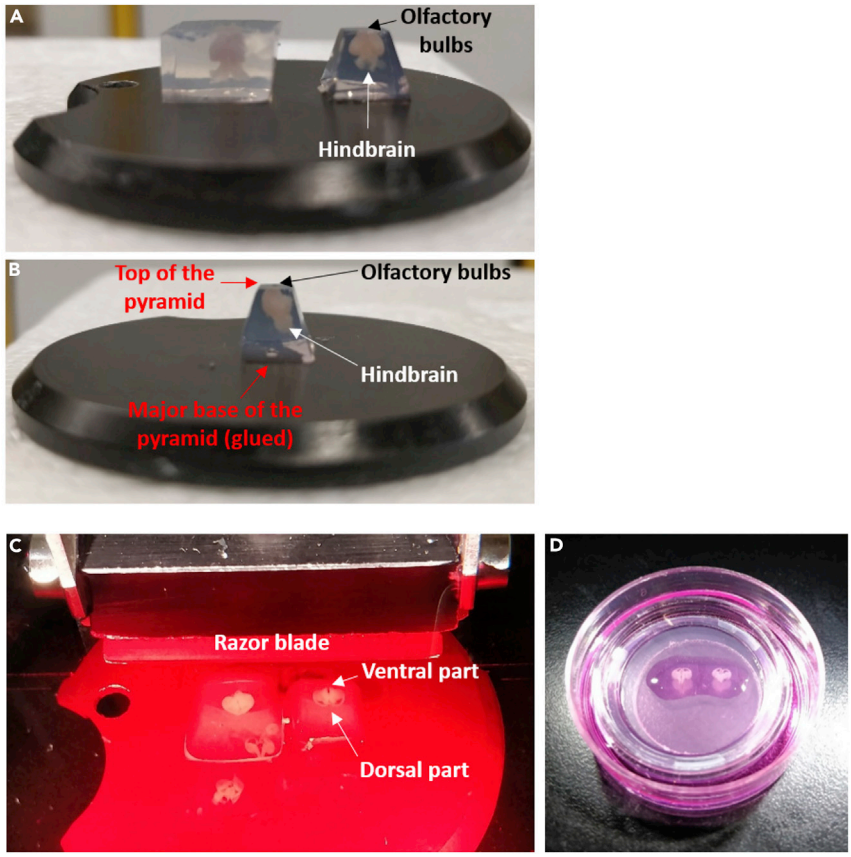

Figure 1. Organotypic brain slice preparation

(A and B) Embedded E13.5 brains. Trim the agarose block into a truncated pyramidal shape so that olfactory bulbs face the smallest edge of the pyramid, the hindbrain facing the major base glued on the vibratome support (see figure A on the right, and figure B).

(C) Vibratome slicing. Place the brains in the vibratome tray containing ice-cold HBSS with the ventral part facing the blade.

(D) Culture of slices. The slices are cultured on a millicell membrane placed in a MatTek® glass-bottom whose bottom is irrigated with the maintenance culture medium to optimize gas exchange in the slices.

Time-lapse microscopy

Timing: 10 h

After 4 to 24 h of recovery in the incubator, the migration of GFP-expressing cortical interneurons can be recorded for few hours at the confocal microscope.

18. Place the dishes in the atmosphere-controlled recording chamber of an inverted confocal microscope (here we used a Nikon A1R microscope) and set the parameters to 37°C and 5% CO_2.
19. Focus on interneuron migratory streams in the cortex using a 20× long-range objective (0.45 mm) (see Figure 4A for a representative image of the region of interest). The two interneuron migratory streams in the marginal zone (MZ) and intermediate zone (IZ) should be clearly recognizable.
20. Acquire time-lapse images. Image at least 5 Z-steps of 2 μm every 5 min for 5 to 10 h. Interneurons should be traceable for at least 3 h.

⚠ CRITICAL: it is very important to set the laser power at a level allowing the visualization of the migrating cell bodies without inducing phototoxicity; fluorescent cells are photosensitive and an over-exposition would impair migration parameters and viability. Dying cells can be recognized by the appearance of large vacuoles or fluorescent protein aggregation, as well as disintegrated cell processes. It should be mentioned that recording with a two-photon microscope is particularly well-suited as it reduces phototoxicity.

