

Relevant Anatomical Details in Rats Regarding Otologic Research

Detalles Anatómicos Relevantes en Ratas para la Investigación Otológica

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SUMMARY: The objective of the study was to provide a detailed anatomical description of the rat's ear anatomy that will prove insightful to different experimental otologic surgical procedures regardless of scope. Three male Wistar rats were enrolled in the study. Candidates were screened for systemic and otologic pathology. External ear canal endoscopy was carried out with a 30° rigid endoscope through an image capture system. Middle ear anatomical elements were analyzed under stereomicroscopy. 3D computer tomography image reconstruction was realized with a micro-CT to describe the anatomy. Image data from all three rats were analyzed. Anatomical annotations and surgical exposure recommendations were added for key elements. The most relevant images from all three rats were selected for representation. Detailed visualization of the structural elements of the tympanic cavity were clearly visible: promontory, round window, stapedia artery, stapes, incus, and tympanic membrane were all constant findings. We describe a step wise ventral surgical approach of the middle and inner ear for which we found that the clavotrapezius muscle was a reliable landmark. For the transtympanic approach the endoscopic transcanal access was an easy and reliable method for which a detailed anatomical representation was depicted. Further, anatomical similarities to humans were observed by stereomicroscopy and Micro-CT imaging reiterating that the rat model is suitable for otologic research. The endoscopic approach to the tympanic membrane is comfortable and less expensive than a microscope. The tendon of the clavotrapezius muscle can be a reliable landmark for discovering the tympanic bulla when considering a ventral approach. 3D Micro-CT reconstruction allows intact evaluation of the samples, simultaneously being a diagnostic and also a learning tool.

KEY WORDS: Ear; Rat; Anatomy; Otologic surgery; Micro-CT.

INTRODUCTION

Experimental models are a key aspect in consolidating fundamental ideas and scaling them further to clinical applications. Although established cell culture lines are expanding research possibilities, experimental animal models remain the primary source of knowledge, when investigating pharmacokinetics and pharmacodynamics of certain drugs and nanomaterials.

Because hearing loss has become one of the leading sensory deficits the need for experimental models in audiology and otology is also growing. Understanding the anatomy and ultrastructure of specific experimental models is crucial for proper evaluation of study feasibility and hypothesis (Elliott *et al.*, 2022).

No animal model is a perfect replica of the human physiology, but some species provide distinct benefits for

certain experimental applications (Albuquerque *et al.*, 2009). The rat stands out as being the leading experimental model to date due to the vast amount of research data already available. Also, the availability of genetic sequencing and established surgical applications combined with low maintenance and rapid growing rates, make them the ideal candidate for experimental research (Ujvary *et al.*, 2022).

In otology, experimental models aided in the exploration of pathways for therapeutic agents administration, including intratympanic drug distribution, cochlear transportation via the round window (RW) or the oval window (OW) (Dindelegan *et al.*, 2022). As cochlear implantation became a standard of care for profound hearing loss, association with different types of drug delivery methods, stem cell therapy as well as gene therapy became the new focal point (Blebea *et al.*, 2022).

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Although there is a vast amount of data that recommends the rat as a robust model for otologic research, only a few articles focus on describing surgical approaches and anatomical landmarks in detail (Li *et al.*, 2015; Bryant *et al.*, 2021). Newcomers to otologic research could encounter difficulties in implementing the needed surgical approach and will likely spend a significant amount of time mastering the required technique. This descriptive study aims to present the main anatomical landmarks of the rat's ear and put them into perspective for different research applications.

MATERIAL AND METHOD

Study and subject details: Three adult male Wistar rats were included and were screened to ensure good general and otological health. One additional rat with otitis media was included just for the purpose of image documentation. All protocols were conducted according to European law and welfare of experimental animals and followed the guidelines established by our institution's ethical committee and the National Veterinary Health and Food Safety Authority (ethical approval nr. 82/07.12.2017). Due to the

descriptive nature of the study, measures were taken to ensure that no rats were sacrificed just for this purpose. In accordance with the 3Rs approach (Fenwick *et al.*, 2009), animals participating in this study were sacrificed after having participated in another study, which did not interfere in any way with the conduct of our research.

