A Galvanotaxis Assay for Analysis of Neural Precursor Cell Migration Kinetics in an Externally Applied Direct Current Electric Field

Robart Babona-Pilipos (/author/Robart_Babona-Pilipos)[1], Milos R. Popovic (/author/Milos+R._Popovic)[2], Cindi M. Morshead (/author/Cindi+M._Morshead)[3]

[1]Institute for Biomaterials and Biomedical Engineering, **University of Toronto** (/institutions/NA-north-america/CA-canada/24824-university-of-toronto), [2]Lyndhurst Centre, **Toronto Rehabilitation Institute** (/institutions/NA-north-america/CA-canada/5151-toronto-rehab), [3]Department of Surgery, **University of Toronto** (/institutions/NA-north-america/CA-canada/24824-university-of-toronto)

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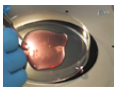
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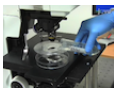
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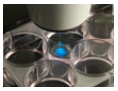
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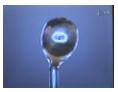
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Abstract

The discovery of neural stem and progenitor cells (collectively termed neural precursor cells) (NPCs) in the adult mammalian brain has led to a body of research aimed at utilizing the multipotent and proliferative properties of these cells for the development of neuroregenerative strategies. A critical step for the success of such strategies is the mobilization of NPCs toward a lesion site following exogenous transplantation or to enhance the response of the endogenous precursors that are found in the periventricular region of the CNS. Accordingly, it is essential to understand the mechanisms that promote, guide, and enhance NPC migration. Our work focuses on the utilization of direct current electric fields (dcEFs) to promote and direct NPC migration

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Information

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- a phenomenon known as galvanotaxis. Endogenous physiological electric fields function as critical cues for cell migration during normal development and wound repair. Pharmacological disruption of the trans-neural tube potential in axolotl embryos causes severe developmental malformations[1]. In the context of wound healing, the rate of repair of wounded cornea is directly correlated with the magnitude of the epithelial wound potential that arises after injury, as shown by pharmacological enhancement or disruption of this dcEF[2-3]. We have demonstrated that adult subependymal NPCs undergo rapid and directed cathodal migration *in vitro* when exposed to an externally applied dcEF. In this protocol we describe our lab's techniques for creating a simple and effective galvanotaxis assay for high-resolution, long-term observation of directed cell body translocation (migration) on a single-cell level. This assay would be suitable for investigating the mechanisms that regulate dcEF transduction into cellular motility through the use of transgenic or knockout mice, short interfering RNA, or specific receptor agonists/antagonists.

Protocol

All procedures involving animal handling were approved by the University of Toronto Animal Care Committee in accordance with institutional guidelines (protocol no. 20009387). The following methods should be performed using sterile tools and techniques, in a laminar flow hood where applicable.

In the protocol text below, the phrase "EFH-SFM" refers to serum free media supplemented with epidermal growth factor, basic fibroblast growth factor and heparin. EFH-SFM is used when investigating the galvanotaxis of undifferentiated NPCs because these mitogens maintain NPCs in their undifferentiated state[4]. When investigating the galvanotaxis of NPCs induced to differentiate into mature cell types, "FBS-SFM" refers to serum free media supplemented with 1% fetal bovine serum. FBS promotes the differentiation of NPCs into mature neural phenotypes[5].

1. Isolation and Culture of Neural Precursors (Not shown in video)

1. Anaesthetize a CD1 mouse (6-8 weeks old) with isofluorane and sacrifice via cervical dislocation.
2. Douse the head in 70% ethanol and decapitate the animal with sharp dissection scissors.
3. While holding the head with surgical forceps, remove the skin on the dorsal surface to expose the skull.
4. Using a scalpel and no. 11 blade, score the skull at the frontal sinus along the mediolateral axis, and also along the sagittal suture in the rostrocaudal direction.
5. Peel the parietal bones away from the head with no. 7 curved forceps, taking care not to pierce the brain tissue.
6. Insert a thin spatula underneath the brain starting from beneath the cerebellum and advancing toward the olfactory bulbs. While holding the skull in place with forceps, gently pull the brain from the skull and immediately place it in ice-cold artificial cerebrospinal fluid (see recipes below).
7. Under a dissection microscope, using sterile dissection scissors and forceps cut the brain in half along the midline. Rotate each hemisphere so that the medial (cut) surface faces upwards.
8. Select a hemisphere, and with the medial surface facing upward, locate the splenium of the corpus callosum (posterior region of the corpus callosum).
9. Make an incision from the surface of the cortex to the splenium of the corpus callosum along the dorsoventral axis.
10. Peel the incised cortex toward the olfactory bulb to expose the medial and lateral walls of the lateral ventricle.
11. Rotate the hemisphere so that the dorsal surface faces upwards, and use curved microscissors to cut out

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Neuroscience (/keyword/neuroscience), Issue 68 (/archive/68), Biomedical Engineering (/keyword/biomedical+engineering), Cellular Biology (/keyword/cellular+biology), Physiology (/keyword/physiology), Molecular Biology (/keyword/molecular+biology), neural precursor cells (/keyword/neural+precursor+cells), galvanotaxis (/keyword/galvanotaxis), cell migration (/keyword/cell+migration), time-lapse imaging (/keyword/time+lapse+imaging), electric fields (/keyword/electric+fields)

and collect the exposed medial and lateral walls, which contain the periventricular region where NPCs reside[6].