Note: Any confocal microscope can be used as long as an atmosphere-controlled recording chamber and a long-range objective are available. The 20× objective is well suited for this experiment as it allows to follow migrating interneurons during few hours before they leave the image field. A 40× long-range objective might work as well.

Protocol 2 – recording and analysis of cortical interneuron branching using MGE explant culture

This protocol consists in a 2D culture of migrating interneurons that exit from MGE explants. Compared to the first protocol, it allows a more detailed analysis of interneuron branching behavior.

Preparation of black sylgard dissection dishes

Timing: 7 h

21. Mix 5 mL of pre-polymer with 45 mL of catalyst coming from the SYLGARD™ 184 Silicone Elastomer Kit (as indicated in the manufacturer's datasheet) in a 50 mL conical tube. Add 3 mL of Chinese ink and mix with a spatula.
22. Place the tube on a 360°C vertical shaker for 30 min. The ink should be homogeneously mixed to the solution.
23. Centrifuge at 120 xg for 5 min.
24. Prepare two Sylgard dishes by pouring 25 mL of the solution in two 100 × 17 mm petri-dishes.
25. Place the dishes in a vacuum bell for 1h to remove the bubbles. Remove the remaining bubbles with a micropipette tip if necessary.
26. Heat the dishes at 60°C for minimum 5 h.

Note: These dishes can be prepared a long time before the day of the experiment. After use, clean them with ethanol 70%; rinse them with water and let them dry. They can be reused for next experiments.

Plasmid preparation

Timing: 3–5 min

27. Prepare the plasmid solution at 1 μg/μL in PBS with 0.05% Fast Green for a total volume of 15 μL.
28. Pull glass microcapillaries by using a P-97 Flaming/Brown type Micropipette Puller. Set the following parameters: heat, 634; pull, 92; velocity, 115; time, 210.

Note: the efficacy of the heating filament from one instrument to another might be slightly different; if needed, adapt the pulling conditions on your instrument.

Preparation of homochronic cortical feeder layer

Timing: 5 h 30

The migration of interneurons out of MGE explants strongly depends on the coating used to cover the glass-bottom dish. An homochronic layer of cortical cells is the most appropriate migration substrate for MGE explants as it recapitulates most properties of cortical interneuron migration in vivo (Bellion et al., 2005). The cortical feeder layer should be prepared the day before culturing the MGE explants.

29. Coat the 4 compartments glass-bottom dish with 0.1 mg/mL poly-L-ornithine (pH=8.4) diluted in sterile water. Add 200 µL per compartment. Incubate the dish in an incubator at 37°C with 5% CO_2 for 45 min.

    Note: Microscopes usually allow imaging one or two petri-dishes at a time. Therefore, the 4 compartments dishes are useful to test different conditions. For experiments with only one condition, the non-compartmented glass-bottom dishes work as well (but adapt the volume to 700 µL per dish).

30. Wash the dish compartments three times with sterile water.
31. Dilute laminin in sterile ice-cold PBS to reach a final concentration of 5 µg/mL. Add 200 µL per compartment and incubate 2 h at 37°C. Alternatively, laminin can be incubated up to 24 h at 37°C.
32. Wash the dish compartments three times with sterile PBS. Place the dish with PBS back to the 37°C incubator.

    Note: Make sure to not let the dish dry as it will disrupt laminin structure and thus impair cell attachment.

33. Sacrifice the E13.5 wild-type pregnant female by cervical dislocation followed by decapitation to confirm death.

    Note: Euthanasia methods may vary from one country to another. Please refer to your local ethical regulations. If needed, anaesthesia can be induced before cervical dislocation using an induction chamber filled with a mixture of 96% air and 4% isoflurane.

34. After sacrifice, disinfect the abdomen of the pregnant female with 70% alcoholic solution and open it with fine scissors under a laminar flow hood. Extract the uterus and excise the embryos from the amniotic sac using forceps. Immediately harvest the embryos in sterile ice-cold HBSS; section the heads and dissect the brains (Figures 2A and 2B). Clean brains from meninges as much as possible without damaging cortices. It is recommended to dissect one embryo per dish plus one extra embryo (i.e. two embryos for one dish, three embryos for two dishes, etc).
35. Separate cortices from ganglionic eminences (Figures 2C and 2D) using forceps to pin ganglionic eminences and hold the tissue, and a micro knife to cut at the border between cortex and ganglionic eminences. Transfer cortices using forceps in one sterile Eppendorf® tube filled with HBSS and keep on ice.
36. Under a culture hood, replace HBSS by 800 µL of cold culture medium and mechanically dissociate the embryo cortices by pipetting up and down with a P1000 micropipette (~20 times).
37. Filtrate the dissociated cells through a sterile 40 µm cell strainer.
38. Replace the PBS from the coated dishes by 200 µL of dissociated cells per compartment.

