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Standardized surgical approaches to ear surgery in rats

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Abstract

Objective: To describe several approaches of ear surgeries for experimental studies in rats.
Methods: Anesthetized rats were prepared for demonstration of various ear surgery approaches designed to optimize experimental outcomes in studies with specific goals and exposure requirements. The surgical approaches included the posterior tympanum, superior tympanum, inferior tympanum and occipital approaches.
Results: The middle ear cavity and inner ear were successfully exposed from different angles via the mentioned surgical approaches. For example, electrode placement for recording of cochlear bioelectric responses was easily achieved through the posterior tympanum or inferior tympanum approach. Alternatively, drug delivery or gene transfection via round window membrane was most easily accomplished using the posterior tympanum approach. Cochlear perfusion of protective or ototoxic drugs was best performed using the inferior tympanum approach. Ossicular chain interruption to induce a prolonged conductive hearing loss was readily achieved using a superior tympanum approach. Lastly, surgical destruction of the endolymphatic sac to induce experimental endolymphatic hydrops was readily performed via an occipital surgical approach.
Conclusion: These standardized surgical approaches can be applied in scientific studies of the ear with different purposes covering electrophysiology, conductive hearing loss, intra-cochlear drug perfusion and experimental studies relevant to Meniere's disease.

Keywords: Rat; Middle ear; Inner ear; Surgical approach

1. Introduction

As the understanding of the rat auditory system and knowledge of the rat genome increase, the use of rats in experimental studies involving the middle and inner ears has also been growing (Liu et al., 2011; Ding et al., 2011a; Wu et al., 2011; Ding et al., 2013, 2014a, 2014b; Yu et al., 2014; Fu et al., 2012; Fu et al., 2013; Ding et al., 2011b, 2012a). Experimental ear studies with rats involve placement of electrodes for recording cochlear bioelectric responses via the posterior and inferior tympanum approaches, local perfusion for inner ear gene transfection or drug delivery via the cochlea or vestibular semicircular canals, interruption of the ossicular chain via a superior tympanum approach to simulate middle ear anomalies, and endolymphatic sac ablation via an occipital approach for hydrops modeling. Knowledge of anatomy of rat temporal bone and neighboring structures is required to performed middle and inner ear surgeries in rats for these studies. This current paper provides a complete description of standardized middle and inner ear surgical techniques in rats to help researchers involved in experimental ear studies using rats.

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2. Anesthesia and pre-operative preparation

Health SD rats weighing about 250 g were selected and anesthetized with intraperitoneal ketamine (100 mg/kg) and chlorpromazine (5 mg/kg). The skin around the ear, neck or occipital area was prepared using iodine and alcohol, respectively, depending on the intended surgical approach. The rat was placed on a heating pad with its head secured in an adjustable frame for appropriate head positions.

3. Posterior tympanum approach via a retroauricular incision

3.1. Purpose of the approach

Along this approach, the following structures can be visualized: the posterior wall of the ear canal, extra-temporal segment of facial nerve, the tympanic bulla, the medial wall of posterior tympanum, round window and cochlear basal turn (Fig. 1). The approach therefore can be used to insert silver wire electrode into the facial canal up to the geniculate ganglion level for recording of cochlear bioelectric activities including cochlear microphonic (CM), auditory nerve compound action potential (CAP) and summit potential (SP) without opening the middle ear (Yu et al., 2014; Ding et al., 2011b, 2012a, 1993a, 1996; Ding and Jin, 1998; Wang et al., 1999a, 1996; Shi et al., 1994; Ding and Zhang, 1995; Qi and Ding, 1997). Upon opening the posterior wall of ear canal, the round wind is visible, where electrodes can be inserted for recording cochlear auditory potentials and drugs can be placed in the round window niche. The approach also allows drilling into the vestibular or tympanic scala at the basal cochlear turn for perilymphatic perfusion. Anatomy of this approach is relatively simple, and the middle ear cavity can be easily closed when needed using the nearby readily available and abundant temporalis flap. For its relative simplicity, minimal invasiveness and satisfactory exposure, this approach is frequently used in experimental animal studies.

