Video Article

# *Ex Utero* Electroporation and Organotypic Slice Cultures of Embryonic Mouse Brains for Live-Imaging of Migrating GABAergic Interneurons

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URL: https://www.jove.com/video/57526
DOI: doi:10.3791/57526

Keywords: Neuroscience, Issue 134, Brain development, confocal microscopy, cortical GABAergic interneurons, e13.5 embryo, electroporation, gene repression, mouse, neuronal migration, organotypic culture, RNA interference, tangential migration, time-lapse imaging

Date Published: 4/20/2018

Citation: Eid, L., Lachance, M., Hickson, G., Rossignol, E. *Ex Utero* Electroporation and Organotypic Slice Cultures of Embryonic Mouse Brains for Live-Imaging of Migrating GABAergic Interneurons. *J. Vis. Exp.* (134), e57526, doi:10.3791/57526 (2018).

## Abstract

GABAergic interneurons (INs) are critical components of neuronal networks that drive cognition and behavior. INs destined to populate the cortex migrate tangentially from their place of origin in the ventral telencephalon (including from the medial and caudal ganglionic eminences (MGE, CGE)) to the dorsal cortical plate in response to a variety of intrinsic and extrinsic cues. Different methodologies have been developed over the years to genetically manipulate specific pathways and investigate how they regulate the dynamic cytoskeletal changes required for proper IN migration. *In utero* electroporation has been extensively used to study the effect of gene repression or overexpression in specific IN subtypes while assessing the impact on morphology and final position. However, while this approach is readily used to modify radially migrating pyramidal cells, it is more technically challenging when targeting INs. *In utero* electroporation generates a low yield given the decreased survival rates of pups when electroporation is conducted before e14.5, as is customary when studying MGE-derived INs. In an alternative approach, MGE explants provide easy access to the MGE and facilitate the imaging of genetically modified INs. However, in these explants, INs migrate into an artificial matrix, devoid of endogenous guidance cues and thalamic inputs. This prompted us to optimize a method where INs can migrate in a more naturalistic environment, while circumventing the technical challenges of *in utero* approaches. In this paper, we describe the combination of *ex utero* electroporation of embryonic mouse brains followed by organotypic slice cultures to readily track, image and reconstruct genetically modified INs migrating along their natural paths in response to endogenous cues. This approach allows for both the quantification of the dynamic aspects of IN migration with time-lapse confocal imaging, as well as the detailed analysis of various morphological parameters using neuronal reconstructions on fixed immunolabeled tissue.

## Video Link

The video component of this article can be found at https://www.jove.com/video/57526/

## Introduction

Cortical GABAergic interneurons (INs) are diverse with regards to their biochemical properties, physiological properties and connectivity, and they mediate different functions in mature networks[1,2,3,4,5]. The specification of different subtypes of cortical INs is tightly regulated through genetic cascades that have been extensively studied[1,2]. The majority (70%) of cortical GABAergic INs originate from progenitors in the medial ganglionic eminence (MGE), a ventrally located embryonic structure, and must migrate across relatively long distances to reach the cortical plate[1,2,6]. While cortical pyramidal cells migrate radially from the ventricular zone (VZ) to the cortical plate along the radial glia scaffold, the tangential migration of INs, which are not attached to such a scaffold, requires a variety of intrinsic and extrinsic cues to attract migrating neurons towards the cortical plate, while guiding them away from non-cortical structures[2,7,8]. After exiting the cell cycle, INs are repelled from the MGE by chemo-repulsive cues expressed within the VZ of the MGE, which triggers tangential migration towards the cortical plate[9,10]. Migrating INs avoid the striatum with the aid of different repulsive cues[11] and, after reaching the cortical plate, they switch from a tangential to a radial migration mode and reach their final laminar position, partly in response to cues from pyramidal cells[12] and other cellular populations[13]. The migration of INs, as for other neuronal populations, involves various dynamic morphological changes to permit the actual movement of the neuron. This so-called neuronal locomotion is characterized by repetitive cycles of three successive steps: the elongation of a leading process, an active anterograde motion of the nucleus (nucleokinesis), and the retraction of the trailing process[14]. IN migration is regulated by numerous intrinsic and extrinsic cues that drive the branching and active remodeling of the leading process to guide INs in the proper direction, determining both orientation and speed of migration[14,15,16].

The determinants regulating cortical IN migration have been extensively studied in recent years[1,2,7,17,18,19,20], and disruption in some of these molecular actors has been postulated to lead to neurodevelopmental disorders, such as pediatric refractory epilepsy or autism spectrum disorders[1,2,21,22,23,24]. Therefore, the development of various *in vitro* and *in vivo* approaches has been pursued to significantly advance our ability

 

to study this dynamic process, as previously reviewed[25]. *In vitro* methods, including the Boyden chamber assay and the Stripe Choice Assay, provide the fastest and most reproducible means of assessing the requirement and cell-autonomous impact of specific genes or proteins during neuronal migration, without the influence of other factors[25]. These assays are particularly useful when combined with live-imaging[8,26,27]. With these techniques, INs are easily retrieved from e13.5 MGE and isolated by enzymatic and mechanical dissociation, after which different signaling pathways and guidance cues can be investigated, as illustrated previously[8,28]. However, these assays take place in an artificial extracellular matrix in the absence of three-dimensional tissue architecture, which may alter neuronal behavior and cell properties, potentially affecting cell migration and/or survival[25]. To circumvent these limitations, *ex vivo* MGE explants have been developed as an alternative tool to quantify the dynamic morphological changes occurring during migration along with parameters such as speed and orientation[14,29]. Generating MGE explants is relatively straightforward and has been extensively described elsewhere[30]. It entails the plating of a small extract of the MGE on a monolayer of mixed cortical cells or in a mixture of matrigel and collagen in the presence of attractive or repulsive cues[25], although the latter are optional[31]. MGE explants allow for high resolution imaging of sparsely labeled cells, simplifying the study of intracellular processes, such as cytoskeletal remodeling during leading process branching, as shown previously[32,33,34] and in the present study. MGE explants have been used successfully to assess dynamic cytoskeletal changes during IN migration in a 2D environment, for instance after specific pharmacological or chemotactic manipulations (see, for example, Tielens *et al.* 2016[33]). However, with this approach, INs migrate within an artificial matrix, and this might alter IN behavior and the reproducibility and significance of the experimental results.

By contrast, *in utero* electroporation enables the genetic manipulation of INs in their native environment and is a widely used method to rapidly and efficiently assess the impact of gain and loss of gene function while circumventing the limitations of costly and time-consuming knockout and knock-in strategies[25,35]. *In utero* electroporation can be biased towards IN progenitors by using cell type specific promoters and by positioning the electrodes towards ventromedial structures, including the MGE[36]. Furthermore, *in utero* electroporation allows for the timely expression of experimental constructs within 1 - 2 days, as compared to the 7 - 10 days required for construct expression using viral vectors[25]. However, *in utero* electroporation of IN progenitors tends to be low-yield. Indeed, although pyramidal cell progenitors located in the dorsal ventricular zone can be efficiently transfected using *in utero* electroporation, targeting more ventrally located structures, such as the MGE, is more technically challenging, especially in small e13.5 embryos, and the high rate of embryonic lethality further reduces the experimental yield[25].