    Note: For the preparation of more than one dish, add the amount of culture medium necessary to the dissociated cell solution.

39. Place the dish back into the incubator set to 37°C and 5% CO_2 and let the cells adhere for 2 h. During that time, bring the temperature of the culture medium to 37°C.

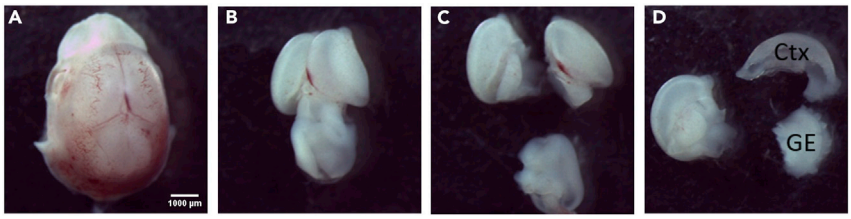

Figure 2. Cortices dissection

(A) Head of E13.5 mice embryo. Hold the brain at the level of the nose or the hindbrain and remove the skin and cartilage around the brain using forceps.

(B) Dissected brain.

(C) Dissected hindbrain (bottom) and hemispheres (top).

(D) Dissected cortex (Ctx) and ganglionic eminences (GE). Cut out the cortex around the ganglionic eminences without damaging cortical cells.

Scale bar, 1000 µm

40. Slowly add 600 µL of heated culture medium to each compartment, making sure to not detach the adhered cells. Maintain the dish in the same conditions in the incubator until the next day.

Electroporation and culture of MGE explants

Timing: 45 min

41. Fill a microcapillary with 10 µL of plasmid solution previously prepared using microloader tips. Cut approximately 2 mm of the capillary tip using small spring scissors.

Note: The tip of a newly pulled microcapillary is closed, and prevents the plasmid solution to enter into the tip. The solution is allowed to reach the microcapillary tip once it is cut to the edge.

Note: The plasmid concentration should be between 0.5 and 1 µg/µL. The electroporation of an excessive amount of plasmid might generate cellular toxicity.

42. Place the capillary on the Femtojet microinjector station and set the following parameters: Pi, 100 hPa; Ti, 1.7s; Pc, 17 hPa.
43. Install the electrodes and set the parameters of the ECM™ 830 electroporator as following: polarity, unipolar; mode, LV; voltage, 50V; pulse length, 50 msec; interval, 1 sec; pulses, 5.
44. Sacrifice the E13.5 wild-type pregnant female by cervical dislocation followed by decapitation to confirm death.

Note: Euthanasia methods may vary from one country to another. Please refer to your local ethical regulations. If needed, anaesthesia can be induced before cervical dislocation using an induction chamber filled with a mixture of 96% air and 4% isoflurane.

45. After sacrifice, disinfect the abdomen of the pregnant female with 70% alcoholic solution and open it with fine scissors under a laminar flow hood. Collect the uterus and excise the embryos from the amniotic sac using forceps. Immediately harvest the embryos in sterile ice-cold HBSS; section the heads and place them in the black Sylgard dissection dish filled with cold PBS.
46. Stabilize the head in the Sylgard dissection dish placing one pin at the level of the nose and another one at the level of the hindbrain (Figure 3A).
47. Using forceps, tear apart the skin and open the skull to reach the forebrain. Then, using two pairs of forceps, gently open the dorsal cortex by making a hole and stretching the tissue in order to visualize the ganglionic eminences located inside the brain, just beneath the dorsal cortex.

