Video Article

Neonatal Subventricular Zone Electroporation

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Abstract

Neural stem cells (NSCs) line the postnatal lateral ventricles and give rise to multiple cell types which include neurons, astrocytes, and ependymal cells[1]. Understanding the molecular pathways responsible for NSC self-renewal, commitment, and differentiation is critical for harnessing their unique potential to repair the brain and better understand central nervous system disorders. Previous methods for the manipulation of mammalian systems required the time consuming and expensive endeavor of genetic engineering at the whole animal level[2]. Thus, the vast majority of studies have explored the functions of NSC molecules *in vitro* or in invertebrates.

Here, we demonstrate the simple and rapid technique to manipulate neonatal NPCs that is referred to as neonatal subventricular zone (SVZ) electroporation. Similar techniques were developed a decade ago to study embryonic NSCs and have aided studies on cortical development[3,4]. More recently this was applied to study the postnatal rodent forebrain[5-7]. This technique results in robust labeling of SVZ NSCs and their progeny. Thus, postnatal SVZ electroporation provides a cost and time effective alternative for mammalian NSC genetic engineering.

Video Link

The video component of this article can be found at http://www.jove.com/video/50197/

Protocol

This procedure is in accordance with Yale IACUC requirements. Scientists should make sure that IACUC guidelines are approved and followed according to their institutional requirements.

1. Section 1. Preparation of DNA, Solutions, and Glass Pipettes

1. Generate high purity (OD 260/280 > 1.80) high concentration (µg/µl > 2.5) endotoxin-free DNA.
2. Prepare 0.9% saline solution and sterile filter through a 22 µm filter.
3. Place 10 cm fire polished borosilicate glass capillary tubes (O.D.: 1.5 mm, I.D.:1.10 mm) into a Model PP-830 Narishige PC-10 glass pipette puller with a kanthal wire. Set puller to a one step weighted pull at 70.5 °C. Break tips with circular forceps and inspect under a dissecting microscope for jagged edges. Bevel tips and place under a UV lamp for 15 min. Pulled pipettes should be marked at 2 mm from the edge of the tip.
4. Prepare 0.1% weight/volume fast green solution by mixing sterile filtered 0.9% saline solution with dry fast green.

2. Section 2. Animal Preparation, Glass Pipette Loading and Plasmid Injection

1. Remove postnatal day 0-1 pups individually from cages, place onto a glass Petri dish pre-chilled to 4 °C, and then place on wet ice.
2. After approximately 5 min, determine the state of anesthetization by utilizing the foot pinch response. If no movement occurs, subject pups to intraventricular injection.
3. It is important to note that the combination of fast green, DNA, and saline solution can result in rapid evaporation, crystallization, and blockade of glass pipettes. Therefore, generate a stock solution and aliquot 1-2 µl onto parafilm immediately prior to injections.
4. Aspirate the entire aliquoted volume with the pulled glass pipette.
5. Hold the head of the pup between your thumb and index finger of your less dominant hand.
6. Turn on lamp and hold head of pup just outside of light beam. If needed, put saline on head to reduce the amount of light reflected by pup. The ventricles should now be illuminated.
7. With your dominant hand, insert the needle into lateral ventricle closest to you (**Figure 1A** and **1B**). The site of injection should be approximately equidistant from the lambdoid suture and eye and 2 mm lateral to the sagittal suture. Insert pulled pipette to the 2 mm mark for a P0 pup, which should ensure penetration into the lateral ventricle. Coincidentally, you may see back filling of CSF into the pipette from

the release of intracranial pressure. To reduce intracranial pressure, which assists in injection of plasmid, loosen your grip on the head of the pup.Step 2.8. Inject approximately 0.5 µl of plasmid into lateral ventricle using an air-pressured injector (Picospritzer, Parker).

3. Section 3. Electroporation and Recovery

1. Set the voltage of the electroporation generator for 5 square pulses, 50 msec/pulse at 100 volts, with 950 msec intervals. The spatial specificity of plasmid electroporation is dictated by the directionality of charge transfer. Close attention should be given to the placement given the heterogeneity of stem cell populations along the SVZ[8]. Differences in the rates of proliferation and cell fate have been identified for ventral-dorsal and rostral-caudal locations[9,10] .
2. Once the plasmid is injected, dip tweezer electrodes into PBS. This step is to maximize charge transfer and to prevent burns caused by resistivity.
3. Place the positive electrode on the dorsal lateral wall of the pup near the ear ipsilateral to the site of plasmid injection (**Figure 1C** and **1D**). Place the negative electrode on the contralateral hemisphere ventral lateral to the pup's snout.
4. Initiate current transfer by pressing the pulse footswitch pedal and sweep the electrodes from dorsal to lateral using ~25 ° angle intervals.
5. Place electroporated pups onto a heating pad for 5 min. Gently rotate pups every 30 sec with mild stimulation.

Representative Results

Neonatal subventricular zone electroporation results in the labeling of nearly all radial glia contiguous with the dorsal, dorsal lateral, and lateral subventricular zone following the "sweeping" movement of the tweezer electrode (**Figure 1E**). However, electroporation can be tailored to the requirement of the respective experiment but not sweeping the electrode and using specific placement and orientation as detailed in the video. For example, since dorsally localized radial glia differ from those lining the lateral section of the ventricle, one can chose to only electroporate a single region[9,10] . Further specificity can also be given using smaller electrodes.