To circumvent some of the technical limitations associated with *in vitro* MGE explant experiments and *in vivo in utero* electroporation, *ex vivo* organotypic slice cultures have been developed[8,37,38,39]. Brain organotypic slice cultures offer the advantage of mimicking *in vivo* conditions, while being less expensive and time-consuming than generating animal models[25]. Indeed, these preparations allow an easy access to the MGE, along with the specific visualization of INs, and can be combined with focal electroporation to investigate specific molecular pathways in INs migrating in a more physiological environment[8,39,40,41]. We have therefore optimized an approach for organotypic cultures[38], which we combined with *ex utero* electroporation and time-lapse confocal imaging, to further assess the morphological and dynamic process occurring during tangential migration of MGE-INs. The present protocol was adapted and optimized from others who have used *ex utero* or *in utero* brain electroporation and organotypic slice cultures to study the migration of pyramidal cells[42,43] and cortical INs[36,39,44]. Specifically, mouse embryos are decapitated and the MGE is electroporated *ex vivo* after the intraventricular injection of the experimental plasmids, allowing more efficient and precise targeting of MGE progenitors than what can be achieved with *in utero* electroporation. The brains are then extracted and sectioned into whole brain coronal slices that can be cultured for a few days, thus allowing continuous tracking and imaging of transfected INs. This approach typically labels 5 - 20 tangentially migrating INs per brain slice, minimizing the number of experimental iterations required to reach statistical significance, while labeling a sufficiently sparse neuronal population to ensure easy separation of individual neurons for reconstruction and fine morphological assessment. Furthermore, compared to MGE explants, organotypic cultures ensure that migrating INs are exposed to a more natural environment, including locally secreted chemokines and inputs from thalamic afferents. This approach is thus well suited to quantify the directionality and migratory path adopted by transfected INs, while offering sufficient anatomical details to allow the characterization of finer dynamic processes such as leading process branching, nucleokinesis and trailing process retraction.

## Protocol

All experiments involving animals were approved by the Comité Institutionnel des Bonnes Pratiques avec les Animaux de Recherche (CIBPAR) at the CHU Sainte-Justine Research Center and were conducted in accordance with the Canadian Council on Animal Care's guide to the Care and Use of Experimental Animals (Ed. 2).

The protocol described here was optimized for electroporation of embryos at embryonic day (e) 13.5, at a time when MGE-derived INs are actively generated, before the peak of CGE-derived INs production[45,46]. Furthermore, to bias the electroporation towards GABAergic INs, we use a promoter selectively expressed in INs (for instance the *Dlx5/6* promoter with its minimal enhancer)[47].

# 1. Preparation of Solutions for Electroporation and Organotypic Slice Cultures

1. Prepare 125 mL of sterile culture medium.
    1. Measure 125 mL of regular neuron-specific culture medium (see **Table of Materials** for formulation) in a sterilized bottle in a previously UV-sterilized biosafety cabinet sprayed with 70% ethanol. Add 2.25 mL of 50x serum-free neuron-specific supplement, 1.75 mL of 200mM glutamine (final concentration of 0.5 mM) and 6.25 mL of heat-inactivated horse serum previously aliquoted under sterile conditions. Mix thoroughly, aliquot in 15 mL sterile conical tubes, and store at 4 °C.
    NOTE: Prepared culture medium can be stored for up to 3 weeks at 4 °C.
    2. Divide a 100X stock solution of Botteinstein's N-2 formulation[48] into 150 µL aliquots under sterile conditions and freeze at -20°C until use.

2. Prepare 1 L of sterile artificial cerebrospinal fluid (ACSF).
    1. Measure 800 mL of distilled water in a 1 L beaker. Add 25.67 g of sucrose, 5.08 g of sodium chloride (NaCl), 2.18 g of sodium bicarbonate ($NaHCO_3$), 1.80 g of glucose, 0.19 g of potassium chloride (KCl), 0.15 g of monobasic anhydrous sodium phosphate ($NaH_2PO_4$), 1 mL of 1M stock $CaCl_2.2H_2O$ and 2 mL of 1M stock $MgSO_4.7H_2O$. Stir to dissolve at room temperature. Add distilled water to reach a total volume of 1 L.

2. Using a 0.22 µm filter, filter the solution into a sterile bottle in a sterilized biosafety cabinet and store at 4 °C for up to 1 month.

3. Prepare a fresh solution of 4% agarose in ACSF before each experiment.
   1. Measure 25 mL of previously prepared ACSF in a 50-mL sterile conical tube and add 1 g of low-melting point agarose.
   2. Heat for 45 s in a microwave oven. To avoid spilling, interrupt heating every 3 - 4 s when boiling starts, open the tube to let the pressure out, close it again and agitate manually to mix the agarose. Repeat until the agarose solution is homogeneous. Keep the agarose solution at 42 °C during the remainder of the experiment to avoid solidification.
      NOTE: Higher temperatures will damage brain tissues.

## 2. Preparation of Plasmids for Injection

1. Pull a glass microinjection pipette
   1. Set the micropipette puller with the proper parameters, secure the glass capillary in the provided space and make sure it is centered with the filament.
   2. Press the pull button.
   3. Carefully remove the newly made microinjection pipettes from the heat puller and place in a box or clean petri dish until further use to avoid damaging the tip.

2. Set-up the biosafety cabinet with all the instruments needed for this experiment (see **Figure 1A**), generously spray all instruments in the biosafety cabinet with 70% ethanol and sterilize the instruments and environment with UV light for 15 - 20 min.
3. During the sterilization step, thaw plasmids on ice (4 °C).
4. Measure 10 µL of the plasmid from a stock solution (4 µg/µL) into a clean 1.5 mL centrifuge tube. Add 0.01% of Fast Green FCF. Mix gently, spin briefly and keep on ice until use.
   NOTE: Maxi-prep DNA should be prepared according to the manufacturer's protocol using an endotoxin-free maxi-prep kit. DNA can be solubilized in TE buffer or nuclease-free $H_2O$ and the preparation of the plasmid with dye solution can, but does not need to be performed under sterile conditions. Plasmids from Maxi-prep should be aliquoted to avoid multiple freeze-thaw cycles. Aliquoted plasmids should be mixed with the dye no more that 2 h before use and should not be refrozen for further use.
5. After sterilization, prepare the nano-injector as follows.
   1. Choose one of the previously prepared glass microinjection pipette stored in a clean box or petri dish and use small tweezers to cut the tip of the pipette in a beveled way to achieve an outer diameter of roughly 15 µm.
      NOTE: The outer diameter given here is an approximate measure to give the user an idea of what we use in our experiments and has been optimized in order to facilitate the piercing of the skull for plasmid injection without damaging the brain, while allowing for the fluid loading and release of the DNA. The outer diameter can be measured by looking at the cut tip of the glass microinjection pipette apposed to a micrometric scale bar under a bright field microscope.
   2. Use a syringe to fill the micropipette with mineral oil from its unpulled end (to expel all the air).
   3. Insert the filled glass micropipette in the nano-injector by following the manufacturer's instructions.
   4. Empty 2/3rds of the glass micropipette (keeping enough oil to prevent air entry).

6. Carefully insert the prepared micropipette in the tube containing the plasmid/dye solution and fill the glass micropipette with the plasmid/dye solution.

## 3. Collection of Mouse Embryos from Pregnant Females

1. Monitor breeding females daily to assess for vaginal plugging, preferably at the same time daily (early afternoon). Day e0.5 corresponds to the first day when a vaginal plug is observed.
   NOTE: The experiments described here can be conducted in wild-type mice. However, to facilitate the identification of the MGE and to label all GABAergic INs (or specific sub-sets such as MGE-derived INs), transgenic animals can be used (e.g.: *GAD67*$^{EGFP}$; *Dlx5/6*$^{Cre}$ with a Cre-reporter allele, etc.[47,49]). In this situation, the experimental plasmid injected should express another fluorophore (for instance *mCherry* or *TdTomato*) to allow visualization of the transfected INs (yellow) that can be compared with non-transfected INs (green).
2. Sacrifice the female at embryonic day e13.5, by neck dislocation.
   NOTE: Anesthetic agents given at the time of sacrifice may impact IN migration and survival[50,51] and should be avoided.
3. Collect embryos by C-section as follows.
   1. Generously spray the female abdomen with 70% ethanol. Pull the abdominal skin up with a pair of sterilized forceps and, with the other hand, use sterilized surgical scissors to cut the skin from the abdomen.
   2. With a second pair of sterilized forceps and scissors, pull the abdominal fascia up and cut it while carefully avoiding the uterus.
   3. Using a third pair of sterilized forceps and scissors, pull the uterine horns and cut them out of the pelvic cavity. Place the dissected uterine horns in a sterile 60-mm petri dish filled with neural-based culture medium supplemented with amino acids, vitamins and inorganic salts (see **Table of Materials** for commercially available product).