3.2. Surgical procedure

The rat was placed in a lateral position on a heating pad and a 2 cm long incision was made along the retroauricular groove. Soft tissues were separated by the layer to expose the muscles. A triangular depression became visible upon pulling the pinna anteriorly and superiorly, which borders the sternomastoid and temporalis muscles and the posterior wall of ear canal (Fig. 1A). Between the temporalis and sternomastoid muscles is the extra-temporal segment of facial nerve, which extends laterally on the surface of bony posterior wall of ear canal until dividing into several branches to supply facial muscles. The shiny tendon of the sternomastoid muscle can be used to guide identification of the facial nerve (Fig. 1B). By following the facial nerve, the opening of facial canal can be exposed behind the tendon. After removing the facial nerve from the canal, a silver wire electrode can be inserted into the canal up to its horizontal segment which is separated from the cochlea by only a very thin bony wall, allowing acquisition of cochlear bioelectric signals in response to acoustic stimulation with

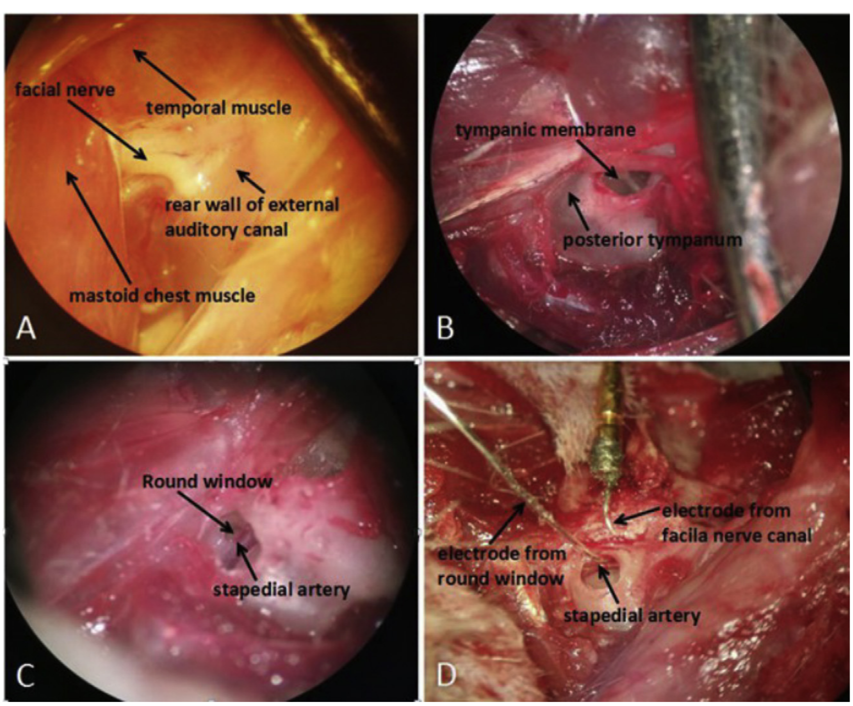

Fig. 1. Posterior tympanum approach via a retro-auricular incision. A. Subcutaneous tissue, muscles and facial nerve exposed via the retro-auricular incision. B. Lateral wall of posterior tympanum exposed following separation of muscles. C. Round window niche visible after opening the lateral wall of posterior tympanum. D. A silver wire electrode inserted into the facial canal and a silver ball electrode placed in the round window niche.