Image acquisition and workflow: All rats were anesthetized via intramuscular injection using 7mg/kg ketamine and 70mg xylazine/kg. External ear canal and tympanic membrane visualization was carried out using a rigid 30°, 2.7 mm diameter endoscope. Images were captured using a Storz® Telecam 20212030 camera system. The endoscopic image acquisition was followed by a retroauricular surgical approach of the tympanic bulla for step wise documentation. One rat was used to demonstrate the procedure of intratympanic injection and cochlear implantation. The animals were sacrificed with isoflurane overdose followed by cervical dislocation. Following the bilateral removal of the temporal bones from all three rats, a seriate dissection was carried out on one temporal bone from each rat to ensure that no anatomical variant was described in favor of the normal anatomy. A stereomicroscope (Olympus® Model SZ2-ILST) was used to provide a step-by-step image documentation of the anatomy.

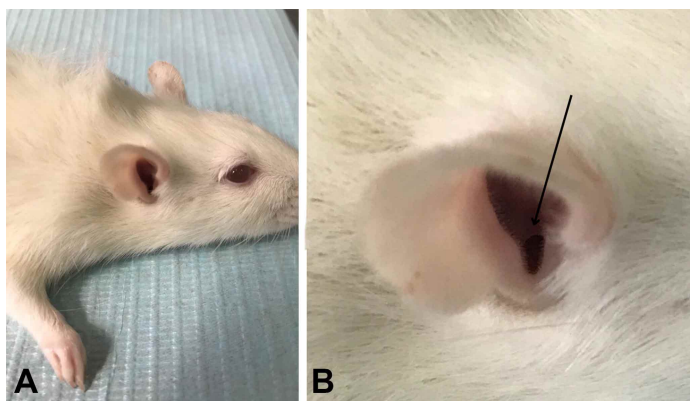


Fig. 1. External ear anatomy. A-the right pinna; B-the isthmus of the right external ear canal (arrow).

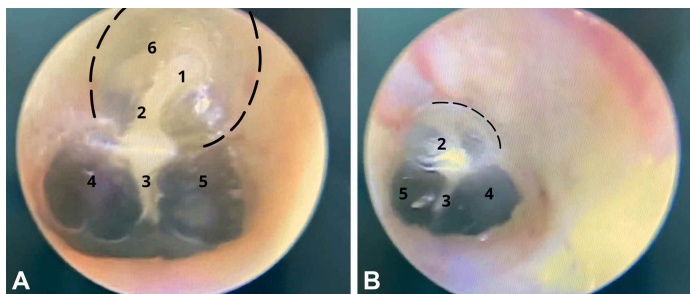


Fig. 2. Endoscopic view of the TM. A-right TM; B-left TM; 1-head of the malleus; 2-neck of the malleus; 3-handle of the malleus; 4-posterior part of pars tensa; 5-anterior part of pars tensa; 6-incus; dotted line-delimitation of pars flaccida.

The three remaining temporal bone specimens were prepared and submitted to micro-computed tomography (Micro-CT) scanning using the Bruker SkyScan® 1272 micro-CT at intermediate resolution at a slice thickness of 13.6 mm. A 3D model rendering was obtained for each of the cochleae and were further processed for anatomical annotations and documentation. Where measurement were performed, two independent operators' average results were considered as final value.

RESULTS

Clinical aspects of the external ear anatomy. The rat's external ear is formed by the auricle, a mobile cartilaginous structure with no relevant anatomical landmarks. It extends further, forming the cartilaginous ring of the external auditory canal that subsequently continues with the bony part and terminates at the level of the tympanic membrane (TM). The external ear canal presents an isthmus between the external one third and medial two thirds, representing the transition between the cartilaginous and the bony ear canal. Anatomical aspects of the external ear and tympanic membrane are illustrated through (Fig. 1 to 3).