4. In a sterile biosafety cabinet, use two pairs of fine tweezers (one in each hand) to dissect the embryos out of the placenta and isolate the heads by decapitation.
5. Bevel-cut the tip of a sterile 3 mL plastic transfer pipette, aspirate the heads and transfer them in a new sterile 60-mm petri dish layered with solidified black wax and filled with the same neural-based supplemented culture medium as above.
   NOTE: This step minimizes the transfer of contaminants (mouse hair, blood). The black wax is used to stabilize the head during dissection. Culture media do not need to be oxygenated during these procedures.

## 4. Intraventricular Plasmid Injections and *Ex Vivo* Electroporation of the MGE

NOTE: The following steps must be performed under sterile conditions in the previously prepared biosafety cabinet.

1. Place the 60-mm petri dish layered with black wax and containing the decapitated heads in neural-based supplemented culture medium under the binoculars in the biosafety cabinet.
2. Stabilize the head, the rostral part facing right, with fine tweezers with the left hand and use the nano-injector in the right hand to inject 1 - 2 µL of the plasmid/dye solution into the right lateral ventricle.
   NOTE: Co-expression experiments can be conducted by co-electroporating a rescue plasmid and a shRNA-expressing plasmid by mixing both plasmids at equimolar concentrations.
3. Electroporate the injected brain.
   1. Place the head between the electrodes with the negative electrode positioned dorsally and parallel to the head and the positive electrode towards the ventral side of the head to target the MGE.
   2. Once the electrodes are well positioned, deliver 4 square pulses of 40 V for 50 ms at 500 ms inter-pulse intervals.
   3. Remove any residual tissue from the electrodes using tweezers already placed in the biosafety cabinet.
      NOTE: These parameters have been optimized specifically for the electroporator used in our experiments. We recommend that the users perform optimizing tests beforehand if using a different type of electroporator.
   4. Repeat steps 4.1 to 4.3 for all remaining brains.
      NOTE: Although this protocol describes the manipulations required for one brain, it is possible to inject up to 4 brains sequentially before electroporating each brain, thus increasing the yield. This strategy is especially advantageous when 2 or more different plasmids are injected sequentially (e.g. control or experimental plasmid) during the same experiment (allowing for comparison between littermates). In addition, it is possible to inject and electroporate simultaneously both sides of the brain to increase the yield, by positioning the electrodes completely parallel to the brain surface.

## 5. Brain Dissection and Organotypic Slice Cultures

1. While still manipulating in the sterile environment of the biosafety cabinet, dissect the brain out of the skull.
   1. Stabilize the head on the layer of black wax by inserting a needle into each eye while carefully avoiding the brain.
   2. Use a pair of fine tweezers to hold the left side of the neck and a second pair of fine tweezers to tear the skin from the skull, from back to front.
   3. While holding the head laterally with tweezers in one hand, use another pair of tweezers in the other hand to carefully cut the skull at the level of the brainstem and gently pull the skull up. With each tweezer, cut the skull in the sagittal plane (midline) towards the front, and then incise laterally to liberate the skull fragments.
   4. Lift the brainstem and carefully cut the meninges and cranial nerves until the brain is completely out of the skull.
      NOTE: All steps described in 5.1 should be performed under stringent sterile conditions in a biosafety cabinet.

2. Embed the brain in 4% low-melting point agarose for sectioning.
   1. Fill a 35-mm petri dish with the agarose solution prepared above (kept liquid at 42 °C).
   2. Quickly transfer an electroporated brain to the agarose-filled dish using the previously cut transfer pipette. Keep the dish at room temperature.
   3. Stir the agarose with a metal stick to keep the brain in the middle of the well (to prevent sinking) and position the brain in a rostro-caudal plane parallel to the dish. Stop stirring when the agarose starts to solidify, to avoid any brain damage.
   4. Use a razor blade to cut the agarose surrounding the brain in order to form a rectangular block, leaving a margin of 1 - 2 millimeters around the brain. Ensure that the rostral part of the brain is perpendicular to the anterior limit of the block to facilitate orientation for the subsequent sectioning steps.
   5. Repeat for each brain.
      NOTE: It is possible to cut more than one brain at a time (maximum 3) by shaping separate agarose blocks while setting each brain at different heights.

3. Vibratome coronal sections and slice culture.
   1. Thaw one aliquot of 100X N-2 supplement (150 µL) on ice and add to 15 mL aliquoted culture medium under sterile conditions.
   2. Transfer 750 µL of culture medium (with 1X N2 supplement) to each well of a 6-well culture plate.
   3. With curved tweezers, place one cell culture insert (30 mm diameter, 0.4 µm pore size, PTFE) in each medium-filled well.
   4. Fill the vibratome bath with continuously oxygenated ACSF. Cool to 4 °C with ice surrounding the bath, or use a refrigerated vibratome.
   5. Set the vibratome speed to 0.150 mm/s and the frequency to 80 Hertz.
   6. Glue the agarose block on the vibratome platform, rostral edge facing down and ventral edge facing the user.
   7. Cut the brain in coronal sections to obtain 250 µm-thick sections (at 4 °C).
   8. With sterilized spatulas, collect 2 - 3 sections containing the MGE and place all brain sections from one animal on a single 30-mm membrane insert, while carefully avoiding overlap between sections. Place the insert in one well of a 6-well culture plate (containing 750 µL of supplemented culture medium, as described above). Alternatively, each section can be placed on a separate 13-mm diameter membrane in a 12-well culture plate filled with 500 µl supplemented culture medium. The amount of culture medium recommended for each well allows the brain sections to be nourished by the media without being submerged.
      NOTE: The steps described in 5.3.6 and 5.3.7 are not carried out under complete sterile conditions, unless the vibratome can be sterilized and used in a biosafety cabinet. Therefore, it is crucial to conduct these steps carefully to avoid any contamination. Appropriate protective equipment (clean mask, surgical gloves and lab coat) should be worn at all times and body parts, even covered, such as hair, face and hands, should never pass over culture plates (with or without culture medium). It is also recommended to spray 70% ethanol frequently on the gloves and spatulas used to collect the brain sections.
   9. Place the culture plate in a ventilated sterile incubator at 37 °C with 60% humidity and 5% $CO_2$ for 48 or 72 h.

NOTE: These incubation times were optimized for time-lapse imaging of MGE-derived INs and confocal imaging of fixed slices, respectively. Optimal incubation times should be tested beforehand for each experimental design. In addition, if the chosen incubation is 72 h and under, there is no need to change the culture medium. For longer incubation times, the culture medium should be changed every 2 - 3 days.

10. After the desired incubation time, transfer the sections of interest to an 8-chambered coverslip and add 3 - 5 µL of culture medium. Place the coverslip in an environmental chamber (37 °C, 60% humidity, 5% $CO_2$) connected to an inverted spinning disk confocal equipped with a computer-assisted acquisition software to set-up the time-lapse imaging session.
NOTE: Alternatively, sections can be fixed with 4% paraformaldehyde (overnight at 4 °C or 2 h at room temperature) and subsequently immunostained with different antibodies for visualization of the morphological features of electroporated INs under a confocal microscope. Although eGFP and mCherry can be visualized by confocal microscopy without any counter-staining procedure, we recommend performing immunohistochemistry against GFP and mCherry to enhance the signal since the fixation process can reduce fluorescence, reducing the detection of finer components of embryonic neurons, such as smaller branches in the leading or trailing processes.

## Representative Results

In this section, we provide representative results obtained following the *ex utero* electroporation of a control plasmid, or an experimental plasmid targeting a gene of interest, in the MGE of e13.5 mouse embryos followed by organotypic slice cultures incubated at 37 °C for 48 h (for time-lapse imaging) or 72 h (for fixation and immunohistochemical labeling) (see **Figure 1B** for schematic protocol). Representative examples of INs migrating from an MGE explant are also illustrated (see **Figure 1C** for schematic protocol) as a comparison to the method described here. The electroporation of a plasmid in MGE progenitors seems to delay the exit of INs from the MGE and culture time must be adjusted accordingly.