12. Repeat steps 1.8-1.11 for the other hemisphere.
13. Pipette the isolated tissue into 7 ml of trypsin solution (see recipe below) in a 15 cc tube, and place the tube on a rocker in a 37 °C incubator for 25 min.
14. Centrifuge the tube at 1,500 rpm for 5 min, aspirate the supernatant, and resuspend the tissue in 2 ml of trypsin inhibitor solution (see recipe below).
15. Gently triturate the tissue with a small borehole Pasteur pipette 30-50 times carefully to avoid air bubbles.
16. Centrifuge the tube at 1,500 rpm for 5 min, aspirate the supernatant, and resuspend in 1-2 ml of SFM (see recipe below) by triturating the pellet 3-5 times.
17. Centrifuge the tube at 1,500 rpm for 3 min, aspirate the supernatant and resuspend in 1 ml of SFM + EFH.
18. Count live cell density with a haemocytometer and plate the cells in a T25 culture flask at a density of 10 cells per µl in SFM + EFH.
19. Allow the culture to grow for 7 days undisturbed to yield free-floating primary neurospheres comprised of NPCs.

2. Galvanotaxis Chamber Preparation

1. Place 3 square glass no. 1 cover slips (22 x 22 x 0.17 mm) in a bottle of 6N hydrochloric acid overnight.
2. The next day, use a diamond-tip glass-cutter to cut 6 rectangular strips (22 x 5 x 0.17 mm) of glass from square no. 1 cover slips.
3. Transfer the acid-washed square slips and rectangular slips into a laminar flow hood. Wash the rectangular and square strips first with 70% ethanol, then with tissue culture-grade autoclaved water, and allow to dry on a Kim Wipe (for added sterility, the glass may be allowed to air dry).
4. Apply vacuum grease to the perimeter of one surface of the square glass slides, and seal them to the base of 60 mm plastic Petri dishes.
5. Apply vacuum grease along the long axis of one surface of the rectangular glass strips, and seal them to opposite edges of the square glass slides (such that they are parallel to each other) in order to create a central trough.
6. UV-sterilize the chambers for at least 15 min in the laminar flow hood.
7. Pipette 250-300 µl of poly-L-lysine onto the central trough of the chambers and incubate at room temperature for 2 hr.
8. Approximately 15 min prior to the end of the incubation period, prepare the Matrigel solution (see recipe below).
9. Aspirate the poly-L-lysine, wash the central troughs with 1 ml of autoclaved water, and pipette 250-300 µl of Matrigel solution onto the central troughs.
10. Incubate the chambers at 37 °C for 1 hr.
11. Aspirate the Matrigel solution and gently wash the central troughs with 1-2 ml of SFM.
12. Pipette 100 µl of EFH-SFM or FBS-SFM onto the central troughs and transfer the galvanotaxis chambers onto the stage of a counting microscope.
13. Pipette 3-4 ml of the neurosphere-containing culture into a 60 mm Petri dish and transfer the Petri dish to the stage of the counting microscope.
14. At a viewing objective of 5x, use a P10 pipette to transfer 5-8 whole neurospheres (up to four at a time) onto the central trough of each galvanotaxis chamber without dissociating them, and carefully spread the

neurospheres around the central trough without disrupting the Matrigel substrate.

15. Add an additional 150-200 µl of EFH-SFM or FBS-SFM onto the central troughs.
16. Transfer the galvanotaxis chambers into a 37 °C, 5% CO_2, 100% humidified incubator for 17-20 hr (if analyzing undifferentiated NPCs) to allow the neurospheres to adhere to the Matrigel substrate and dissociate into single cells as shown in **Figure 1**. If analyzing differentiated NPCs, the incubation period should be extended to 69-72 hr to allow the differentiation of the cells.