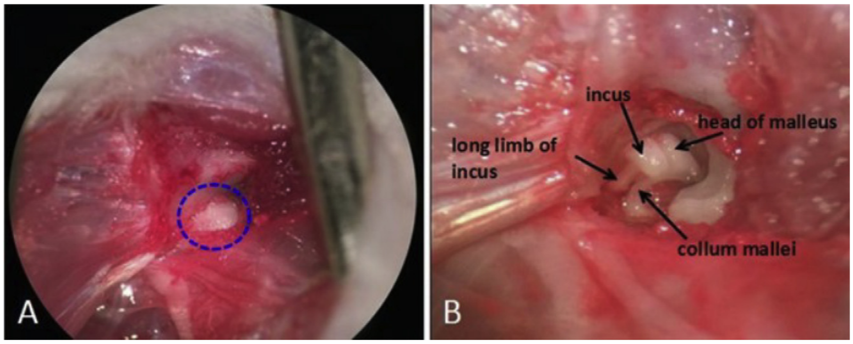

Fig. 2. Superior tympanum approach via a supra-auricular incision in rat. A. Lateral wall of epitympanum exposed following separation of muscles (the blue circle marks the location for opening the bony lateral wall). B. Ossicular chain exposed once the epitympanum is open.

high signal/noise ratios (Fig. 1C) (Yu et al., 2014; Ding et al., 1993a, 1996; Ding and Jin, 1998; Wang et al., 1999a; Shi et al., 1994; Wang et al., 1996; Ding and Zhang, 1995; Qi and Ding, 1997). Separating muscle attachment on the tympanic bulla inferiorly, posteriorly and superiorly, most lateral wall of the bulla, as well as the posterior, inferior and superior walls of bony and cartilaginous ear canal can be exposed. An opening was drilled open about 2 mm below the opening of the facial canal, which allowed visualization of structures inside the middle ear cavity, including the stapes artery traveling posteriorly and inferiorly on the medial tympanic wall and an oval shadow on the same wall superior and posterior to the artery, which is the round window where a round window niche electrode can be placed and drugs can delivered to penetrate the round window membrane (Fig. 1D) (Ding et al., 2012b, 2011c; Wu et al., 2012; Zhou et al., 2009; Li et al., 2004; Wang et al., 1999b; Ding et al., 2010a, 2001, 1987).

4. Superior tympanum approach via a supra-auricular incision

4.1. Purpose of the approach

The epitympanum in rats is small and houses the malleus, malleoincudal joint and incus, with no signs of the lateral or superior semicircular canals, which is different from humans, chinchilla and guinea pigs (Li et al., 2004; Ding et al., 2010a, 2001, 1987; Ding and Jiang, 1989). Therefore, the superior