In a successful experiment, organotypic slices appear healthy under the confocal microscope, i.e. cortical layers are easily distinguishable using the bright field mode and there is no visible contamination on the slice surface (recognizable by intense autofluorescence and the presence of fluorescent filaments visible under epifluorescence). In healthy chronic organotypic slices, approximately 20% of cells undergo apoptosis after 72 h in culture (**Figure 2**), as previously described in chronic organotypic cultures (e.g. postnatal cultures)[52]. Nonetheless, gross cytoarchitectural brain structure remains well defined, as illustrated here using DAPI staining (**Figure 2A**). Our protocol for *ex vivo* electroporation of the MGE yields an average of 50 - 100 transfected cells per slice (**Figure 3A,B**), of which 5 - 20 cells will be seen migrating dorsally after 72 h in culture. After fixation and immunohistochemical staining, INs migrating tangentially towards the cortical plate can be easily identified with confocal microscopy as they present an elongated/oval cell body, the occasional trailing process, and a leading process oriented tangentially. The leading process usually gives rise to one or two branches and is recognizable by the presence of an occasional swelling in front of the nucleus (housing the centrosome before nucleokinesis) and growth cones at the end of each branch. (**Figure 3C-G**).

This protocol was designed for the observation of migrating MGE-derived INs at the single-cell level using time-lapse imaging. For this particular experiment (**Figure 4**), coronal organotypic brain slices were generated from *Dlx5/6*$^{Cre}$;*RCE*$^{EGFP}$ embryos electroporated at e13.5 with a plasmid expressing an experimental shRNA (targeting a gene of interest) and the *TdTomato* cassette. The slices were incubated for 48 h, transferred to an 8-well chambered coverslip dish (1 slice per chamber floating on a thin layer of supplemented culture medium) and imaged every 3 minutes for 6 - 8 h using a 20x air (0.70 NA) objective on an inverted microscope equipped with a spinning disk confocal head, a computer-assisted acquisition software and a stage-top environmental chamber. During imaging, the slices were kept at 37 °C and were continuously oxygenated and humidified in the environmental chamber (5% $CO_2$ and 60% $H_2O$). Electroporated MGE-derived INs were identified as such by their expression of eGFP, confirming their GABAergic IN identity, and their expression of *TdTomato*, confirming that they express the experimental plasmid (**Figure 4A,B**). Electroporated INs are easily identifiable and well isolated, as seen in **Figure 4**, allowing for the finer analysis of migration dynamic parameters, such as speed and distance traveled. In addition, labeled INs are seen migrating tangentially towards the cortical plate, while in their natural environment, enabling us to identify dynamic morphological changes such as branching of the leading process and nucleokinesis (**Figure 4C-F**).

The *ex vivo* electroporation of MGE-derived INs can also be combined with MGE explants to enable high resolution imaging of dynamic cytoskeletal processes occurring during migration, as a complement to the study of directionality and migratory dynamics in organotypic cultures. As an example, different phases of neuronal locomotion reminiscent of those occurring during tangential migration of INs in organotypic slice cultures can be observed in electroporated INs migrating from an MGE explant 48 h after electroporation, such as leading neurite extension and nucleokinesis (**Figure 5B-E**). Various cytoskeletal processes can then be studied at high resolution in isolated cells migrating from an MGE explant, for instance by staining F-actin structures with phalloidin (**Figure 6A**), which cannot be achieved optimally in organotypic slices given the high cellular density (and thus of cytoskeletal elements) in a native environment. These cytoskeletal processes, and in particular F-actin remodeling, can further be studied dynamically by combining Lifeact expression with time-lapse imaging. For instance, we show an example of high-resolution time-lapse imaging of an IN derived from an MGE explant obtained from a *Nkx2.1*$^{Cre}$;*RCE*$^{EGFP}$ mouse brain carrying a targeted deletion of a gene of interest in INs. This IN was transfected with the mCherry-Lifeact-7 plasmid under the control of the CMV promoter (gift from Michael Davidson), using the method schematized in **Figure 1C**, allowing for the tracking of F-actin remodeling occurring in real-time (**Figure 6B**). Thus, while organotypic cultures are required to properly assess directionality or migration path of genetically modified INs, MGE explants can complement such studies by providing better access to isolated cells for high-resolution imaging of dynamic cytoskeletal processes.

Technical pitfalls may result in failure of the experiments described above. For instance, embedding the brains in an agarose solution warmer than 42 °C can result in tissue destruction and neuronal death, revealed by a loss of translucency of the brain or slices, which become opaque. However, the temperature should not be kept too low, as a solidifying agarose solution can destroy the fragile embryonic brain during the embedding process. Secondly, contamination can significantly reduce the yield of the experimental approaches described here. Thus, all steps should be carried out with care, under stringent sterile conditions as much as feasible. All solutions and equipment should be sterilized before use and equipment should be frequently sprayed with 70% ethanol to avoid contamination from either bacteria or mold. Contamination can be visible directly in the culture wells, as the culture medium becomes yellow or opaque. Contamination may go unnoticed when the culture medium remains clear and fluid. However, a layer of mold on the slice surface can usually be observed or the slices become excessively fragile during the fixation and staining steps. When such slices are visualized under the microscope, contamination may manifest as a layer of autofluorescence over labeled neurons or be revealed by the presence of long fluorescent filaments. Organotypic slices kept in contaminated wells should be thrown out, but the slices cultured in the other wells of the same plate can be kept for further steps. Bacterial contamination is quite rare but should be taken seriously, meaning that all equipment, including shared incubators, and culture room should be manually cleaned and sterilized.