3. Live Cell Time-Lapse Imaging

1. Allow the live cell imaging system to equilibrate at 37 °C, 5% CO_2 for a minimum of 30 min prior to initiation of the time-lapse recording.
2. Cut two 12 cm pieces of 1 mm diameter Silver wire, coil them from one end, and place them in Clorox bleach for 20 min to form Ag/AgCl electrodes.
3. Transfer the galvanotaxis chambers onto the stage of a counting microscope and select which chamber will be used for live-cell imaging migration analysis based on the following criteria: i) the neurospheres should be almost completely dissociated into single cells and ii) the cells should possess round morphologies with little-to-no processes extending from the cell bodies.
4. Transfer the selected galvanotaxis chamber into a laminar flow hood, along with a separate square no. 1 glass cover slip and vacuum grease.
5. Wash the cover slip first with 70% ethanol, then with autoclaved water, and apply a strip of vacuum grease on two parallel edges of the cover slip.
6. Aspirate the culture media from the central trough of the chamber, then quickly place the cover slip (grease-side facing down) onto the chamber such that the grease strips rest on the two parallel rectangular glass strips, effectively creating a roof to the chamber.
7. Pipette 100 µl of fresh EFH-SFM or FBS-SFM into the central trough via capillary action.
8. Use vacuum grease to create borders for pools of culture media on each end of the central trough, as shown in **Figure 2**.
9. Cut two 15 cm pieces of PVC tubing, and use a 10 cc syringe with an 18 gauge needle to carefully inject agarose solution into the tubing, ensuring no bubbles form in the tubes, and allow the gel to solidify for 5 min.
10. Transfer the galvanotaxis chamber to the live cell imaging system, along with the agarose gel tubes, Ag/AgCl electrodes, and a pair of empty 60 mm Petri dishes that will be used as culture media reservoirs and will contain the Ag/AgCl electrodes. Allow the galvanotaxis chamber to rest within the 37 °C, 5% CO_2 environment for 20-30 min.
11. During this time, prepare the lids of the 2 empty Petri dishes and the lid of the galvanotaxis chamber's Petri dish by drilling holes into them with a Dremel or similar tool as shown in **Figure 3**.
12. Pipette 1-1.5 ml of EFH-SFM or FBS-SFM onto either side of the central trough, and 7-8 ml of SFM into each empty Petri dish. Place one Petri dish on each side of the galvanotaxis chamber's central trough and place one Ag/AgCl electrode into each dish. Bridge the gap between the galvanotaxis chamber and the Petri dishes to establish electrical continuity with the agarose gel bridges, as shown in **Figure 4**.
13. Connect the Ag/AgCl electrodes to an external power supply, with an ammeter in series to measure electrical current, and turn on the power supply. Use a voltmeter to measure the strength of the electric field directly across the central trough, and adjust the output of the power supply until the desired electric field strength is achieved (the assays performed in this lab utilize a dcEF strength of 250 mV/mm with electrical current between 1 and 1.5 mA).

14. Initiate the time-lapse module on the live cell imaging system, and allow the experiment to run for the desired amount of time. After completion of the assay, fix the cells in 4% paraformaldehyde for standard immunostaining analysis.

Representative Results

Kinematic analysis reveals that in the presence of a 250 mV/mm dcEF, undifferentiated NPCs exhibit highly directed and rapid galvanotaxis toward the cathode (**Figure 5A, Movie 1**). In the absence of a dcEF, random movement of the cells is observed (**Figure 5B, Movie 2**). At this field strength, > 98% of undifferentiated NPCs migrate for the entire 6-8 hr for which they are imaged, and since dead cells do not migrate this suggests that they remain viable during this period in the absence or presence of a dcEF.

Differentiated phenotypes undergo negligible migration both in the presence and the absence of a dcEF (**Movies 3, 4**). Subsets of differentiated cells extend processes that tend to align perpendicular to the direction of the dcEF, but no noticeable cell body translocation is observed.

Immunostaining verifies that NPCs maintain positive expression of the neural precursor marker nestin after 6 hr of dcEF exposure (**Figure 6A**). In the assays involving NPCs induced to differentiate into mature phenotypes, the majority of cells express the mature astrocyte marker glial fibrillary acidic protein (GFAP) after 6 hr of dcEF exposure (**Figure 6B**).

The primary antibodies used in these analyses were as follows: mouse monoclonal anti-nestin (1:400, Millipore, Canada), and rabbit polyclonal anti-GFAP (1:500, Sigma, Canada). The secondary antibodies used in these analyses were as follows: goat-anti-mouse conjugated with Alexafluor 568 (1:400, Invitrogen-Gibco, Canada), and goat-anti-rabbit conjugated with Alexafluor 488 (1:400, Invitrogen-Gibco, Canada).