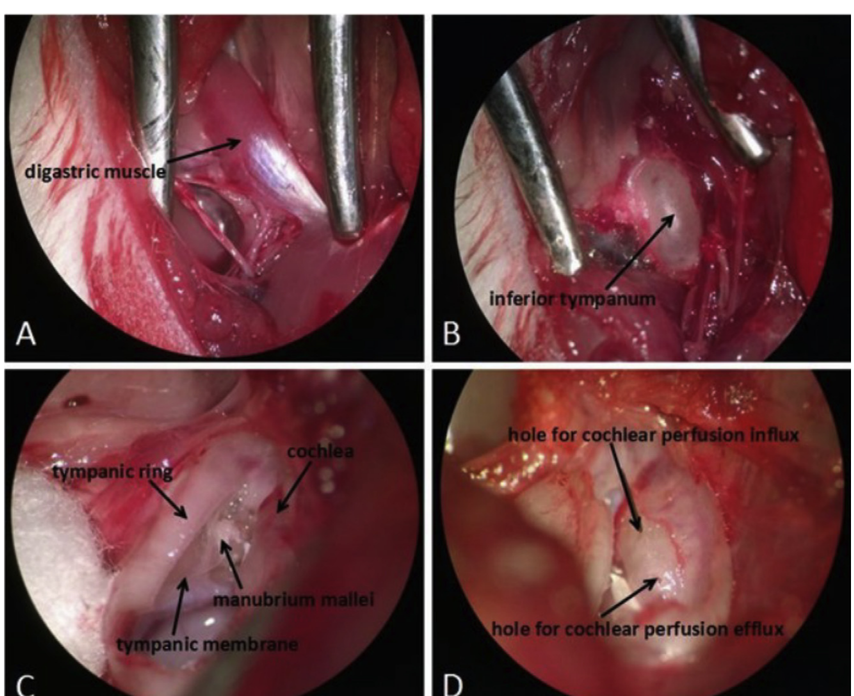

Fig. 3. Inferior tympanum approach via a ventral mid-line cervical incision. A. Neck muscles exposed. B. Bony wall of inferior tympanum exposed after removal of the digastric muscle. C. Tympanic membrane and annulus on the lateral side and cochlea on the medial side exposed following opening the bony wall. D. Openings have been made in the apical and basal turns of the cochlea, respective, for perilymphatic perfusion.

tympanum approach is used in rats mainly for interruption of the ossicular chain, while leaving the tympanic membrane and inner ear intact (Fig. 2).

4.2. Surgical procedure

The anesthetized rat was put in a lateral position on a heating pad and a 1.5 cm long incision extending from the retro-auricular groove or superior and parallel to the pinna was made. The temporalis muscle was exposed by separating soft tissues by layers (Fig. 2A). The lateral wall of the epitympanum became visible after lifting the temporalis muscle. This wall was opened at the junction of the tympanic bulla wall and squamous portion of the temporal bone. An opening of 2 mm is sufficient for exposure of the malleus, malleoincudal joint and incus (Fig. 2B).

5. Inferior tympanum approach via a ventral cervical incision

5.1. Purpose of the approach

The pneumatized middle ear cavity in rats expands inferiorly and anteriorly. The inferior tympanum approach via a ventral cervical incision allows removal of a large area of the inferior tympanic wall and thus exposure of middle ear structures when viewed upwards, including the tympanic membrane, annulus, malleus, tendon of the tensor tympani, most of lateral wall of the cochlea, long process of incus, incudostapedial joint, stapedial suprastructure, stapedial artery and the rim of round window niche. The relatively empty space in the inferior tympanum also provides adequate room for surgical maneuvers. This approach is therefore ideal for a number of experimental studies (Fig. 3). Examples include recording of cochlear endolymphatic potential, cochlear perilymphatic and endolymphatic perfusion, as well as placement of round window niche electrodes and interruption of the ossicular chain (Ding et al., 2010b; Ding et al., 1993b, 2010c, 2002; Ding and Jin, 1996; Chen et al., 2013). Compared to the posterior tympanum approach, this approach presents relatively more complex anatomy features, requires maintaining of an extended head position and demands a high awareness of major vessels in the neck. It is thus usually used in acute experiments.

5.2. Surgical procedure

The rat was supine on a heating pad with the head extended. A mid-line incision was made to expose neck muscles (Fig. 3A). The digastric muscle on the intended study side was severed and a retractor was used to expose the entire ventral aspect of the tympanic bulla (Fig. 3B). Once the bulla