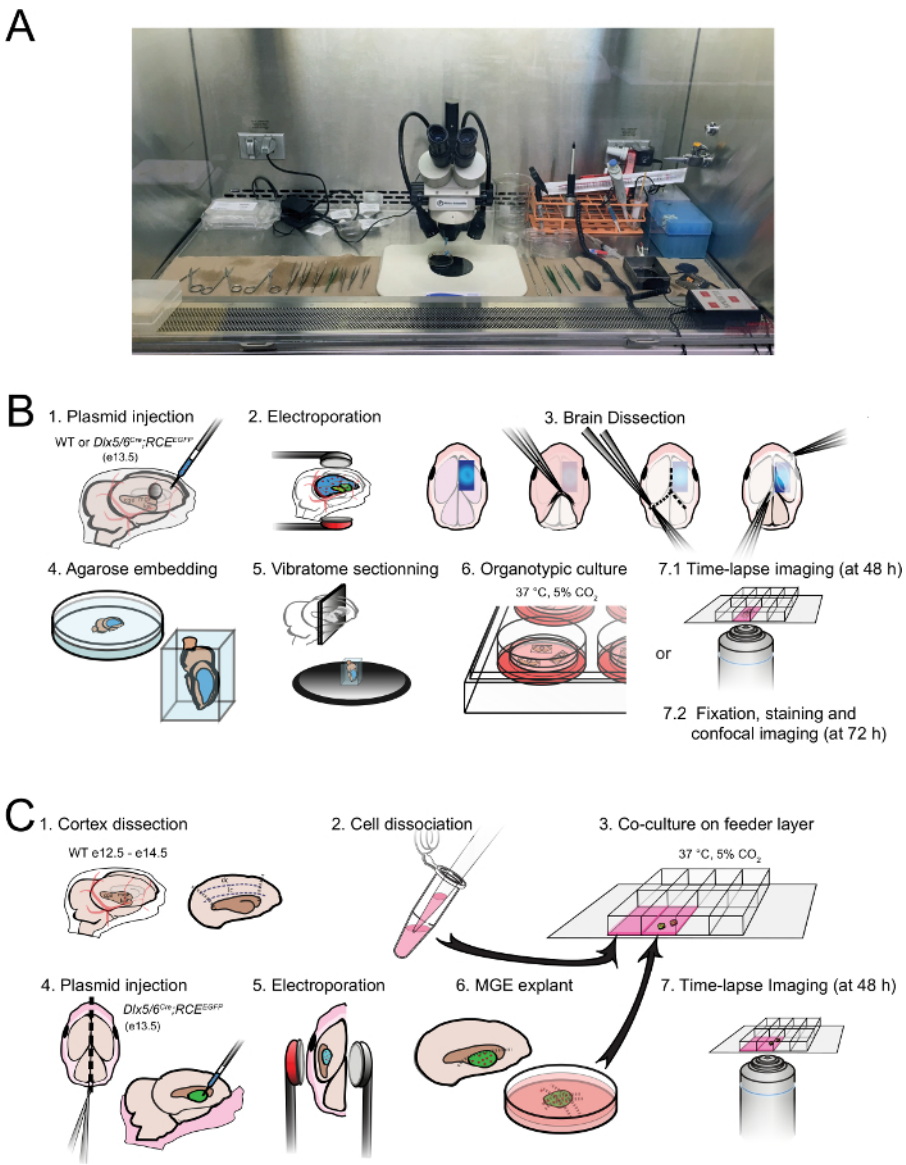


**Figure 1: Schematic representations of the protocols for *ex utero* electroporation followed by organotypic slice culture or MGE explants. A.** Example of a biosafety cabinet setup with all sterilized instruments needed for the protocol described here. **B**. Schematic representation of the protocol used for the *ex utero* electroporation and organotypic slice cultures. Briefly, the embryos are decapitated, the experimental plasmid is injected in the lateral ventricle (1) and electroporated in the MGE (2). The brain is microdissected out of the skull (3) and embedded in agarose (4). Organotypic sections are generated on a vibratome (5) and placed in culture at 37 °C(6) for 48 h for time-lapse microscopy (7.1) or 72 h for cell reconstructions (7.2). **C.** Schematic representation of the protocol adapted from Myers *et al.* 2013[53] and used for the generation of MGE explants. Briefly, dorsolateral cortices are first dissected out from e12.5 to e14.5 wild-type mouse embryos (1) and dissociated mechanically in supplemented culture medium (2). Cortical cells are plated at a density of 5.25 x $10^5$ cortical cells/$cm^2$ and cultured for 2 h at 37 °C in a collagen/poly-L-lysine-coated 8-chambered coverslip (3). Then, e13.5 *Dlx5/6$^{Cre}$;RCE$^{EGFP}$* embryos are processed for *ex utero* electroporation of the MGE (4, 5), and the MGE is manually isolated from the brain and cut in 100 $\mu m^2$ fragments (6). These explants are then placed on top of the previously prepared cortical feeder layer, covered with culture medium and co-cultured for 48 h before time-lapse imaging (7). Please click here to view a larger version of this figure.

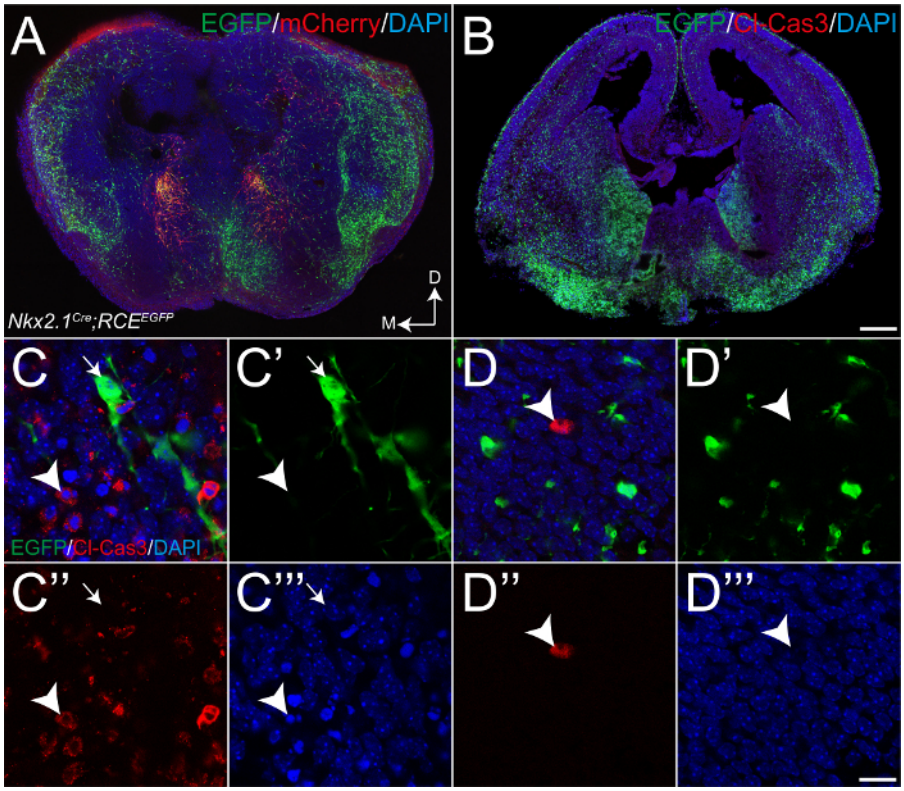


**Figure 2: Preserved cytoarchitecture in healthy organotypic slices.** The generation of organotypic slice cultures after *ex vivo* electroporation of *Nkx2.1*$^{Cre}$*;RCE*$^{EGFP}$ mouse embryos (**A**) does not result in significant tissue damage, as revealed by preserved cytoarchitecture (DAPI (blue) and Cre-reporter (GFP)), when compared to an 18 µm-thick cryosection from a *Nkx2.1*$^{Cre}$*;RCE*$^{EGFP}$ embryo transcardially perfused with 4% PFA at e15.5. D and M indicate dorso-ventral and latero-medial axes, respectively. (**B**). High magnification images taken with an inverted confocal microscope equipped with a 63x/1.4 oil objective after staining with an anti-Cleaved-Caspase 3 antibody (Cl-cas3) reveal that approximately 20% of the cells in the cortical plate undergo apoptosis (white arrowheads) after 72 h in culture, while GFP-positive INs stay healthy (white arrow), as exemplified in the photomicrographs in **C-C'''**. By comparison, cellular apoptosis is almost completely absent from the thin cryosection from a perfused animal (**D-D'''**). Scale bars: 250 µm (A-B) and 15 µm (**C-D'''**). Please click here to view a larger version of this figure.