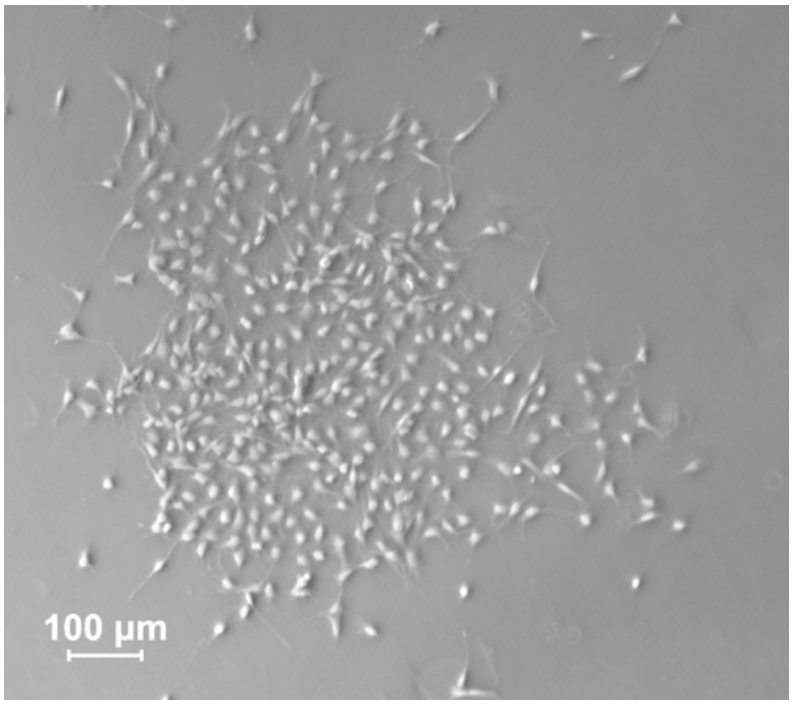

Figure 1. Neurospheres adhere to the Matrigel substrate and dissociate into single cells following 17 hr of incubation at 37 °C/5% CO_2, 100% humidity in EFH-SFM.

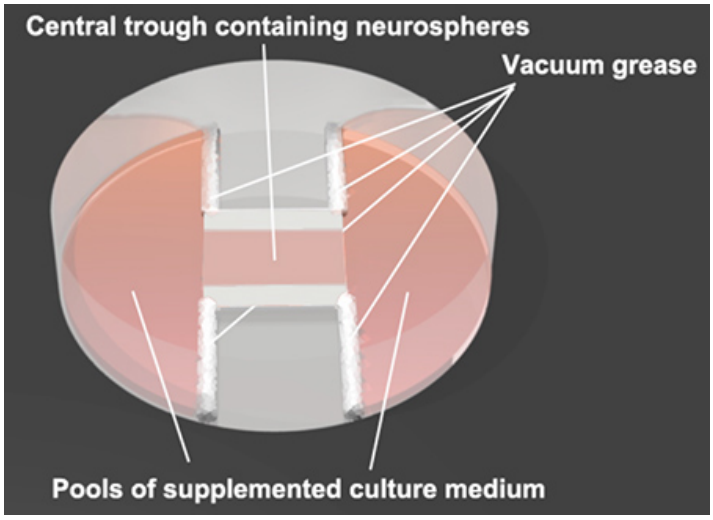

Figure 2. Illustration of galvanotaxis chamber. Strips of vacuum grease are used to create a pool of culture media on either side of the central trough.

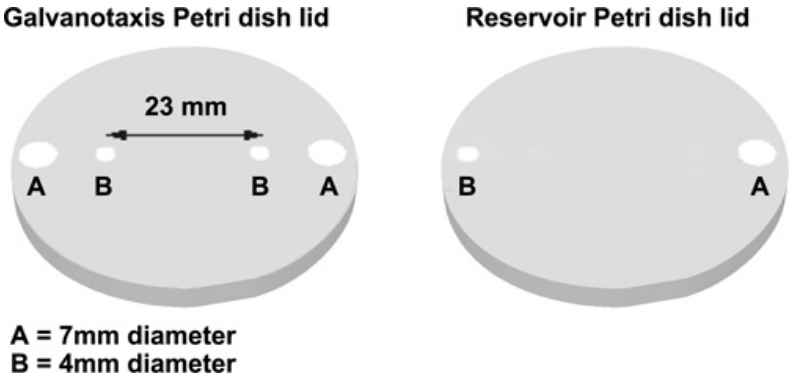

Figure 3. Schematic of holes that should be drilled into the lids the galvanotaxis chamber and the culture medium reservoirs. The 7 mm diameter holes in both lids are used to insert the agarose gel tubes. The 4 mm diameter holes in the lid of the galvanotaxis chamber is used to measure the electric potential directly across the central trough using a voltmeter. The 4 mm diameter hole in the lid of the culture medium reservoir is to allow the Ag/AgCl electrode to protrude from the lid of the dish for connection to the power supply.