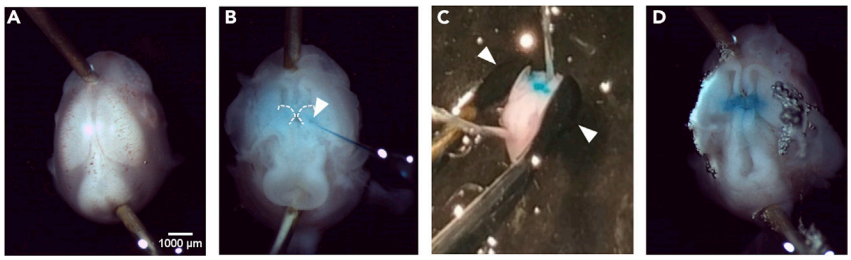

Figure 3. MGE electroporation

(A) Pinned head of E13.5 mice embryo. Put one pin in the nose (top) and one pin in the hindbrain (bottom).

(B) Injection of plasmid solution in the MGEs. The white arrowhead shows the microcapillary tip. The white dotted lines represent MGE borders.

(C) Electroporation of the brain. The white arrowheads show the electrodes placed from either side of the head.

(D) Pinned head after injection of plasmid solution in the MGEs and electroporation. It is possible to distinguish the injected MGEs thanks to the Fast Green contained in the plasmid solution.

Scale bar, 1000 µm

Make sure to not pin your forceps too deeply in the tissue as you would damage the ganglionic eminences.

48. Using the microcapillary and the injector, inject the plasmid of interest in both MGEs, directly into the tissue (Figure 3B). Perform up to ten injections in each MGE, making sure to not completely damage the tissue.

Note: We usually inject around 0.1 µL plasmid solution per MGE in total. By cutting 2 mm of the microcapillary tip, the injection volume does not strongly vary. However, the volume ejected from the microcapillary when pressing the "injection" button can be determined if necessary. To do so, continuously press the injection button of the injector until 1 µL of the solution is ejected from the microcapillary. The number of injection is automatically counted by the injector. The volume ejected from the microcapillary when pressing the injection button corresponds to 1 µL divided per the number of injections performed. Using this method, it is possible to determine the exact volume injected in the MGE. Adapt the number of injections according to that volume.

The volume remaining in the microcapillary at the end of the experiment can be retrieve using a microloader tip, and kept at −20°C.

49. Electroporate the brain by placing the tweezer platinum electrodes from either side of the head at the level of the MGE (Figures 3C and 3D).

⚠ CRITICAL: Do not touch the metallic pins with the electrodes during electroporation.

Note: The relative positions of positive and negative electrodes are not important as the plasmid solution is injected in multiple regions of the MGE tissue. Using this method, both hemispheres are electroporated, and MGE cells around the injection sites incorporated the plasmid.

50. Micro dissect the electroporated MGE and cut it in multiple small blocks (300–500 μm^3) with forceps or with a micro knife. Each piece is considered as an explant.
51. Take the dish coated with cortical feeder out of the incubator and remove the culture medium. Only leave a thin layer of medium so that the cortical feeder does not dry.
52. Place the electroporated explants on the cortical feeder. Transfer the dish in the incubator for approximately 10 min to allow the explants to adhere to the cortical feeder.

53. Slowly add 800 μL of culture medium to each compartment of the dish, making sure to not detach the explants.

Time-lapse microscopy

Timing: 10 h

Time-lapse recordings of explant culture are usually performed after 24 h of culture, when the cortical interneurons have migrated out of the explants. Explant cultures can be kept healthy for up to 3 days. Half of the culture medium should be replaced every day.

54. Place the dishes in the recording chamber of an inverted confocal microscope (here, Zeiss LSM 880) and set the parameters to 37°C and 5% CO_2.
55. Focus on the edge of the migratory front to record individual migrating interneurons. This makes the analysis of the branching parameters easier. Use a 20× long-range objective.
56. Acquire time-lapse images; image at least 5 Z-steps of 2 μm each 5 min for 5 to 10 h.

⚠ CRITICAL: it is very important to set the laser power at a level that allows visualizing the leading process branches without inducing phototoxicity; cells expressing fluorophores are photosensitive and laser over exposure would impair migration parameters and viability. Dying cells can be recognized by the appearance of large vacuoles or fluorescent protein aggregation, as well as disintegrated cell processes.