Fig. 3. Endoscopic view of a rat's left ear presenting otitis media. Left TM with opacity and retraction of pars flaccida, denoting the presence of chronic otitis media.

The tympanic membrane can be visualized after advancing beyond the isthmus. The tympanic membrane separates the external ear canal from the middle ear cavity and is composed of pars tensa and a pars flaccida, similar to humans. The pars flaccida occupies a larger surface area of the tympanic membrane compared to humans and permits the visualization of the incudomalleolar joint. The short handle of the malleus points posteriorly, which is the exact opposite than found in humans. When examining the rat through external contention care should be taken not to apply pressure so that the breathing becomes heavy. The pars flaccida presents a hypermobility distending outwards considerably making it hard to properly visualize the pars tensa.

Evaluation of the TM's condition is a preliminary step that is mandatory before including any participants for otologic research. The screening must be performed to rule out subjects with middle ear disease. The subjects with otitis media can easily be identified through otoendoscopy (Fig. 3).

Good knowledge as well as visualisation of the tympanic membrane anatomy is crucial when performing transtympanic injections. A wide endoscopic view of the eardrum allows for a controlled transtympanic drug delivery into the middle ear (Fig. 4). The needle's tip should always point downwards to avoid injury of the stapedial artery.

Middle ear elements. The middle ear is formed by a main air space, which is the tympanic bulla, and two smaller air spaces, the anterior and posterior epitympanic recesses. The epitympanic recesses lie in the superior portion of the tympanic cavity and contain the head of the malleus and the body of the incus. To best document this detail, the middle ear was split in two through a sagittal plane, parallel with

the TM, depicting the inner as well as the lateral wall of the tympanic cavity (Fig. 5).

The middle ear (ME) is a miniature three-dimensional biomechanical system with a volume of approximately 30 to 50 μ L (Zou *et al.*, 2010) with no mastoid cells as they are replaced by a sole air cell. As in humans, the tympanic membrane (TM) attaches to the malleus. Articulated to the malleus is the incus followed by the stapes. Together with their supporting ligaments and muscles they constitute the ossicular chain. The head of malleus and the body of the incus are all easily accessible at the level of the epitympanic recess (Fig. 5-7).

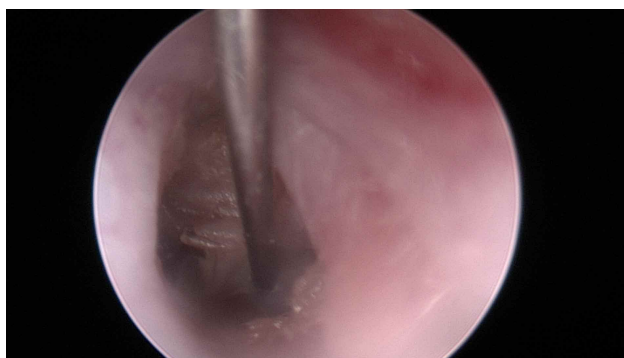


Fig. 4. Intratympanic injection. Left ear intratympanic injection under endoscopic control performed in the posterior part of pars tensa with a 31G needle.