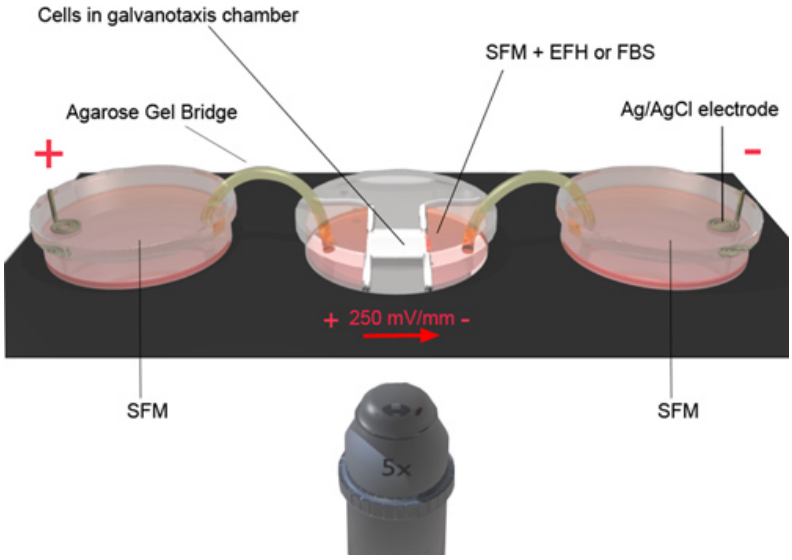

Figure 4. Illustration of galvanotaxis chamber assembly for time-lapse imaging. The galvanotaxis chamber is the central Petri dish, in which the cells are plated. The media inside the central Petri dish housing the galvanotaxis chamber is either SFM + EFH or SFM + 1% FBS. The Petri dishes on either side of the galvanotaxis chamber are filled with SFM, and also contain the Ag/AgCl electrodes. These electrodes are

connected to an external power supply, and bridging the three Petri dishes with agarose-gel bridges forms a complete circuit.

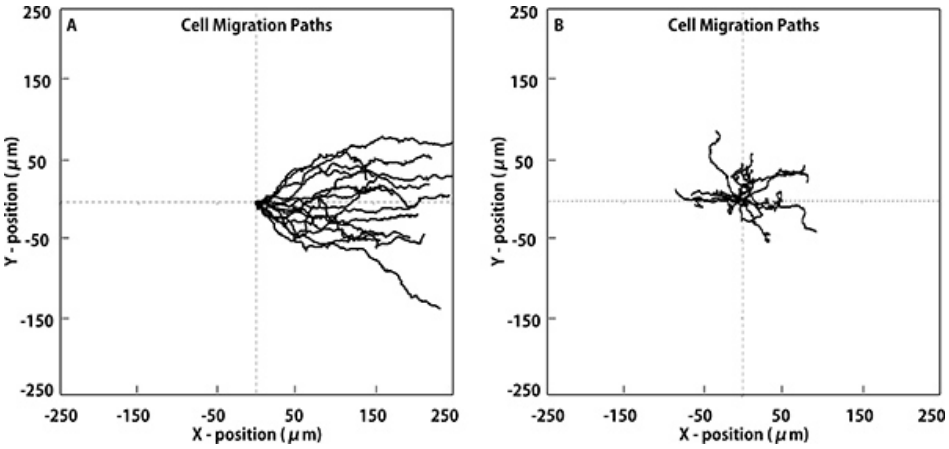

Figure 5. In the presence of a dcEF undifferentiated NPCs undergo rapid galvanotactic migration toward the cathode (A), whereas in the absence of a dcEF the cells undergo random radial migration (B).

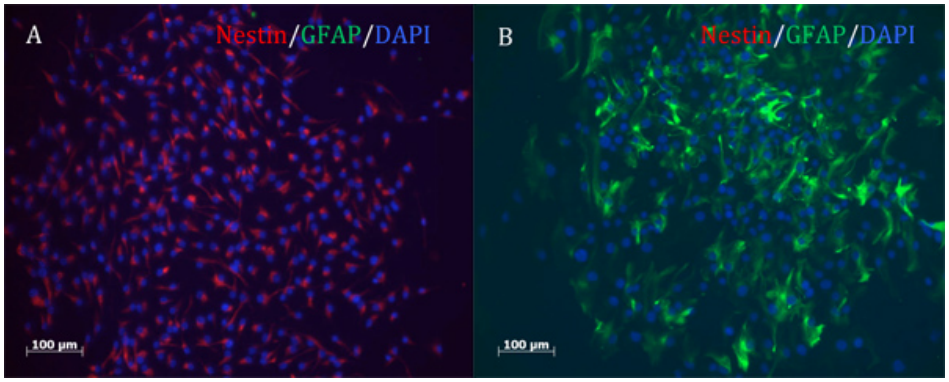

Figure 6. Immunostaining verifies that NPCs remain nestin-positive undifferentiated precursors after 6 hr of dcEF exposure (A), and that NPCs induced to differentiate mostly express the mature astrocyte marker GFAP after 6 hr of dcEF exposure (B).