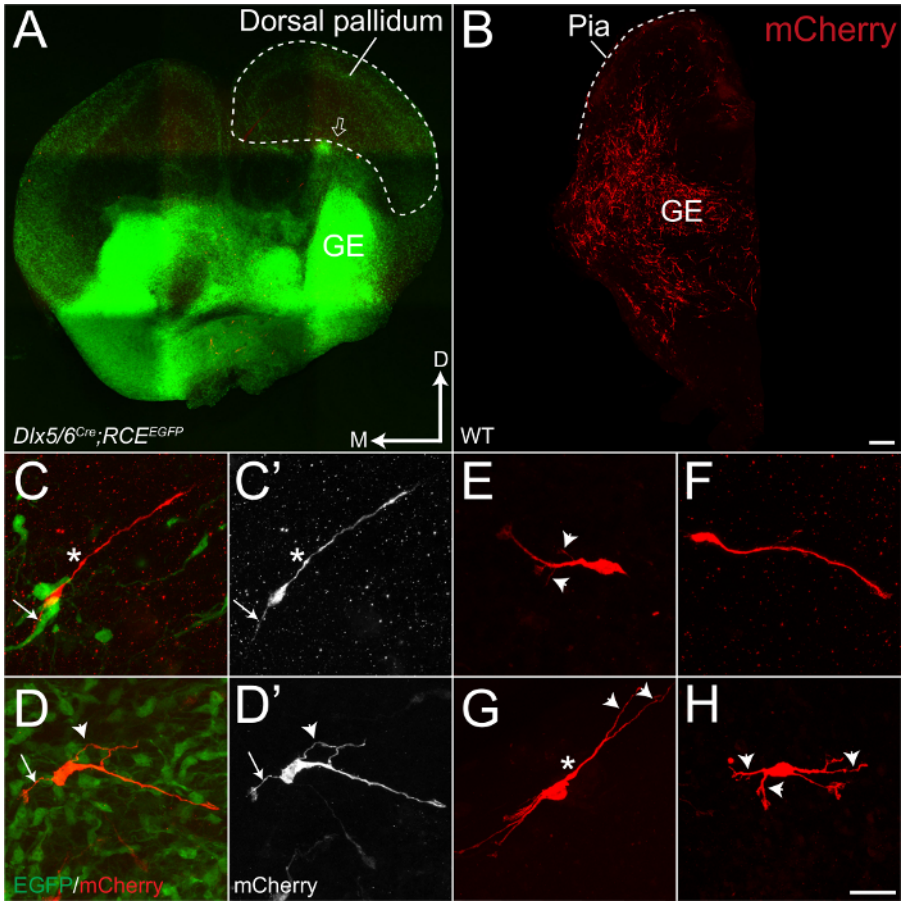


**Figure 3: Organotypic slices of mice brain embryos and high magnification photomicrographs of electroporated interneurons (INs).** **A.** An organotypic slice culture from a *Dlx5/6*$^{Cre}$*;RCE*$^{EGFP}$ mouse brain (in which all INs are green) electroporated with a *Dlx5/6::shRNA*$^{scrambled}$*-IRES-TdTomato* plasmid. The slice was fixed with 4% PFA after 72 h in culture, and immunostained for GFP and mCherry. Electroporated INs were imaged in 3D (50 - 60 µm-thick z-stacks with optical sections taken every 0.5 µm) using a confocal microscope equipped with a 63x/1.3 NA oil objective. INs migrating tangentially in the sub-ventricular zone of the dorsal pallium are readily identifiable (red, open arrow). D and M indicate dorso-ventral and latero-medial axes, respectively. **B.** A representative organotypic slice culture from a wildtype (WT) embryo electoporated with a control *Dlx5/6::shRNA*$^{scrambled}$*-IRES-TdTomato* plasmid, fixed at 72 h and immunostained for mCherry only. Electroporated INs (red) are seen migrating dorsally towards the cortical plate. Electroporated INs are identified by their co-expression of *TdTomato* and *EGFP* in *Dlx5/6*$^{Cre}$*;RCE*$^{EGFP}$ mice (**C-D**), or by their expression of *TdTomato* only in WT mice (**E-H**), and by their typical morphology, i.e. an oval cell body, a branched leading process (white arrowheads, **D-H**), an occasional trailing process (white arrow, **C-D**) and an occasional swelling of the leading process housing the centrosome before nucleokinesis (white star in **C-C'** and **G**). Scale bars: 250 µm (**A-B**) and 25 µm (**C-H**). Please click here to view a larger version of this figure.

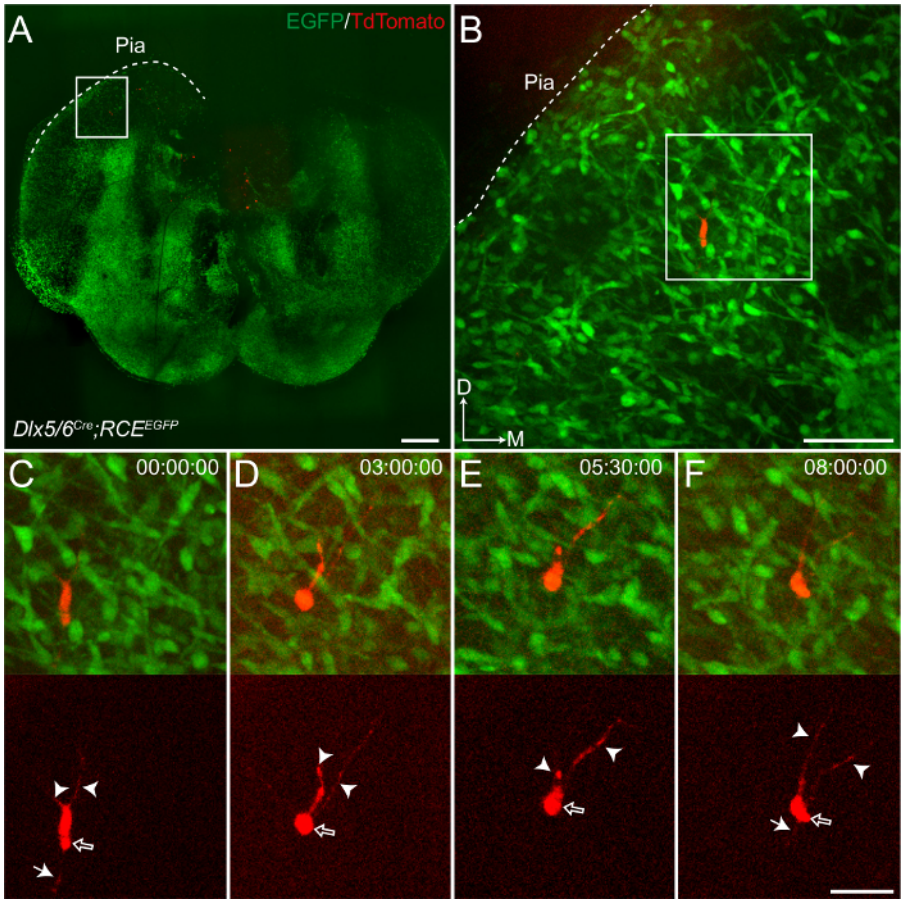


**Figure 4: Time-lapse live-imaging of electroporated INs in organotypic slices cultured for 48 h.** INs electroporated at e13.5 with an experimental plasmid expressing the *TdTomato* cassette and a shRNA targeting a gene of interest are seen in tangential migration in an organotypic brain slice obtained from a *Dlx5/6*$^{Cre}$*;RCE*$^{EGFP}$ mouse embryo after 48 h of culture (**A,** white square). In **B**, the same electroporated IN (white square) is seen in the process of nucleokinesis, while migrating tangentially in the cortex, i.e. parallel to the pial surface,and is followed in time-lapse imaging every 3 minutes for 8 h using a 20x/0.85 NA air objective (enlarged boxes, **C-F**). The neuron initially displays an elongated cell body (open arrow) in the process of nucleokinesis (**C**), as well as a trailing process (white arrow) and a branched leading process (white arrowheads). After 3 h of live-imaging, the nucleokinesis is completed, the trailing process has retracted, and the leading process is extending two long branches (**D**, white arrowheads). After 5 h 30 min, one of the leading process branch has retracted (white arrowheads) and the neuron cell body (open arrow) has moved forward about 10 µm (**E**). Finally, after 8 h of imaging, migration has paused, the neuron has extended its leading process again and a trailing process has appeared (white arrow), but the nucleus (open arrow) has not yet moved forward (**F**). Scale bars: 250 µm (A), 70 µm (B) and 30 µm (C-F). Please click here to view a larger version of this figure.