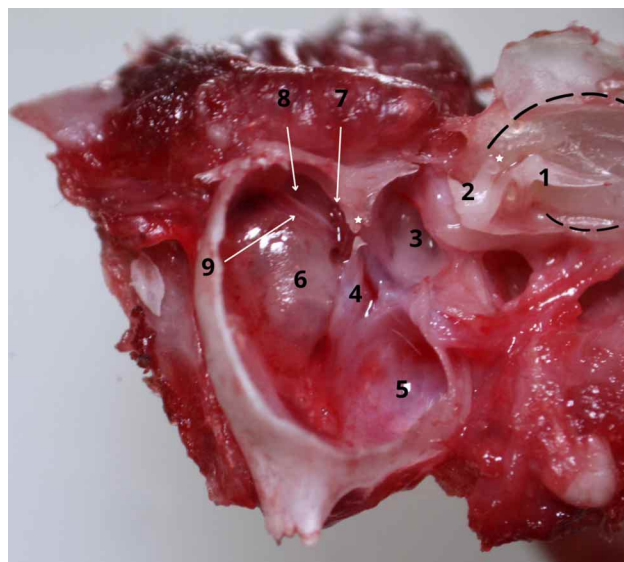


Fig. 5. Elements of the middle ear. Right middle ear showing the lateral and medial wall elements (microphotography, 50X magnification). 1-malleus; 2-incus; 3-anterior epitympanic recess; 4-tensor tympani muscle; 5-middle ear cavity (bulla); 6-cochlea; 7-stapes covering the OW; 8-RW; 9-stapedial artery going through the stapes' cruras; dotted line-limits of the TM as seen from the middle ear cavity (annulus); white stars-articulation surface between incus and stapes.

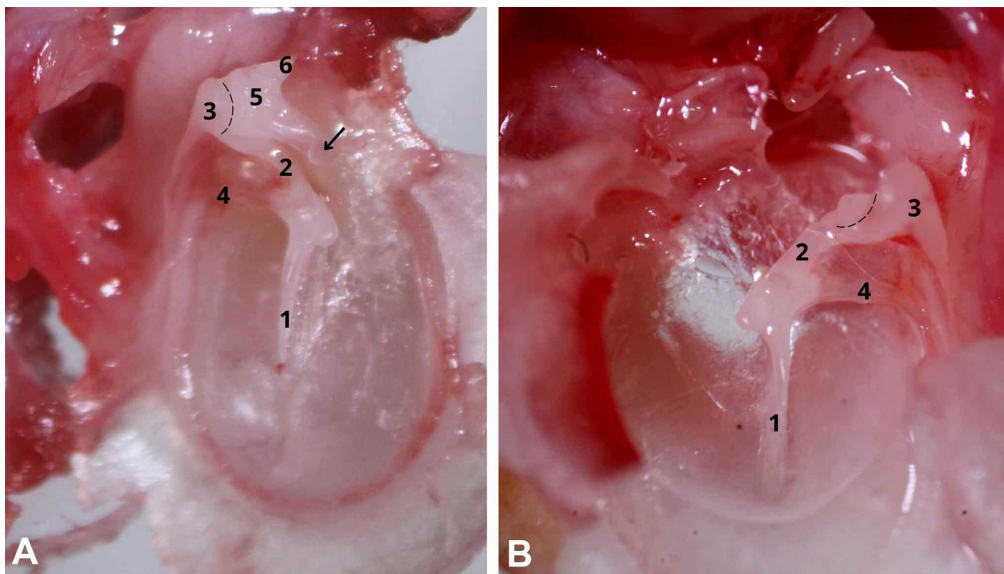


Fig. 6. Elements of the ossicular chain. A-right tympanic membrane medial view; B-left tympanic membrane medial view;(microphotography, 60X magnification). 1-malleus handle; 2-malleus neck; 3-malleus head; 4-malleus anterior process; 5-incus body; 6-short process of incus; dotted line-articulation surface between malleus and incus; arrow-incus articulation surface with the stapes.