Note: Any confocal microscope can be used as long as an atmosphere-controlled recording chamber and a long-range objective are available. The 20× objective is well suited for this experiment as it allows to follow migrating interneurons during few hours before they leave the image field. A 40× long-range objective might work as well.

EXPECTED OUTCOMES

The two protocols described here are meant to study the migration of interneurons during cortical development. The first protocol relies on the use of transgenic mouse models to visualize cortical interneuron migration in living brain slices. This procedure allows the study of some interneuron migration parameters (e.g., velocity and nucleokinesis) in an environment closer to *in vivo* condition. The second protocol consists in a 2D culture of interneurons migrating from MGE explants. This method represents a good complement to the first one, as it allows the fine analysis of interneuron branching behavior.

As an example for the first protocol, we used Dlx5/6-Cre-IRES-EGFP transgenic mice (Stenman et al., 2003) to record the migration of post-mitotic interneurons in organotypic brain slices using the GFP reporter (Figure 4A and Methods video S1). The organotypic slices were obtained from E13.5 embryos and recordings were performed at the exit of ganglionic eminences (GE). At this stage, cortical interneurons migrate tangentially in the IZ and MZ migratory streams. Cell bodies can be tracked during the recordings, allowing the measurements of the average speed of interneuron migration and the nucleokinesis events (Figure 5).

To illustrate the branching analysis here, we electroporated the MGE from E13.5 embryos with the pCX memb-mCherry plasmid (Figure 4B and Methods video S2). By labeling cell membrane, it allows proper monitoring of interneuron processes. Branch remodeling can be manually tracked over time (Figure 6 and Methods video S2) and include measurements of growth cone splitting frequency, leading process length, life duration of neuritic branches, or average number of neurite types.

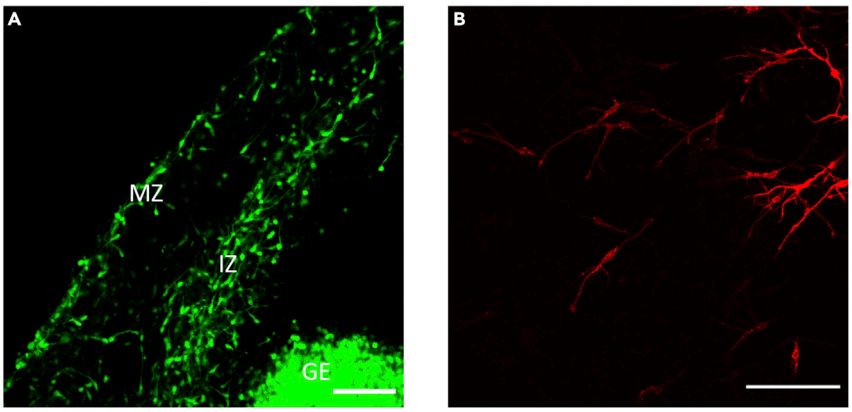

Figure 4. Representative images of migrating interneurons in an organotypic brain slice or in an explant culture
(A) Organotypic brain slice of E13.5 Dlx5/6-Cre-IRES-EGFP embryo. MZ=Marginal Zone; IZ=Intermediate Zone; GE=Ganglionic Eminences. 20× objective, scale bar,100 μm. See also Methods video S1.
(B) Migrating interneurons in an explant culture from MGE electroporated with the pCX memb-mCherry plasmid. 20× objective, scale bar,100 μm. See also Methods video S2

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis of migration parameters

1. Download and install the FIJI/ImageJ plugin mTrackJ.
2. Open the time-lapse recording in FIJI/ImageJ.
3. Make sure to use a file format that contains the metadata. If not, adjust the scale depending on the objective used [Analyze / Set scale] as well as the frame interval [Image / Properties / Frame Interval].
4. Open mTrackJ plugin and track the cell bodies for at least 3 h of video recording (36-time frames when acquiring images each 5 min) (Figure 6).
5. The "Measure" button gives all the data necessary to analyze the migration parameters.
6. Export the measurements into an Excel sheet.
7. Using the "Time", "Length" and "D2P" (displacement between two following time frames) measurements, we usually analyze the Average speed of migration (μm/h), the Frequency of nucleokinesis (number/h), the Amplitude of nucleokinesis (μm) and the Total time of nuclear pausing (min/h). We commonly consider a nucleokinesis as a nuclear movement larger than 5 μm (D2P).