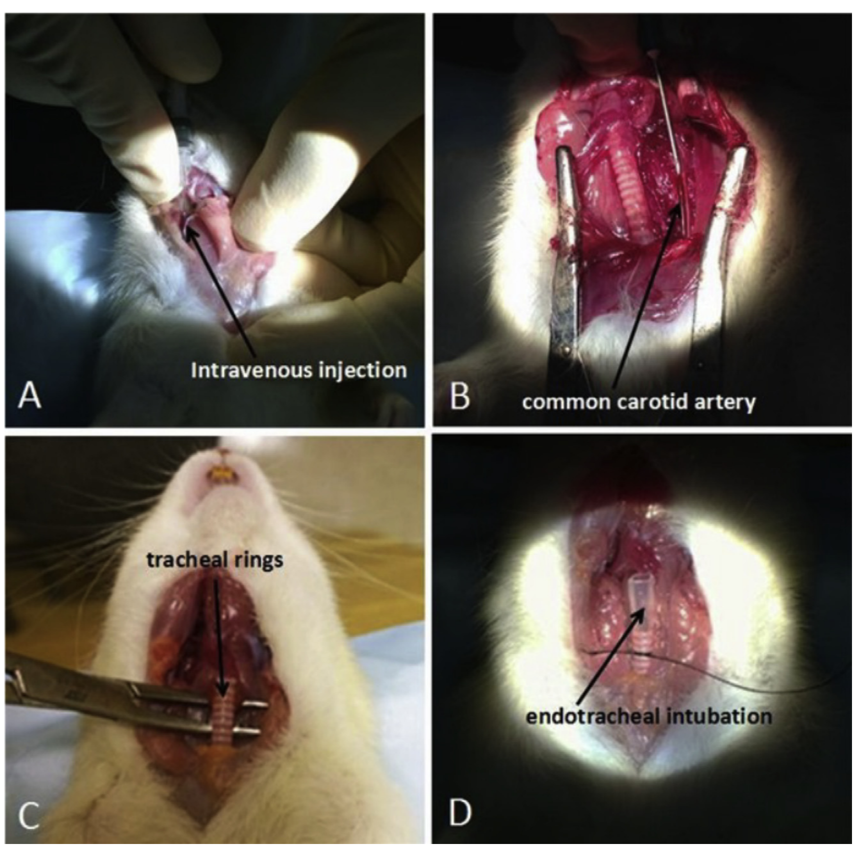

Fig. 4. Exposure of the carotid artery, jugular vein and trachea via the ventral mid-line cervical incision. A. Injection into the jugular vein. B. Inserting a catheter into the carotid artery. C. Trachea exposed. D. Intubation through tracheotomy.

was open, the cochlea on the medial side and tympanic membrane on the lateral side became visible. Also visible were the annulus, malleus and its manubrium, tendon of the tensor tympani, most of lateral wall of the cochlea, long process of incus, incudostapedial joint, stapedial suprastructure, stapedial artery and the rim of round window niche (Fig. 3C). An opening in the middle turn of the cochlea would allow insertion of glass micro-electrode through the spiral ligament and stria vascularis to record endolymphatic potential, or endolymphatic perfusion, or delivery of drugs, markers or other transfection agents. Perilymphatic perfusion can be performed by making a small opening into the tympanic and vestibular scalae (Fig. 3D). Transfection agents can be directly placed in the round window niche, which can also house a silver wire electrode for recording sound induced cochlear potentials. In addition to all these, an advantage of this approach is it also provides access to the carotid artery and jugular vein for intravenous drug injection and arterial gas analysis, as well as access to the trachea for respiratory control (including hypoxia simulation) and the convenience of harvesting the chemoreceptor tissues (Fig. 4) (Ding and Jin, 1998, 1996).

6. Occipital approach to the endolymphatic sac

6.1. Purpose of the approach

This approach provides a direct access to the opening of the endolymphatic sac on the lateral side of the dura. The sigmoid venous sinus in the close vicinity tends to cover the endolymphatice sac opening located in a bony fissure, making the occipital approach the only approach passing through the narrow space between the sigmoid sinus to the sac. This approach is useful for measuring endolymphatic sac potentials and destruction of the sac for obstructive endolymphatic hydrops modeling (Fig. 5) (Ding et al., 2010d; Guo and Jin, 1991; Xu et al., 1991; Wu et al., 1993; Luo and Jin, 1991).