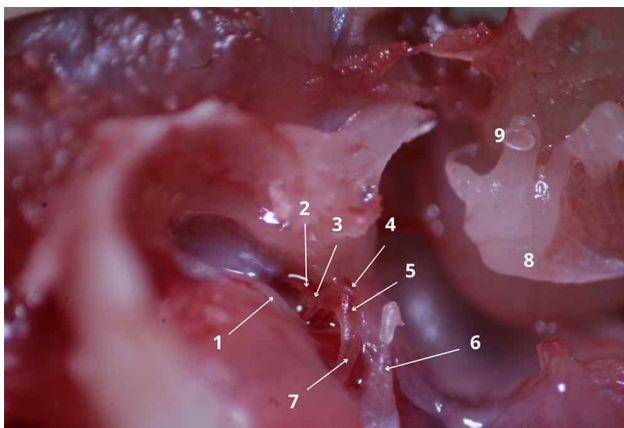


Fig.7. Elements of the stapes. 1-stapedial artery; 2-stapedian muscle; 3-posterior crus of the stapes; 4-head of the stapes; 5-neck of the stapes; 6-tensor tympani muscle;7-anterior crus of the stapes; 8-malleus head; 9-incus articulation surface with the stapes; (microphotography, 60X magnification).

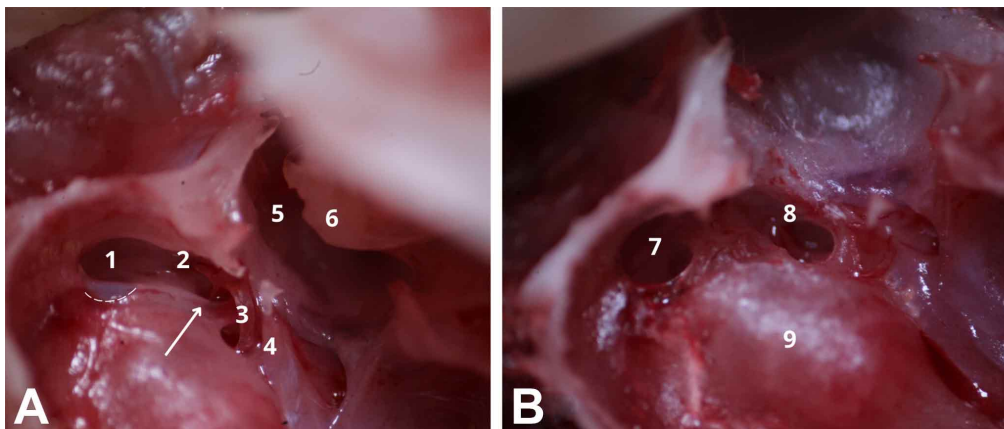


Fig. 8. Images of the RW and OW. A-microphotography, 60X magnification;1-RW; 2-stapedian muscle; 3-stapes; 4-tensor tympani muscle; arrow-stapedial artery; dotted line-margin of the RW seen through transparency of the stapedial artery; B-microphotography, 70X magnification after removing the stapes, the stapedial artery and muscle and the bony ridge; 7-RW; 8-OW; 9-cochlear promontory.