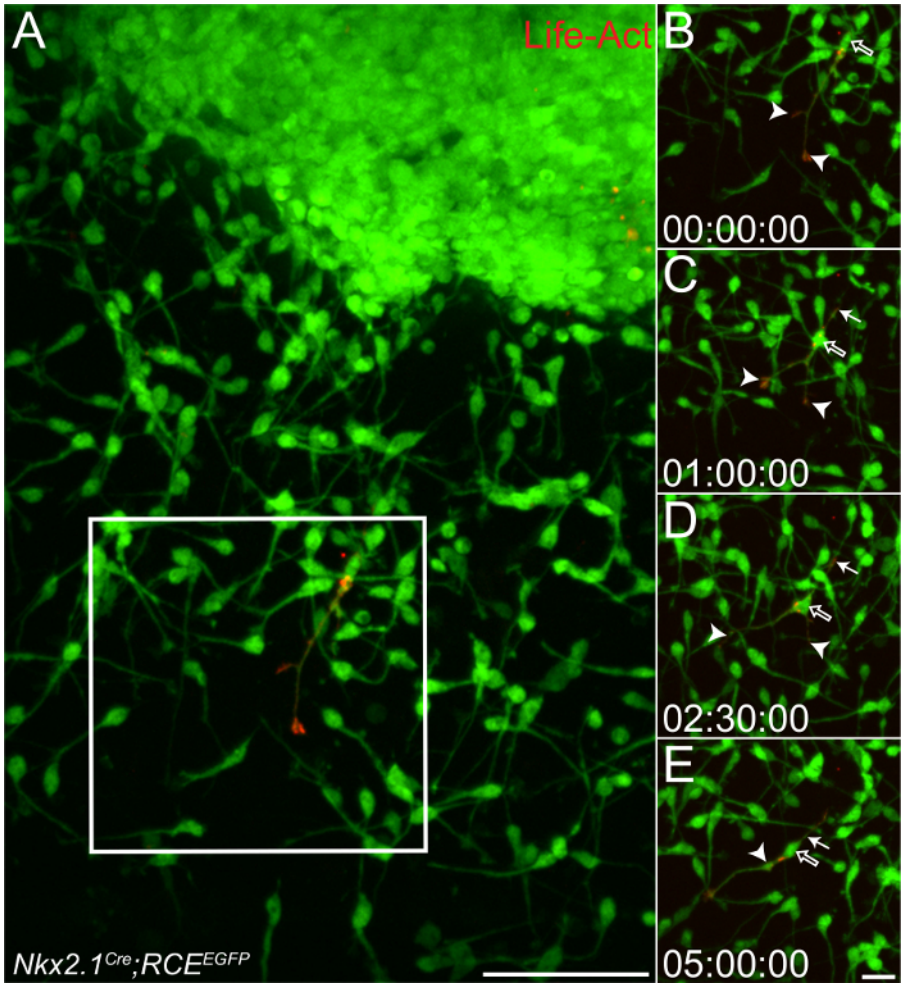


**Figure 5: Time-lapse imaging of INs derived from an MGE explant cultured for 48 h.** INs are seen migrating out of an MGE explant obtained from a *Nkx2.1*$^{Cre}$;*RCE*$^{EGFP}$ mouse (in which all MGE-derived INs express eGFP) electroporated with the *CMV::mCherry-LifeAct-7* plasmid at e13.5, using the method adapted from Myers *et al.* 2013[53] and schematized in **Figure 1C**, and cultured for 48 h (**A**). INs were visualized using time-lapse live-imaging for 5 h (**B-E**). In this example, one IN co-expressing eGFP and LifeAct is seen initially in the process of nucleokinesis (open arrow) and extends two leading process branches (white arrowheads; **B**). This IN completes nucleokinesis after 1 h (see open arrow), as the cell body has moved forward and a trailing process has appeared at the rear of the cell body (white arrow), and still displays one leading process with two branches (white arrowheads; **C**). A second nucleokinesis is under way after 2 h 30 min (open arrows) and the IN is now endowed with two leading processes originating from the cell body and a longer trailing process (white arrow; **D**). Finally, after 5 h, the IN retracts one neurite while translocating the centrosome in the remaining leading process branch (white arrowhead) in preparation for a third nucleokinesis, with a trailing process (white arrow) still present at the rear of the cell body (**E**). Scale bars: 70 µm (**A**) and 20 µm (**B-E**). Please click here to view a larger version of this figure.

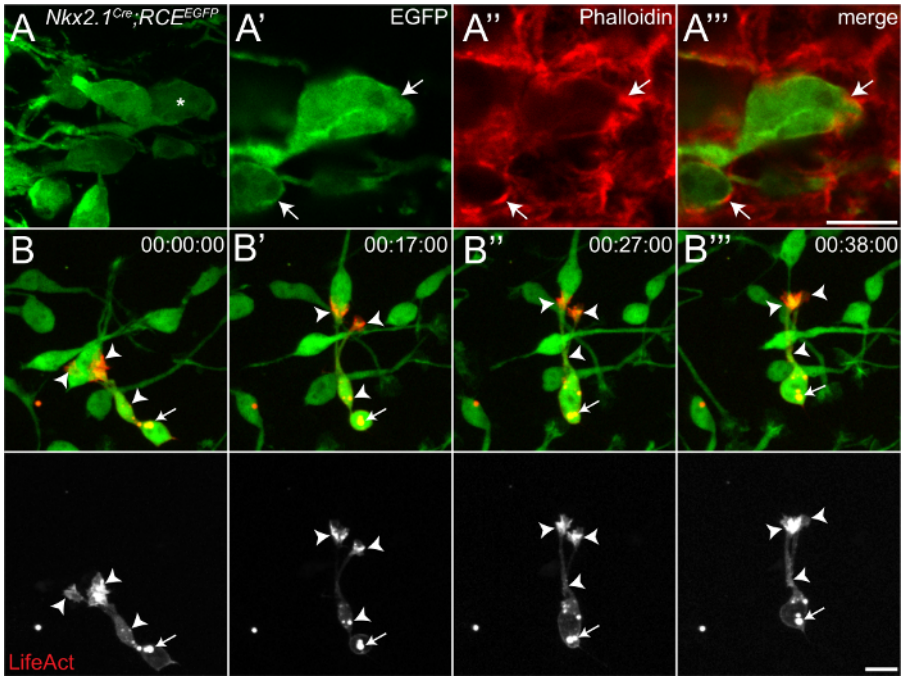


**Figure 6: High-resolution time-lapse imaging of actin remodeling in INs from an MGE explant cultured for 48 h. A.** An organotypic slice from a *Nkx2.1*$^{Cre}$*;RCE*$^{EGFP}$ mouse brain is fixed at e15.5, stained with Alexa-594 Phalloidin, allowing for the visualization of filamentous actin. INs are imaged using a 63x/1.3 NA oil objective on a confocal microscope. In healthy INs, filamentous actin is seen cupping the rear of the cell body following the retraction of the trailing process after completion of nucleokinesis (white arrow, **A'-A'''**). **B.** An MGE explant is obtained as above from a *Nkx2.1*$^{Cre}$*;RCE*$^{EGFP}$ mouse brain carrying a targeted deletion of a gene of interest and electroporated with a plasmid expressing mCherry-Lifeact-7 under the control of the *CMV* promoter. Time-lapse live imaging is performed after 48 h of culture, and electroporated INs are followed every 3 minutes for 3 h using a 40x/0.85 air objective. Active remodeling of the actin cytoskeleton occurs in the IN transfected with LifeAct, as exemplified by the movement of red (white in the black and white images) fluorescent dots within the leading process and growth cones (white arrowheads) and at the rear of the cell body during nucleokinesis (**B-B'''**, white arrows). Scale bars: 7 µm (**A-A'''**) and 10 µm (**B-B'''**). Please click here to view a larger version of this figure.

## Discussion

In this article, we provide a reliable method for performing *ex utero* electroporation of the mouse MGE at e13.5 and for the generation of organotypic cultures of embryonic brain slices. Although *in vitro* methods, such as the Boyden Chamber Assay, are relatively easy to perform and can be used to assess the specific roles of different genes and proteins without the interference of other factors, they preclude the investigation of IN migration dynamics with regards to directionality and migration path[25]. MGE explants provide a useful means to study the dynamic cytoskeletal changes occurring during IN migration, as we show here, but they are devoid of most endogenous cues and often require the addition of guidance cues to promote neuronal migration (although this is significantly improved when MGE explants are cultured on cortical feeder layers)[25,54]. Nonetheless, assays based on MGE explants can fail to detect the implication of molecular cues involved in more subtle mechanisms such as those guiding the specific directionality or migration path of INs[29]. This problem was eventually circumvented by *in utero* electroporation[25], which allows for the specific labeling of MGE-derived INs migrating in their native environment[13,29,41]. However, that technique is skill-challenging, due to the surgical procedures involved, and its yield is limited by the low survival rate of embryos and the fact that the MGE is difficult to target *in utero*, often requiring the use of multiple litters to reach significance[55]. Hence, *ex utero* electroporation of the MGE followed by organotypic culture of mice brain embryos provides a low-cost, time-efficient, and reliable method for investigating the migration dynamics and the morphology of MGE-derived INs in their natural environment, while circumventing the surgical procedures of *in utero* electroporation and the need for guidance cues in MGE explants. In addition, this technique allows an easier access to the MGE, which can be targeted by directly apposing the positive electrode under the neck and the negative one on top of the head, a configuration more difficult to adopt *in utero*. Alternatively, the MGE can be focally injected and electroporated directly after generating organotypic slices[13,25,29], but that technique has proved more difficult and less effective in our hands than the protocol described here. By following the steps described in this article, one will obtain 50 - 100 electroporated MGE-derived INs per organotypic slice, 5 - 20 of which will be seen migrating tangentially out of the MGE after 72 h. Transfected INs can be visualized live with time-lapse imaging or imaged and reconstructed after fixation and immunohistochemical labeling.

