Video Article

Posterior Semicircular Canal Approach for Inner Ear Gene Delivery in Neonatal Mouse

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Abstract

Inner ear gene therapy offers great promise as a potential treatment for hearing loss and dizziness. One of the critical determinants of the success of inner ear gene therapy is to find a delivery method which results in consistent transduction efficiency of targeted cell types while minimizing hearing loss. In this study, we describe the posterior semicircular canal approach as a viable method for inner ear gene delivery in neonatal mice. We show that gene delivery through the posterior semicircular canal is able to perfuse the entire inner ear. The easy anatomic identification of the posterior semicircular canal, as well as minimal manipulation of the temporal bone required, make this surgical approach an attractive option for inner ear gene delivery.

Video Link

The video component of this article can be found at https://www.jove.com/video/56648/

Introduction

Inner ear gene therapy is a rapidly developing field of investigation. It has been applied in various animals models to combat ototoxicity, noise trauma, and hereditary hearing loss[1]. Several recent studies have shown functional recovery of hearing and balance functions in mutant mice after inner ear gene therapy delivery[2,3,4,5,6,7]. One of the key factors in determining the success of inner ear gene therapy is the surgical approach used to access the inner ear. Ideally, the surgical approach would be easy to perform, the anatomic landmarks would be consistent and easy to identify, and the resulting transduction of targeted cell types would be high.

In a recent study, we showed that when viral gene therapy was injected through the posterior semicircular canal of the whirler mutant mouse (a model of hearing loss and vestibular dysfunction), efficient transduction of sensory hair cells was seen in the vestibular organs as well as in the cochlea[5]. The high efficiency of sensory hair cell transduction resulted in improvement of auditory and vestibular functions in these mutant mice.

In this article, we describe in detail the posterior semicircular canal approach to access the neonatal mouse inner ear.

Protocol

All animal procedures were approved by the Animal Care and Use Committee at the National Institute on Deafness and Other Communication Disorders (NIDCD ASP1378-15).

1. Procedure Setup and Preparation

1. Sterilize all instruments by ethylene oxide in the beginning of the experiment. Between animals, clean instruments using bead sterilization.
2. Load the solution containing viral gene therapy into a micropipette on the micro-injector. The viral vector used in this study was AAV2/8-whirlin (1 x 10^{13} genome copies per milliliter, see the table of materials).
   NOTE: Typically, 1.1 µL total volume is loaded into the micropipette.

2. Anesthesia

NOTE: The mouse strain used in this study is the whirler mouse. Both homozygous mutants ($Whrn^{wi/wi}$) and heterozygous littermates ($Whrn^{+/wi}$) were used.

1. Place the mother in a separate cage (separate from the litter).

2. Place the home cage containing the litter (P0 - P5 pups) on a recirculating heat pad (set at 37.5 °C) to keep the mice warm.
3. Cut out the thumb portion of a latex glove, and place a pup in it.
4. Place the pup in latex glove thumb into a bucket of ice for ~2 min.
5. Place the anesthetized pup on a large square commercial plastic freeze pack with a 4" x 4" gauze between the pup and the pack's surface.
6. Fill a heavy-duty latex glove with crushed ice, and place the ice glove around the pup.
7. Check to see if the pup is adequately anesthetized by the complete lack of any response to various stimuli (including a firm toe pinch). Leave the pup on the ice pack for the duration of the surgery (approximately 5 - 10 min).
   NOTE: We recommend leaving the pups on the ice pack for no more than 15 min during the surgery.