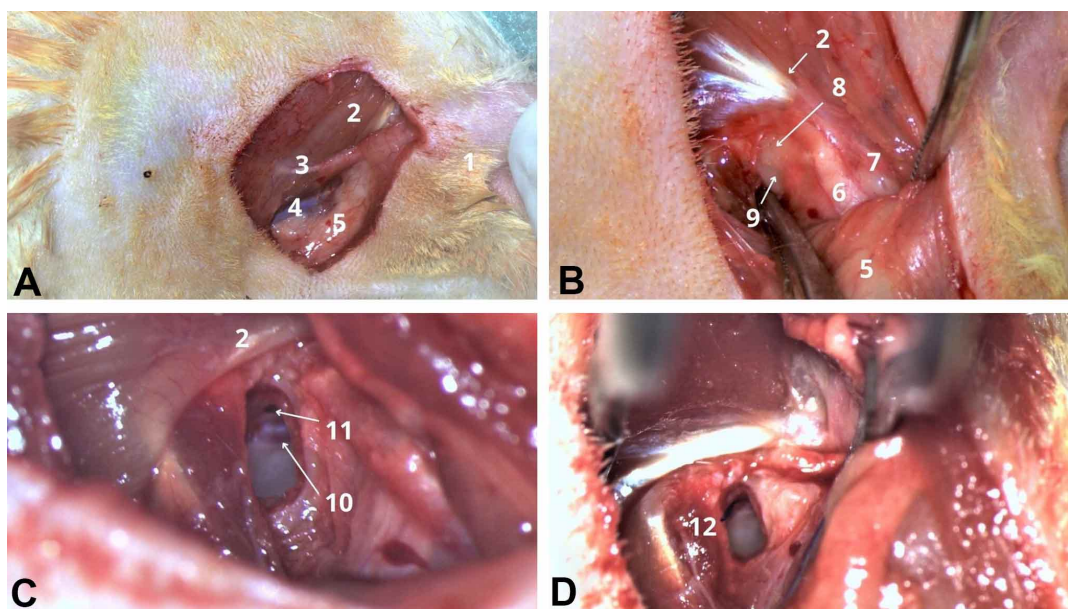


Fig. 9. Surgical steps in RW approach. A-retroauricular incision (6x magnification); 1-right ear pavilion retracted anteriorly; 2- clavotrapezius muscle tendon; 3-great auricular nerve; 4- posterior facial vein - branch of external jugular vein; 5-parotid gland; B-identification of the bulla (16x magnification); 6-facial nerve; 7-cartilaginous external ear canal; 8-bony external ear canal; 9-bulla; C-bullostomy with RW identification;10-stapedial artery; 11-RW; D-cochlear implant; 12-cochlear implant dummy.

Access to the middle ear can be obtained through a bone defect at the postero-lateral side of the tympanic bulla. By using a retroauricular approach of the middle ear, a good visualization of the cochlea, RW and OW can be obtained. This approach is less utilized for middle ear drug delivery, as the intratympanic approach is less aggressive and technically simpler to perform. The retroauricular approach is useful when performing cochlear implant through the RW, cochleostomy or perilymphatic perfusion through the vestibule or tympanic

scala (Li *et al.*, 2015). The following image series (Fig. 9) document the most important landmarks to help perform the retroauricular approach of the middle ear.

Micro-computed tomography imaging of the middle and the internal ear. Detailed anatomical measurements that are not possible through microscopy can be achieved through 2D micro-CT measurements as is the case of the round and oval window diameter (Fig. 10).

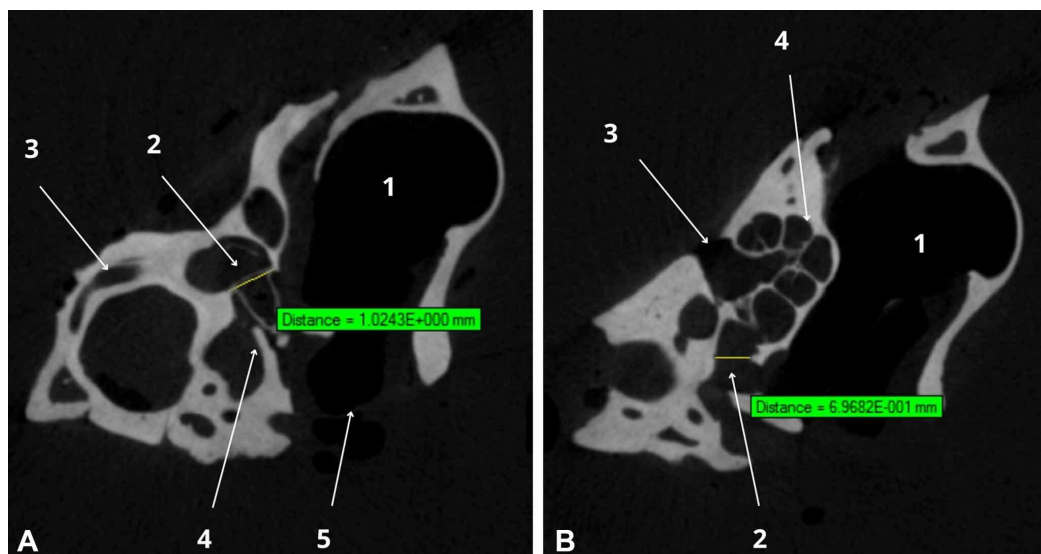


Fig. 10. 2D micro-CT. A-level of OW; 1- tympanic bulla; 2- footplate of stapes; 3- superior semicircular canal; 4-bony ridge at the level of epitympanic recess; 5- external auditory canal. B- level of RW; 1- tympanic bulla; 2-RW; 3-internal auditory canal; 4-cochlear apex.