The protocol described here was optimized to study IN migration *ex vivo* and was inspired by various published protocols describing *in utero* electroporation of MGE-derived INs, *ex vivo* MGE injection and electroporation, or the generation of chronic organotypic brain slice cultures[36,38,39,42,43,56,57,58]. Our approach limits potential damage to MGE progenitors by using lower pulse intensity (40 V) and intraventricular plasmid injections rather than intra MGE injections with high voltage pulses (100 V), as described elsewhere[39,56,57,58]. Furthermore, while others use a combination of penicillin, streptomycin and gentamycin to prevent bacterial contamination[39,56,57,58], we have opted to avoid antibiotics in our culture medium since they can theoretically affect IN migration. In particular, penicillin is an antagonist of the $GABA_A$ receptor[59,60], and $GABA_A$ receptor activation activates and later stops IN migration depending on intracellular chloride gradients and KCC2 expression at various phases of IN migration[61]. However, using the various precautions stated in the current protocol, contamination can be effectively avoided even in the absence of antibiotics.

Despite the efficiency of this approach in our hands, *ex utero* electroporation also has its disadvantages. While providing a natural three-dimensional environment for cells to migrate, it can be difficult to perform pharmacological assays in organotypic slices in the context of IN migration. Indeed, the migration of MGE-derived INs mostly depends on extracellular cues[2,13,19] and the application of pharmacological inhibitors or activators on organotypic slices does not allow the precise dissociation of cell autonomous effects from global effects on the overall slice health or activity. For such experiments, MGE explants grown over mixed dissociated cortical cells might be preferable to organotypic slice cultures, since INs migrating out of the explant can be more readily isolated and exposed selectively to specific compounds[62]. Furthermore, although *ex utero* electroporation is easier to perform than *in utero* electroporation, it can still be technically challenging at the embryonic ages illustrated here given the small size and fragile state of embryonic brains at e13.5. Investigators will need a few trials to efficiently extract the electroporated brains at e13.5 without damaging the brain surface. In addition, as opposed to postnatal slices, embryonic organotypic slices cannot be kept in culture over long periods of time. Although embryonic organotypic slice cultures can be kept for at least 7 days *in vitro*[63], this was not combined with electroporation and the experiments described here have been tested for up to 3 days *in vitro* with reliable results in our hands. Therefore, investigators should perform optimization tests beforehand if longer incubation times are needed. Further, the investigation of IN development at late embryonic or early postnatal stages is not recommended with this technique when using e13.5 embryos[25], but can be achieved with *in utero* electroporation, where successfully electroporated embryos are put back into the uterine cavity and can be left to be born and analyzed at later stages[21,64]. Finally, *ex utero* electroporation followed by organotypic slice cultures allows for the analysis of both the morphology and the migration dynamics of developing INs. However, as it has been previously pointed out for the *in utero* electroporation technique[36], *ex utero* electroporations yield 50 - 100 electoporated cells per brain slice and are thus not suitable for population analysis. Such investigations are better conducted in animal models carrying either a full or conditional deletion (or knock-in) of the gene of interest. When available, such models can then be efficiently used to generate organotypic slice cultures or MGE explants to visualize the actual dynamics of IN migration, as demonstrated here (see **Figure 5** and **Figure 6**).

Many steps in this protocol should be carried out carefully in order to obtain optimal results. For instance, it is primordial that all the steps are performed under stringent sterile conditions as contamination can occur quite easily. Gloves and instruments used outside the biosafety cabinet should be sprayed with 70% ethanol frequently during the entire procedure. In addition, solutions such as culture media and artificial cerebrospinal fluid should be kept sterile and cold (4 °C), and made fresh every 2 - 3 weeks. To label MGE-derived INs more specifically, plasmids that will be used in such experiments should be cloned under the control of an IN-specific promoter (ex: *Dlx5/6*[49], *Lhx6*[65]), instead of simply relying on the anatomical targeting of the MGE. The generation of organotypic slices requires the brain to stay alive. Thus, to preserve cell health and survival, this experiment should be conducted in less than 3 hours from the moment that the embryos are retrieved until the organotypic slices are put in culture. For time-efficiency, culture plates can be pre-filled with homemade culture medium just before starting the experiment and put in the 37 °C cell culture incubator until use. Furthermore, the brains should be carefully dissected out of the skull without any damage to the cortical surface in order to achieve proper embedding in the agarose solution and subsequent vibratome-sectioning. For instance, damage to the cortical surface, even minimal, may result in the detachment of the 250 µm-thick sections from the surrounding agarose gel or in degradation of the sections during vibratome sectioning. For neuronal survival, it is also critical that the agarose solution temperature remains close to 42 °C, as higher temperatures lead to tissue damage and cell death, whereas lower temperatures will preclude the proper embedding of the brain (or damage it during the embedding process). Once in culture, to avoid other contamination sources, the slices should not be manipulated until they are transferred to the microscope for imaging. These steps should be carried out in a sterilized environment and attention must be paid not to contaminate the plates after these manipulations, especially if they need to be put back in culture for subsequent re-imaging. Finally, we do not recommend recuperating a DNA aliquot mixed with loading dye from previous experiments for future use as we observed a considerable reduction in the yield of electroporated neurons with such material.

In conclusion, the *ex utero* electroporation and organotypic slice culture protocol described above allows for the specific labeling of MGE-derived INs at the single-cell level and can be used to genetically manipulate specific molecular pathways to study their role in regulating the morphological changes and migration dynamics of tangentially migrating INs. This method allows for the rapid and low-cost early functional investigation of novel candidate genes identified through single cell RNA sequencing of migratory INs[66,67] or through Next Generation Sequencing of patients with neurodevelopmental disorders[68,69,70]. This technique has been extensively used to study migration in other cell populations, including pyramidal cells in the cortex and hippocampus[70,71,72,73]. Once mastered, it could potentially be used to image the recycling and trafficking of membrane proteins, such as receptors and channels using GFP-tagged proteins; the activation of specific cellular signaling cascades using biosensors[74]; or even the monitoring and manipulation of cellular activity using calcium imaging coupled to optogenetics in cortical INs, but also in other brain areas, such as the hippocampus, the amygdala or even the striatum.

## Disclosures

The authors have nothing to disclose. The views expressed herein do not necessarily represent the views of the Minister of Health or the Government of Canada.

## Acknowledgements

This work was supported by operating grants from the Savoy Foundation and the CURE Epilepsy Foundation and by equipment grants from the Canadian Foundation for Innovation to E.R (confocal microscope) and G.H (spinning disk confocal microscope). E.R. receives a career award from the *Fonds de recherche du Québec-Santé* (FRQ-S; Clinician-scientist Award) as well as from the Canadian Institutes for Health Research (CIHR; Young Investigator Award). G.H. is a senior scholar of the FRQ-S. L.E is the recipient of the Steriade-Savoy postdoctoral training award from the Savoy Foundation, the CHU Sainte-Justine Foundation postdoctoral training award and the FRQ-S postdoctoral training award, in partnership with the Foundation of Stars. This project has been made possible by Brain Canada through the Canada Brain Research Fund, with the financial support of Health Canada, awarded to L.E.

 

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