3. Surgical Approach (Figure 1)

1. Clean the skin behind the ear with an iodine wipe and an alcohol wipe once the animal is anesthetized.
2. Make a postauricular incision ~2 mm behind the ear using micro-scissors, and divide the sternocleidomastoid muscle with micro-scissors.
3. Identify the facial nerve and the bulla. The bulla is cartilaginous and semi-transparent at this age and it lies medial to the facial nerve. The stapedial artery can be seen through the bulla at this age, which is a useful landmark.
4. Follow the facial nerve superiorly and posteriorly to locate the posterior semicircular canal (PSCC). Remove the muscle fibers and soft tissue overlying the posterior semicircular canal using micro-scissors.
   NOTE: The PSCC is cartilaginous at this age.
5. Penetrate the PSCC using a glass micropipette (~10 µm in diameter) on the micro-injector.
6. Inject viral gene therapy into the inner ear.
   NOTE: Typically, a total of 20 injections of 49 nL of the gene therapy are delivered into the posterior semicircular canal over ~40 s (total volume ~1 µL). The viral titer used was 1 x 10^{13} genome copies per mL.
7. Close the skin incision using a 5-0 polyglactin suture.

4. Postoperative Care

1. Place the pup on a warming pad to restore a normal body temperature during recovery from anesthesia with constant manual stimulation/ rolling with gloved human fingers.
2. Once the pup is awake, place it back into its home cage.
3. Caress each pup with a cotton swab that has been exposed to the home cage bedding.
   NOTE: The purpose of this is to have the mice smell as they did prior to surgery, which increases the likelihood of the mother re-accepting her litter post-surgery. If possible, urine from the mother can be collected and rubbed on the pups using a cotton swab to further decrease the likelihood of rejection.
4. Apply mineral oil to the mother's nose to desensitize her[8], and reintroduce the mother into the home cage.

Representative Results

Injection of AAV8-whirlin gene therapy into neonatal whirler mice through the posterior semicircular canal resulted in whirlin expression (green) in utricular hair cells (**Figure 2**), with the overall infection efficiency of 53.1% (SD 38.1, n = 28)[5]. Transduced hair cells had elongated stereocilia (red) compared to hair cells from contralateral non-injected ears (5.35 ± 2.11 µm vs. 3.20 ± 0.34 µm, respectively)[5].

Posterior semicircular canal injection of AAV8-whirlin also resulted in transduction of cochlear hair cells in the whirler mice (**Figure 3**). The average inner hair cell infection efficiency was 77.1% (SD 12.7, n = 8)[5]. Transduced hair cells expressed whirlin (green) at stereocilia tips and had elongated stereocilia (red) compared to hair cells from contralateral non-injected ears (5.04 ± 0.72 µm vs. 1.01 ± 0.08 µm at the cochlear apex, respectively)[5].

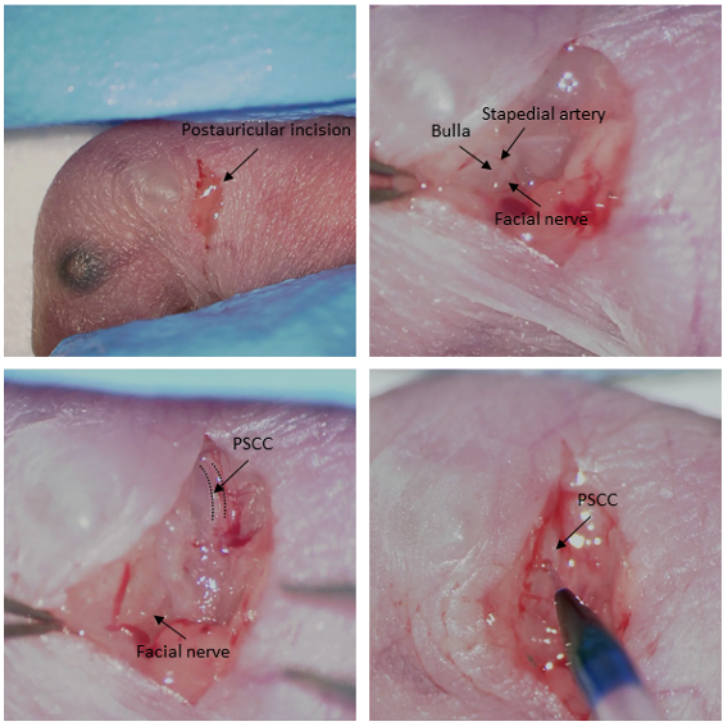

Figure 1: Intraoperative images.
Intraoperative images showing surgical access to the posterior semicircular canal (PSCC) in a P0 mouse.The left ear is shown. The PSCC is outlined in dashed black lines. Please click here to view a larger version of this figure.