The measured long axis diameter of the oval window was 1 mm and was constant in all cochleae ($0.99 \text{ mm} \pm 0.07$). The measured diameter of the round window was 0.68 mm ($0.61 \text{ mm} \pm 0.02$) and was as well constant through all measurements.

The possibility of 3D micro-CT reconstruction offers substantial information about anatomical landmarks, with a comprehensive depiction of the middle ears biomechanical system, that otherwise would not be easily accessible without dissection (Fig. 11).

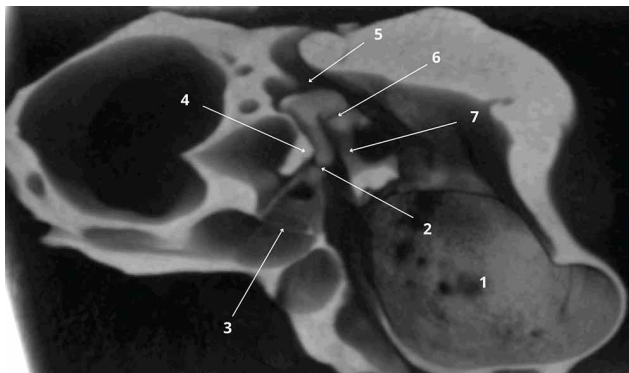


Fig. 11. 3D micro-CT reconstruction of the middle ear. 1-tympanic bulla; 2-incudo-stapedial joint; 3-footplate of stapes; 4-bony ridge of the epitympanic recess; 5-epitympanic recess; 6-malleus and incus articulation; 7-neck of the malleus.

DISCUSSION

Parenteral drug administration requires high systemic concentrations to pass the blood-labyrinth barrier and to reach therapeutic effect at a cochlear level (Dindelegan *et al.*, 2022). Due to the high concentrations required, these compounds may present severe side effects that can significantly reduce the number of animal subjects enrolled in a study. Thus, understanding the rat's middle and internal ear anatomy is of clinical importance and is required to focus drug delivery through intratympanic injections and limit systemic side effects of therapeutic agents that previously were administered via a parenteral route.

When considering instrumentation, using a 2.7 mm, 30° rigid endoscope will allow good visualization of the tympanic membrane and facilitates the passing through the curved external ear canal. The endoscope's small diameter permits the simultaneous passing of instruments (perforator, needle, etc.) with adequate room left for precise manipulation. When considering endoscope length, the 11 cm seems an optimal choice due to its stability when handling, good depth perception and field of view. A 2.7 mm diameter endoscope supports the external ear canal, allowing the operator different

visualization angles of the tympanic membrane as well as different working distances, also limiting the need for additional assistance. All these features cannot be attained using a microscope.

Another essential utility of the endoscope is the possibility of rapidly inspecting the eardrum and establish the membrane's condition, before including the animal in a study. No general anesthesia is needed for this procedure as it can be performed with animal contention. Determining the condition of the tympanic membrane and rapidly identifying otitis media (Fig. 3) warns researchers not to proceed with any audiological measurements.

One procedural detail is particularly important when considering intratympanic injections and it is due to the similarities compared to the human middle ear. The posteroinferior quadrant is the safest region to perform an intratympanic injection. Secondly, the high position of the OW and RW needs to be considered. The volume of the injected substance ought to be approximately equal with the volume of the middle ear so that it reaches the level of the windows. As the middle ear cavity is filled with air, performing a second tympanic membrane perforation may be advisable before injecting any substances. This second perforation will facilitate the elimination of air bubbles formed inside the tympanic cavity but also create a pressure valve and limit possible damage to the window's membranes. Also, a second perforation acts as a valve and limits the retrograde spillage of the substance through the injection tympanotomy (Dkhar *et al.*, 2020).