Movie 1. Time-lapse video of undifferentiated NPCs exposed to a dcEF. NPCs plated onto galvanotaxis chambers for 17 hr in SFM + EFH, and then exposed to a 250 mV/mm dcEF exhibit rapid and directed migration toward the cathode. 1 second of video = 15 minutes real time. Click here to view movie (http://www.jove.com/files/ftp_upload/4193/4193movie1.mp4).

Movie 2. Time-lapse video of undifferentiated NPCs in the absence of a dcEF. NPCs plated onto galvanotaxis chambers for 17 hr in SFM + EFH, and then imaged in the absence of an applied dcEF undergo random migration. 1 second of video = 15 minutes real time. Click here to view movie (http://www.jove.com/files/ftp_upload/4193/4193movie2.mp4).

Movie 3. Time-lapse video of differentiated NPCs exposed to a dcEF. NPCs plated onto galvanotaxis chambers for 69-72 hr in SFM + FBS, and then exposed to a 250 mV/mm dcEF exhibit very little migration in any direction. 1 second of video = 15 minutes real time. Click here to view movie (http://www.jove.com/files/ftp_upload/4193/4193movie3.mp4).

Movie 4. Time-lapse video of differentiated NPCs in the absence to a dcEF. NPCs plated onto galvanotaxis chambers for 69-72 hr in SFM + FBS, and then imaged in the absence of an applied dcEF exhibit very little migration in any direction, similar to differentiated NPCs that are exposed to a dcEF. 1 second of video = 15 minutes real time. Click here to view movie (http://www.jove.com/files/ftp_upload/4193/4193movie4.mp4).

Discussion

This protocol has been adapted from the well-established methods of previous studies[7-9]. Galvanotactic chambers can be constructed using a variety of different techniques, including the construction of a separate glass well for confinement of cell seeding, or using CO_2 laser ablation for microfabrication of the central trough[10,11]. Some techniques may be more laborious or costly than others. We have described a simple and cost-effective assay for constructing a NPC galvanotaxis chamber using materials commonly found in most cell biology laboratories. Our protocol includes a heated, humidified, CO_2 regulated incubator that surrounds the live cell imaging system to maintain the cells under optimal conditions for continuous long-term imaging. The lack of such apparatus is a limitation of some previously published techniques[9,12].

Recent work by another group demonstrated similar galvanotactic behavior of cells from a hippocampal cell line[13], using a different protocol and a more labor-intensive galvanotaxis chamber design. Our assay is particularly elegant in that it permits an analysis of the identical starting population of cells - primary cell cultures of undifferentiated neurosphere derived NPCs - that have been exposed to distinct conditions thereby permitting a comparison of the migratory properties of both undifferentiated and differentiated cells, simply by modifying the factors used to supplement the culture media within the galvanotaxis chamber.

The assay presented in this protocol is a powerful tool for long-term tracking and analysis of NPC galvanotaxis, and possibly other cell types that undergo galvanotactic migration[14,15]. As with any protocol, nuances in the preparation and execution of certain steps in this protocol may lead to unsuccessful results. The following guidelines and suggestions should assist in the successful execution of this protocol:

- To prevent the cells from undergoing electrical current-associated heat death, the dimensions of the galvanotaxis chamber have been calculated to minimize the effects of Joule heating[16]. The heat generated in the chamber is proportional to the square of the current flowing through it, which is in turn proportional to the cross-sectional area of the galvanotaxis chamber. We advise maintaining the cross-sectional area of the chamber as 2.04 mm^2 (12 mm x 0.17 mm).
- After 7 days of culture neurospheres can be collected, dissociated into single cells and replated in growth-factor conditions (a process referred to as 'passaging') to yield secondary neurospheres. We obtain our best results using neurospheres that have undergone less than three passages (maximum 21 days in culture), with a visible decline in migratory capabilities when using neurospheres that have been passaged a greater number of times. This is consistent with our previous work demonstrating that longer-term cultures alter cell kinetics[17].
- When selecting a galvanotaxis chamber containing NPCs for observation, it is important to select a chamber in which the neurosphere derived cells have dissociated well in order to permit accurate kinematic analyses. If the neurospheres have not dissociated well (NPCs are adhered to each other and cannot be delineated), analysis of individual cell migratory behaviour will become near impossible. The cultures should be allowed more time to dissociate prior to observation. In addition, cells that are in close proximity to each other can physically obstruct the migration of neighboring cells. Our analyses have shown that cells positioned at least one cell body away from their nearest neighboring cell migrate at significantly greater velocities than cells that are clustered together near the center of the dissociated neurosphere, albeit with equal directedness.
- If there is an abundance of processes extending from the NPCs, this is an indicator that the cells have begun to differentiate and therefore they are not a true representation of an undifferentiated population. A benefit of preparing the galvanotaxis chambers in triplicate is that the remaining two chambers that are not selected for

time-lapse imaging can be fixed immediately following their incubated plating period (Step 2.16), and immunostained to verify the differentiation profile of the cells.