Plasmid DNA is rapidly diluted within the first three weeks[11]. Therefore, identifying genomically modified cells using plasmid DNA is not recommended. To circumvent this limitation, CRE recombinase expressing plasmids are introduced along with GFP into mice which have a stop sequence flanked by lox P sites upstream of a genomically expressed fluorescent protein (tdTomato)[12]. Upon recombination, the stop sequence is removed and the fluorescent protein is expressed (**Figure 1F**). By four weeks post-electroporation few GFP plasmid expressing cells are seen in the subventricular zone whereas many more tdTomato positive cells are seen. Upon sectioning the olfactory bulb it is also apparent that in comparison to tdTomato expressing cells, far fewer GFP expressing cells are present, however both have acquired features of mature granule cells (**Figure 1G**).

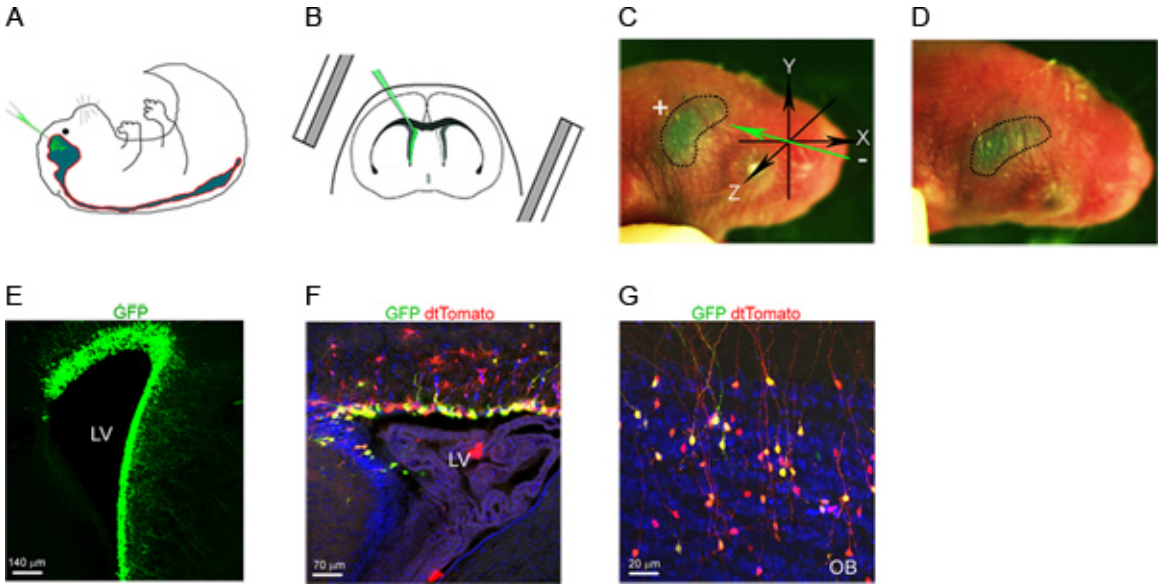

Figure 1. Neonatal Subventricular Zone Electroporation. (A) Schematic diagram of a P0-P1 pup with ventricular system and spinal cord containing cerebrospinal fluid (teal) highlighted injected with enhanced GFP (eGFP) encoding plasmid (Green). **(B)** Coronal section showing orientation of paddles relative to injection site and lateral ventricle. **(C)** Orientation of negative (-) and positive (+) electrodes with respect to XYZ axis on injected pup. **(D)** Horizontal view of a pup following electroporation. **(E)** Image of the lateral ventricle 24 hr following electroporation of a eGFP-encoding vector (Green). **(F)** Image of the lateral ventricle 28 days after electroporation of CRE recombinase- and eGFP-encoding plasmids into mice carrying a stop codon upstream of tdTomato. Recombination induces expression of tdTomato (Red). Successive dilutions of plasmid result in few remaining eGFP positive SVZ cells whereas genomically expressed tdTomato is permanently expressed. **(G)** Dilution of plasmid DNA (green) is apparent given that more cells in the granule cell layer of the olfactory bulb express the genomic marker, tdTomato (red). LV: lateral ventricle; OB: olfactory bulb. Click here to view larger figure.

Discussion

Here we detail the technique of neonatal SVZ electroporation, a technique to rapidly and robustly label and manipulate SVZ stem cells and their progeny. There are several advantages that electroporation has in comparison to other techniques. First, given the focal labeling of cells, one is able to discern cell autonomous and non-cell autonomous effects. Second, genetic manipulation using inducible systems allows one to compare effects prior to or after synaptic integration. Furthermore, one can bypass the use of multiple plasmids by electroporating CRE expressing mice with plasmids containing loxP sites. Fourth, gene reporter plasmids can be used to study transcriptional transactivation. In this case, transcriptional activity can be measured only in cells that are manipulated; added specificity can be introduced when subregions are microdissected. Fifth, the transient labeling of transit amplifying cells due to continued proliferation and dilution of the plasmid can be used as "birthdating" method similar to labeling with thymidine analogs[11]. Sixth, although the transient labeling of cells could be seen as a disadvantage, the technique should be amenable to transposase-transposon pairs to induce genomic integration[13]. Alternatively, ongoing neurogenesis can be altered with CRE recombinase to induce genomic modification of mice containing alleles flanked by loxP sites[7,11] . Using this protocol, newborn neurons were identified up to four months post-electroporation in Rosa26R-Stop-tdTomato mice in which the stop cassette is flanked by loxP sites[12]. Taken together, neonatal SVZ electroporation is a cost effective and time efficient minimally invasive tool for the transient or permanent genetic manipulation and labeling of forebrain neural stem cells and newborn neurons.

Disclosures

The authors have no disclosures to make.

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