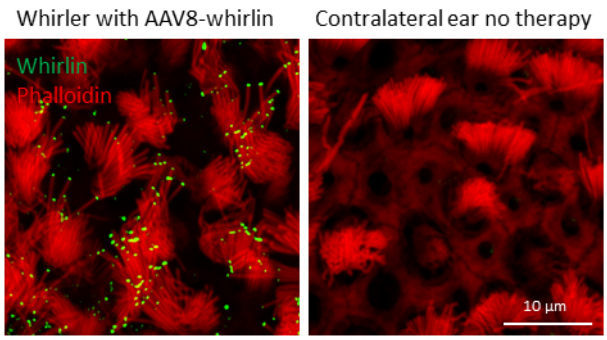

Figure 2: Vestibular hair cells are transduced using PSCC gene delivery.
AAV8-whirlin delivered via the PSCC approach resulted in high levels of utricular hair cell infection. Please click here to view a larger version of this figure.

Figure 3: Cochlear hair cells are transduced using PSCC gene delivery.
AAV8-whirlin delivered via the PSCC approach resulted in high levels of cochlear hair cell infection. Please click here to view a larger version of this figure.

Discussion

Several surgical approaches have been described to access rodent inner ears. Cochleostomy and round window approaches are most commonly used to access the cochlea, whereas the posterior semicircular canal and endolymphatic sac approaches are typically used to access the vestibular organs[1]. In a recent study, we showed that posterior semicircular canal injections of viral gene therapy resulted in high efficiency of hair cell transduction in both the vestibular organs and the cochlea[5]. In fact, cochlear hair cell transduction was higher through the posterior semicircular canal injections, when compared with round window injections in the same mouse model of hereditary hearing loss and vestibular dysfunction[5,9]. Given the anatomic proximity between the round window and the cochlea, it seems paradoxical that round window injections may lead to lower cochlear hair cell transduction compared to posterior semicircular canal injections. This finding may be explained by the fact that the round window is located close to the cochlear aqueduct. Therefore, when viral gene therapy is injected through the round window, its concentration may be diluted by the cerebrospinal fluid coming from the cochlear aqueduct[10].

The finding that posterior semicircular canal injection results in cochlear and vestibular hair cell transduction has also been reported by other studies[11,12]. In the study by Okada *et al.*, transduction of cochlear and vestibular hair cells by AAV-GFP was reported. However, numerical quantification was not performed. The study by Suzuki *et al.*, reported high levels of cochlear and vestibular hair cell transduction with the AAV-Anc80-GFP using the posterior semicircular canal approach in adult mice. In adult mice, cannulation of the posterior semicircular canal with a small catheter is preferable, since the bony covering of the posterior semicircular canal is completely ossified[12]. Catheterization of the posterior semicircular canal in adult mice can help to minimize injection backflow, which can decrease transduction efficiency. This step is not required in neonatal mice, since the bony covering of the posterior semicircular canal is still cartilaginous at that age.

One of the drawbacks of the posterior semicircular canal injection is the fact that one cannot be certain whether the injected gene therapy is delivered into the perilymph or endolymph. Despite this shortcoming, the posterior semicircular canal is anatomically easy to locate and requires minimal manipulation of the temporal bone for its identification. This decreases the chance of inner ear damage caused by surgical trauma. The posterior semicircular canal injection is an attractive option for inner ear gene therapy delivery.

Disclosures

The authors have no relevant disclosures to make.

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