- For optimal results the culture media should be freshly prepared and supplemented on each day that it is required.
- The time-lapse images and subsequent kinematic analyses are highly sensitive to perturbation of the sample. The live-cell imaging microscope should be placed on an air table to minimize vibrations, and the system should not be perturbed during experimentation.

Our lab routinely images NPC galvanotaxis for 6-8 hr, although up to 15-hr analyses have been performed. Over the 15-hr periods, the dcEF across the chamber declined from 250 mV/mm to 227 mV/mm (9.2% decrease). Longer imaging periods inevitably modify the pH of the media in the chamber, further modifying the electrical field. Thus it is recommended to replace the media approximately every 6 hr. However, we have performed 8-hr NPC galvanotaxis assays without media replacement and have not observed any noticeable cell death (dead cells do not migrate) or decline in cell motility; the cells maintain their velocity of migration throughout the experiment. The investigator may also reduce the depth of the chamber to increase the stability of the dcEF[10].

Following imaging, single-cell kinematic tracking analysis is performed on a subset of the imaged cells using Zeiss Axiovision software's tracking module. Cells are selected for analysis if they are localized closer to the edge of the dissociated neurosphere and at least one cell body apart from its nearest cell. The rationale behind this is to minimize the likelihood of cells overlapping each other during tracking, which would make tracking individual cells near impossible. We analyze the following four parameters of migration:

1. X-axis displacement - the distance a cell migrates in the direction of the axis, which is parallel to the direction of the dcEF.
2. Velocity - the straight-line distance between initial and final cell positions divided by elapsed time.
3. Directedness - x-axis displacement divided by the straight-line distance between the initial and final cell positions.
4. Tortuosity - the total path distance travelled by the cell divided by the straight-line distance between initial and final cell positions.

The latter two parameters are indicators of how straight the migration pathway is in a particular direction.

Upon grasping the methods in this protocol, the investigator may wish to modify some aspects for broader applications. For example, the assay may also be modified to investigate the migratory behavior of other cell types. Genetic knockout models or siRNA transfection techniques could be used to target genes of interest that may play a role in cell migration or the transduction of a dcEF into cell motility. In our lab, we have modified the galvanotaxis chamber to permit continuous cross-perfusion of fresh culture media within the central trough during experimentation[18]. An appropriate substrate and culture media would need to be selected for other cell types, and the duration of cell seeding prior to imaging should be adjusted as necessary. Notably, the dimensions of the chamber may need to be modified depending on the thickness of the tissue being examined (for instance, if a tissue slice is placed in the chamber). The investigator should bear in mind that for a set electrical field, the current flow through the chamber is directly proportional to the cross-sectional area of the chamber, and therefore significant enlargements to the chamber's dimensions may result in increased Joule heating-related cell death.

The techniques described in this report provide a powerful tool for investigating the important, but not widely

studied, phenomenon of galvanotaxis. The ability of cells to respond to dcEFs has direct therapeutic implications. Electrical stimulation (deep brain stimulation) has already proven useful in the treatment of Parkinson's disease, and has been shown to promote hippocampal neurogenesis in a mouse model[19,20]. A complete understanding of the cellular mechanisms responsible for dcEF transduction into cellular motility may eventually lead to the use of electrical stimulation as a clinical means for enhancing and directing endogenous precursor migration to sites of injury or disease to facilitate the repair process. The methods described above provide a simple and robust way of investigating these processes.

Disclosures

No conflicts of interest declared.

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Materials

Name	Company	Catalog Number	Comments
Neural Precursor Cell Isolation			
2M NaCl	Sigma	S5886	11.688 g dissolved in 100 ml dH_2O
1M KCl	Sigma	P5405	7.456 g dissolved in 100 ml dH_2O
1M $MgCl_2$	Sigma	M2393	20.33 g dissolved in 100 ml dH_2O
155 mM $NaHCO_3$	Sigma	S5761	1.302 g dissolved in 100 ml dH_2O
0.5M Glucose	Sigma	G6152	9.01 g dissolved in 100 ml dH_2O
108 mM $CaCl_2$	Sigma	C7902	1.59 g dissolved in 100 ml dH_2O
Penicillin-streptomycin	Gibco	15070	
Bovine pancreas trypsin	Sigma	T1005	
Sheep testes hyaluronidase	Sigma	H6254	
Kynurenic acid	Sigma	K3375	
Ovomucoid trypsin inhibitor	Worthington	LS003086	
DMEM	Invitrogen	12100046	
F12	Invitrogen	21700075	