6.2. Surgical procedure

The rat was put in a prone position on a heating pad. Its neck was padded to produce a flexed head position. An incision was made on the back side of the neck to expose neck muscles, which were cut at the junction between the occipital and parietal bones. The periosteum was elevated posteriorly to the foramen magnum and then extended laterally toward the occipital condyle and vicinity area. Superior and lateral to the cross of a line through the superior border and a line through the lateral border of the occipital condyle is the site to open the occipital bone (Fig. 5A). Using a drill, the occipital bone was thinned down until the sigmoid sinus became recognizable underneath. A surgical pick was used to remove the thin layer of bone to expose the sinus (Fig. 5B). The sinus was gently pushed aside with a normal saline soaked patty to visualize the bony fissure over the endolymphatic sac (Fig. 5C). For recording endolymphatic potentials, a microelectrode can be maneuvered with a controller to be inserted into the sac. To model obstructive hydrops, the sac can be ablated by inserting

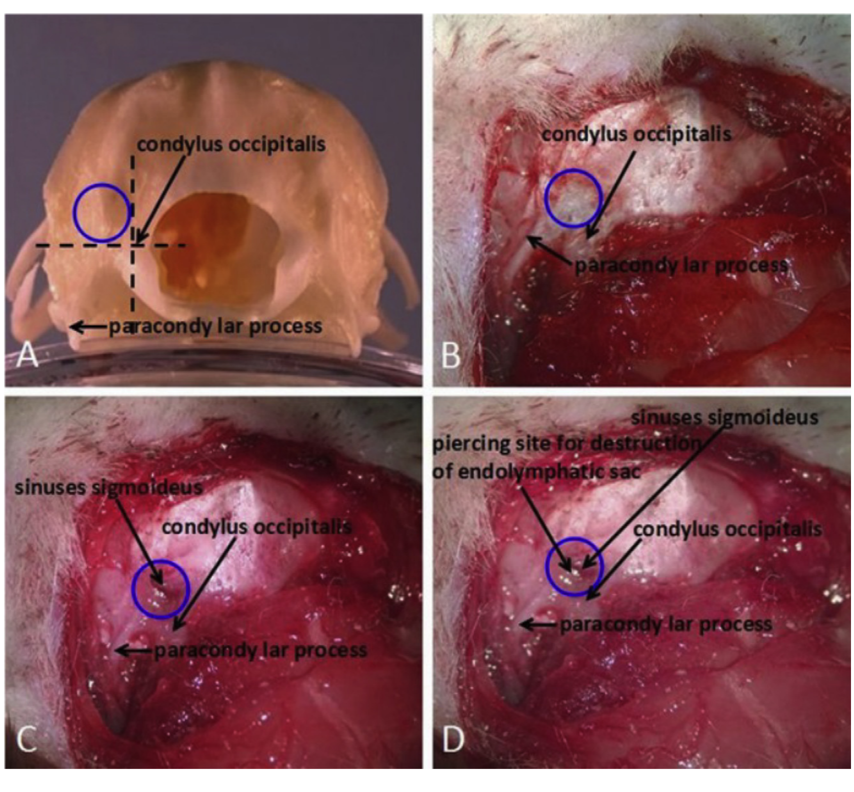

Fig. 5. Approach to endolymphatic sac via occipital incision. A. Landmark for opening the occipital bone (blue circle). B. Occipital bone exposed after separating muscles. C. Sigmoid sinus exposed following opening the occipital bone at a location superior to the occipital condyle. D. Destruction of the endolymphatic sac by inserting a needle into the bony fissure posterior to the sac and lateral to the sigmoid sinus.

a needle into the sac followed by repeated rotation (Fig. 5D), which causes damage to the sac and neighboring bony walls followed by scar tissue growth that block endolymphatic circulation (Ding et al., 2010d; Xu et al., 1991; Wu et al., 1993; Luo and Jin, 1991).

7. Summary

The surgical approaches described in this paper are based on the authors' experiences of many years in animal experimental auditory studies. These middle and inner ear surgery approaches can be used for cochlear drug perfusion or inner ear transfection, electrode placement for recording cochlear bioelectric responses, ossicular interruption to simulate conductive hearing loss in animals, and ablation of the endolymphatic sac for models of obstructive endolymphatic hydrops, and more. In combination with the "Rat Temporal Bone Anatomy" that was previously published, we are hoping that the information will help improve animal ear surgical skills among researchers engaged in experimental ear studies.

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