Branching analysis

8. Open the time-lapse recording in FIJI/ImageJ.
9. Make sure to use a file format that contains the metadata. If not, adjust the scale depending on the objective used [Analyze / Set scale].
10. We manually assess Growth cone splitting frequency (number/h), which corresponds to the emergence of a secondary order branch from the growth cone (the mean length of a secondary order branch is ~20 μm). The life- duration of neuritic branches (min) corresponds to the number of frames during which a branch is visible. Leading process length (μm) and average number of primary, secondary, and tertiary neurites (number of neurite type/cell) can also be quantified (Figure 6).

LIMITATIONS

During the past years, time-lapse imaging helped to describe the morphological changes that occur during neuronal migration (Bellion et al., 2005; Nadarajah et al., 2001). Many studies took advantage of this approach to investigate the role played by certain genes in the migration process. By

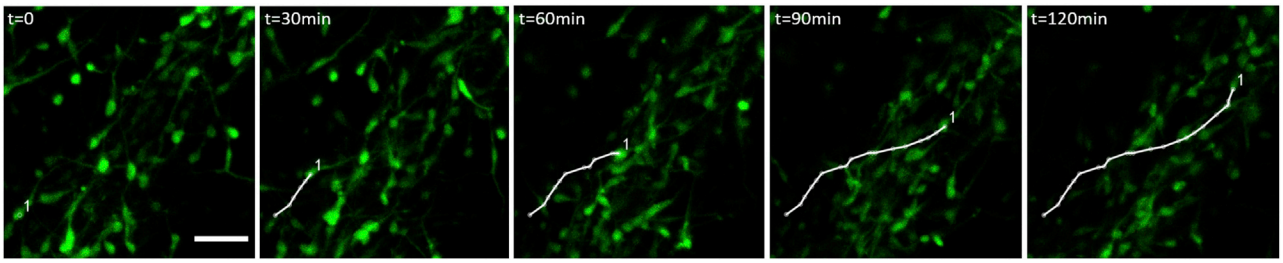

Figure 5. Representative images of migrating cortical interneuron tracking with mTrackJ plugin

Organotypic brain slice of E13.5 Dlx5/6-Cre-IRES-EGFP embryo. The white line represents the manual tracking of a migrating interneuron moving in the cortex. 20× objective, scale bar, 50 μm

combining the two protocols described here with mutant mouse models, our laboratory showed how p27 or CCP1 influence the migration of interneurons (Godin et al., 2012; Silva et al., 2018).

Organotypic brain slices allow the analysis of typical interneuron migration behavior (e.g., average speed of migration, frequency, and amplitude of nucleokinesis) in conditions close to *in vivo* situation, which is a considerable advantage. However, maintenance of the structure and viability of the tissue for a long period of time is a major issue. In a few hours to a few days, the tissue deteriorates and physiological conditions are altered. We usually perform time-lapse recordings between 4 and 48 h after brain slicing, 24 h being the most common timing. Moreover, interneurons are numerous and the dynamics of cellular processes make the branching analysis difficult and inaccurate.

Compared to organotypic brain slices, MGE explant culture represents a less physiological system to culture interneurons; however, the cell migration parameters are comparable between the two techniques (Bellion et al., 2005). Explant culture allows the recording of individual cells in 2D which facilitates the branching analysis as well as morphological changes that occur during interneuron migration (Bellion et al., 2005).

TROUBLESHOOTING

Problem 1

(See steps 5 to 9 in the step-by-step detailed protocol 1)

Damage of the brain occurs during dissection or agarose embedding.

Potential solution

First, make sure to practice your dissection skills as a damaged brain would prevent ad hoc culture for time lapse recording.

If the agarose solution is too cold and polymerize too quickly, it might be difficult to orient the brain without damaging it. It is important to keep the agarose solution at 37°C until the brains are dissected and ready to be embedded. As soon as the agarose solution is poured in the petri-dish, the brains should immediately be transferred and orientated to their final position. It is recommended to place the petri-dish on a polystyrene box during the positioning of the brains as its surface might be less cold than the bench surface. Do not put more than three brains per dish, otherwise orienting the brains before polymerization may be difficult. Do not forget to remove the excess of cold HBSS from the spoon using Tork® paper before transferring the brains into the agarose solution.