30% Glucose	Sigma	G6152	
7.5% $NaHCO_3$	Sigma	S5761	
1M HEPES	Sigma	H3375	23.83 g dissolved in 100 ml dH_2O
L-glutamine	Gibco	25030	
EGF	Invitrogen	PMG8041	Reconstitute in 1 ml of hormone mix and aliquot into 20 µl units.
FGF	Invitrogen	PHG0226	Reconstitute in 0.5 ml of hormone mix and aliquot into 20 µl units.
Heparin	Sigma	H3149	
Apo-transferrin	R&D Systems	3188-AT	0.1 g dissolved into 4 ml dH_20
Putrescine	Sigma	P7505	Dissolve 9.61 mg into Apo-transferrin solution
Insulin	Sigma	I5500	Dissolve 25 mg into 0.5 ml of 0.1N HCl and add to 3.5 ml of dH_20
Selenium	Sigma	S9133	
Progesterone	Sigma	P6149	
Standard Dissection Tools	Fine Science Tools		
Dissection microscope	Zeiss	Stemi 2000	
Galvanotaxis Chamber Preparation			
Square glass cover slides	VWR	16004	
6N Hydrochloric Acid	VWR	BDH3204-1	
High vacuum grease	Dow Corning		
60 mm Petri dishes	Fisher Scientific	0875713A	
Poly-L-lysine	Sigma	P4707	
Matrigel	BD Biosciences	354234	Thaw and aliquot into 150 µl units
FBS	Invitrogen	10082139	Only use if inducing NPC differentiation, otherwise use SFM + EFH culture media as indicated above
Counting microscope	Olympus	CKX41	
Live Cell Time-Lapse Imaging			
Silver wire	Alfa Aesar	11434	
UltraPure Agarose	Invitrogen	15510-027	

Heat Inactivated FBS	Sigma	16140071	
PVC tubing	Fisher Scientific	80000006	3/32"ID x 5/32"OD
Bleach	Clorox		
10 cc syringe	BD	309604	
18 gauge needle	BD	305195	
Dremel drill	Dremel	Model 750	
Inverted microscope equipped with humidified, incubated chamber	Zeiss	Axiovert-200M	

Recipes

Item	**Volume**
2M NaCl	6.2 ml
1M KCl	0.5 ml
1M $MgCl_2$	0.32 ml
155mM $NaHCO_3$	16.9 ml
1M Glucose	1 ml
108 mM $CaCl_2$	0.09256 ml
Penicillin-streptomycin	1 ml
Autoclaved water	74 ml

Artificial cerebrospinal fluid

Item	**Volume or Mass**
Artificial cerebrospinal fluid	30 ml
Bovine pancreas trypsin	40 mg
Sheep testes hyaluronidase	22.8 mg
Kynurenic acid	5 mg

Trypsin Solution

Item	**Volume or Mass**
SFM	15 ml
Ovomucoid trypsin inhibitor	10 mg

Trypsin Inhibitor Solution

Item	**Volume**

Autoclaved water	37 ml
10X DMEM/F12	10 ml
30% Glucose	2 ml
7.5% $NaHCO_3$	1.5 ml
1M HEPES	0.5 ml
Transferrin, Putrescine solution	4 ml
25 mg insulin solution	4 ml
Selenium	100 µl
Progesterone	100 µl

Hormone Mix (100 ml total, store at -20 °C)

Item	**Volume**
Autoclaved water	37.5 ml
10X DMEM/F12 (3:1)	5 ml
30% Glucose	1 ml
7.5% $NaHCO_3$	0.75 ml
1M HEPES	0.25 ml
Hormone mix	5 ml
L-glutamine	0.5 ml
Penicillin-streptomycin	0.5 ml

Serum Free Media EFH-SFM: add 10 µl of EGF, 10 µl of FGF, and 3.66 µl of Heparin FBS-SFM: add 0.5 ml FBS

Item	**Volume**
Matrigel	150 µl
SFM	3.6 ml

Matrigel Solution Matrigel aliquot should be placed in a box of ice and allowed to thaw slowly over 4-5 hours to form a viscous liquid before mixing with SFM. This will ensure the formation of a smooth layer of Matrigel substrate. If not thawed slowly, the resulting substrate will contain clumps of Matrigel, possibly hindering cell migration.

Item	**Volume or Mass**
UltraPure Agarose	300 mg in 10 ml ddH_20
SFM Heat Inactivated FBS	8 ml 2 ml

Matrigel Solution Mix 8 ml of SFM with 2 ml heat inactivated FBS in a 15 cc falcon tube. Mix

agarose with 10 ml ddH_20 in an Erlenmeyer flask, and heat in a microwave for 30 sec in 10-sec intervals, ensuring to remove the solution from the microwave after each 10-sec interval and thoroughly mix. Following the final 10-sec microwave period, mix the agarose solution with the SFM/FBS solution and store in a 57 °C water bath.

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