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Surgical Methods for Inner Ear Gene Delivery in Neonatal Mouse

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Abstract

Inner ear gene therapy offers great potential as a treatment for hearing loss and dizziness. The surgical method used to deliver gene therapy into the inner ear is a critical step in determining the success of inner ear gene therapy. Here we describe two commonly used surgical methods for gene delivery in neonatal mouse inner ear: the round window approach and the posterior semicircular canal approach. Both of these approaches are effective at delivering gene therapy to the neonatal mouse inner ear.

Keywords

Gene therapy; Inner ear; Posterior semicircular canal; Round window; Hearing loss; Dizziness

1 Introduction

Inner ear gene therapy is an exciting area of investigation as a potential treatment for hearing loss and dizziness. Several studies have shown functional recovery of hearing and balance functions in mutant mice after inner ear gene therapy delivery [1–7]. 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 this article, we describe the steps involved with two commonly used surgical approaches for gene delivery in neonatal mouse inner ear. Both of these approaches are effective at delivering gene therapy to the neonatal mouse inner ear. The pros and cons of these two approaches are described.

2 Materials

1. Micro-forceps (#5 and #55 Dumont).
2. Micro-scissors.
3. Heating pad.
4. Nanoliter 2000 micro-injector (Nanoliter2000, World Precision Instruments, Sarasota, FL).
5. Glass pipette.

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6. 5–0 vicryl sutures.
7. Dissecting microscope or operating microscope.
8. Viral vectors.
9. Glass bead sterilizer.

3 Methods

All instruments should be sterilized by ethylene oxide in the beginning of the experiments. Instruments should be cleaned using bead sterilization in between animals.

3.1 Anesthesia

Hypothermia is used to anesthetize neonatal mice (*see* Note 1). This method is used to anesthetize neonatal mice and is approved by American Veterinary Medical Association [2, 3].

1. Prior to surgery, the mother is placed in a cage separate from the litter. During this time, the home cage containing the litter is placed on a recirculating heat pad to keep the mice warm.
2. The first mouse is placed in a heavy-duty glove finger and placed into a bucket of ice for ~2–5 min.
3. After the pup is anesthetized, it is transferred onto a large square commercial plastic freeze pack with a 4 × 4 gauze between the pup and the pack's surface.
4. A heavy-duty glove filled with crushed ice is then molded and placed over the pup. The pup is judged to be under hypothermic anesthesia by the complete lack of any response to various stimuli (including a firm toe pinch). Animals remain on the ice for the duration of the surgery (approximately 5–10 min).

3.2 Surgical Approaches (Fig. 1)

1. Once the animal is anesthetized, the postauricular area is prepped several times, first with iodine, then with alcohol wipes to sterilize the area.
2. A postauricular incision is made ~2 mm behind the pinna with micro-scissors, and the underlying soft tissues are retracted and divided using both #5 Dumont micro-forceps and microscissors.
3. The facial nerve is identified. The facial nerve is a key landmark as it lies lateral to the bulla, which is cartilaginous and semitransparent at this age. The stapedial artery can often be seen through the bulla at this age, which is also a useful landmark.
4. For round window delivery, the round window niche is often visible through the semitransparent bulla, adjacent to the stapedial artery. If the round window niche is clearly visible, gene delivery can be performed through the bulla as described

¹It is important to ensure that the total duration of hypothermia is kept below ~15 min to increase the pup survival.

- below. If the round window niche is not visible, then a #55 micro-forceps is used to peel the bulla open to visualize the round window *see* Notes 2 and 3).
5. For posterior semicircular canal delivery (*see* Note 4), the facial nerve is followed superiorly and posteriorly to locate the posterior semicircular canal (PSCC). The facial nerve runs medial to the sternocleidomastoid muscle, which should be divided with micro-scissors to expose the PSCC. The soft tissue overlying the posterior semicircular canal is removed with microscissors.
6. Solution containing viral vectors is loaded into a glass micropipette mounted onto a micro-injector (*see* Note 5).
7. For round window delivery, if the round window is clearly visible through the bulla, the glass micropipette can penetrate through the bulla and into the round window. If the round window is not clearly visible, it is advisable to gently remove the bulla wall over the round window, and the round window membrane can be penetrated with the glass micropipette.
8. For posterior semicircular canal delivery, the PSCC is cartilaginous at this age and it is penetrated using a glass micropipette.
9. Viral gene therapy is injected into the inner ear using the micro-injector system. Typically ~1 μ L of viral gene therapy is injected into the neonatal mouse inner ear (*see* Note 6).
10. The glass micropipette is carefully withdrawn.

3.3 Post-operative Care

1. After surgery, mice are placed in a cage on a warming pad to maintain body temperature during recovery from anesthesia, with constant manual stimulation/rolling with warm thinly gloved human fingers. Warming is successful when the pup begins to breathe in approximately 5 min.
2. Typically it takes an additional 5 min of manual stimulation and warming before the pup is responsive.

²In our experience, both the posterior semicircular canal approach and the round window approach are capable of delivering viral vectors to the mouse inner ear (Fig. 2). In the past, the round window approach was most commonly used to access the cochlea, whereas the posterior semicircular canal was typically used to access the vestibular organs [9]. We have shown that the 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]. This may be due to the relatively large cochlear aqueduct in rodents, which is located at the cochlear base, adjacent to the round window. Therefore, viral vectors injected through the round window could be diverted into the cochlear aqueduct, reducing its ability to perfuse the entire inner ear [10].

³When the bulla is transparent, the round window can be easily located and viral vectors can be delivered without opening the bulla. However, the injection micropipette needs to penetrate through two barriers (bulla wall and the round window membrane), which can potentially make the gene delivery less consistent. If the bulla is not transparent, it will need to be opened in order to visualize the round window membrane directly, which increases the risk of hearing loss.

⁴While our published data showed higher cochlear hair cell infection efficiency with the posterior semicircular canal approach compared to round window approach, other inner ear gene therapy studies have also achieved excellent results with the round window approach [3, 4].

⁵When injecting the viral vectors into the mouse inner ear, it is important to ensure the tip of the micropipette is not blocked by debris and the viral vector solution flows properly into the inner ear.

⁶In our lab, we typically inject a total of 1 μ L of viral vector solution into the neonatal mouse inner ear. In our hands, this volume is sufficient to perfuse the entire mouse inner ear without causing noticeable damage. However, various volumes have been used in other studies for inner ear therapy [1, 3, 4].

3. Once the pups recover from anesthesia, they are placed in their home cage as a group (also set upon a warming pad) (*see* Note 7).
4. Prior to reintroducing the mother, each pup should be caressed with a cotton swab that has been exposed to the home cage bedding. 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.
5. If possible, urine from the mother can be collected and rubbed on the pups using the cotton swabs to further decrease the likelihood of rejection.
6. Mineral oil is rubbed on the mother's nose using a cotton swab to desensitize her, which increases the likelihood of pup acceptance after surgery [8].

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⁷After the surgeries are completed and the pups are placed back into the home cage with the mother, it is important to observe their interactions for ~10–15 min to ensure the mother accepts the pups, in order to decrease the probability of cannibalism.

Fig. 1.
Intraoperative images showing surgical access to the round window (RW) and posterior semicircular canal (PSCC) in a P0 mouse. The left ear is shown

Fig. 2. Injection of AAV8-GFP into neonatal mouse inner ear through the round window and posterior semicircular canal resulted in GFP expression in cochlear inner hair cells. Hair cells are labeled with anti-Myo7a antibody (shown in magenta)