Problem 2

(See step 16 in the step-by-step detailed protocol 1)

The brain slices detach from the surrounding agarose.

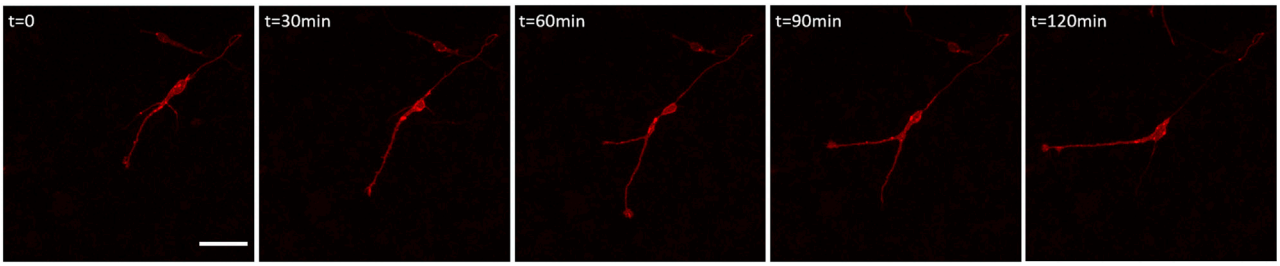

Figure 6. Displacement of a migrating interneuron from an MGE explant
Explant culture of MGE electroporated with the pCX memb-mCherry plasmid. 20× objective, scale bar, 10 μm.

Potential solution

Maintaining the agarose around the brain slices is critical to avoid the spreading of the tissue. To avoid detachment of the agarose, make sure to embed the brains in a cooled agarose solution. Always maintain the agarose solution at 37°C for at least 20 min before embedding. A second possible solution to this problem is to make sure that agarose blocks are maintained in a cold HBSS solution during the slicing. Always add ice around the vibratome tray during the whole procedure as the ice thaws. Once the slides lay on the millicell membrane, a drop of 1% agarose diluted in HBSS can be added inside the ventricles to avoid tissue collapse.

Problem 3

(See step 16 in the step-by-step detailed protocol 1)

The slices are not properly cut or are damaged during slicing at the vibratome.

Potential solution

Check the angle and sharpness of the blade; change the blade during the experiment if necessary. Try to change the cutting parameters by lowering the speed and the frequency. Remove meninges that are detaching from the tissue and may be blocking the slicing. Check if glue does not hinder the blade.

Problem 4

(See step 56 in the step-by-step detailed protocol 2)

The interneurons do not migrate properly or die.

Potential solution

If the explants are too large, interneurons might not be able to migrate out properly. A diameter between 300 and 500 μm is optimal. The use of a tissue biopsy punch might help to obtain explants with a reproducible size.

Areas not covered by cortical feeder may prevent interneuron migration and artificially increase cellular branching. To avoid this problem, dissect and dissociate cortical cells as quickly as possible in sterile conditions. When dissociating the cells however, gently pipette up and down in order to not damage the cells. Make sure to filter the cells with a 40 μm cell strainer as a solution of well dissociated cells is primordial to obtain a homogeneous cell layer.

Interneurons might die or not move during the image acquisition for multiple reasons. Defaults in CO_2 and in temperature supply strongly inhibit interneuron migration. Phototoxicity and culture medium contamination are causes to consider if cells are not viable.

Problem 5

(See step 20 and 56 in the step-by-step detailed protocol 1 and protocol 2 respectively)

The image plan drifts with time during time-lapse recordings.

Potential solution

Drifting of the z-plan can occur during the movie acquisition. This is mainly due to variations of the temperature of the metallic components of the microscope stage or loss of humidity control within the recording chamber. It is recommended to warm the microscope stage few minutes before acquisition and verify the water levels to create a humid atmosphere. If the z-plan drifts during acquisition, the movie may be stopped and the z corrected.

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be addressed to Laurent Nguyen (lnguyen@uliege.be).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This protocol includes all datasets generated or analyzed during this study. Supplemental information can be found online at https://doi.org/10.1016/j.cell.2018.01.031.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100467.

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AUTHOR CONTRIBUTIONS

This protocol relies on experiments done by F.L. and C.G.S. and has been written by F.L., C.G.S., and L.N.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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