A postauricular surgical approach is suitable for reaching the cochlea or the RW. Rapid identification of the tympanic bulla reduces the anesthesiologic risk for the animal and improves recuperation time. Some authors consider that identifying the facial nerve posteroinferior to the external auditory meatus is key for reaching the tympanic bulla. We on the other hand, consider the tendon of the clavotrapezius a convenient and easy-to-detect landmark as the posterior part of the bulla lies anteriorly and inferiorly to it. Other authors are also using the clavotrapezius muscle as a landmark to identify the facial nerve as it can easily be confused with the external ear canal (de Faria *et al.*, 2006).

Modern imaging techniques, such as Micro-CT, provide accurate data collection regarding bony structures and can also differentiate tissue density when using a radio-dense contrast agent (phosphotungstic acid). 3D Micro-CT image reconstruction offers a non-invasive perspective of the middle and inner ear's architecture (Wang *et al.*, 2019) and can also aid in metal nanoparticle distribution studies due to its micron-scale resolution (Zou *et al.*, 2015; Mahan & Doiron, 2018).

CONCLUSIONS

When conducting research that implies intratympanic injection, endoscopic control offers easy visualization of the whole tympanic membrane with a sharp image with high resolution. It implies lesser cost and higher mobility than a microscope. Clavotrapezius muscle insertion can be a landmark for the facial nerve, and identifying the facial nerve is considered an important step when approaching the tympanic bulla. We can consider future research comparing which of these two landmarks hinders a more rapid identification of the tympanic bulla.

Three-Dimensional Micro-CT reconstruction allows the samples to be evaluated intact, being a diagnostic and learning tool simultaneously.

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RESUMEN: El objetivo de este trabajo fue proporcionar una descripción anatómica detallada de la anatomía del órgano vestibulococlear de la rata que resultará útil para diferentes procedimientos quirúrgicos otológicos experimentales, independientemente del alcance. En el estudio se usaron tres ratas Wistar macho. Los ejemplares fueron evaluados por patología sistémica y otológica. La endoscopia del meato acústico externo se realizó con un endoscopio rígido de 30° a través de un sistema de captura de imágenes. Los elementos anatómicos del oído medio se analizaron bajo estereomicroscopía. La reconstrucción de la imagen de tomografía computarizada en 3D se realizó con un micro-CT para describir la anatomía. Se analizaron los datos de imagen de las tres ratas. Se agregaron anotaciones anatómicas y recomendaciones de exposición quirúrgica para elementos clave. Las imágenes más relevantes de las tres ratas fueron seleccionadas para su representación. La visualización detallada de los elementos estructurales de la cavidad timpánica era claramente visible: promontorio timpánico, ventana coclear, arteria estapedial, estapedio, yunque y membrana timpánica eran hallazgos constantes. Describimos un abordaje quirúrgico ventral escalonado del oído medio e interno para el cual encontramos que el músculo clavotrapeziano era un punto de referencia confiable. Para el abordaje transtimpánico, el acceso transcanal endoscópico fue un método fácil y confiable para el cual se describió una representación anatómica detallada. Además, se observaron similitudes anatómicas con los humanos mediante estereomicroscopía e imágenes Micro-CT, lo que reitera que el modelo de rata es adecuado para la investigación otológica. El abordaje endoscópico de la membrana timpánica es cómodo y menos costoso que un microscopio. El tendón del músculo clavotrapeziano puede ser un punto de referencia fiable para descubrir la bulla timpánica cuando se considera un abordaje ventral. La reconstrucción 3D Micro-CT permite la evaluación intacta de las muestras, siendo simultáneamente una herramienta de diagnóstico y también de aprendizaje.

PALABRAS CLAVE: Oído; Rata; Anatomía; Cirugía otológica; Micro-CT

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