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Anatomical studies of canine vascular and ligamentous ear structures with revelance to acute-onset deafness

Cathryn Kay Stevens-Sparks

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ANATOMICAL STUDIES OF CANINE VASCULAR AND LIGAMENTOUS EAR
STRUCTURES WITH RELEVANCE TO ACUTE-ONSET DEAFNESS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Interdepartmental Program in
Veterinary Medical Sciences through the
Department of Comparative Biomedical Sciences

by

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B.S., Louisiana State University, 1993
M.S., Louisiana State University, 1999
August 2012

DEDICATION

Many years of undergraduate and post-graduate education have led to the culmination of this work, which would never have been possible without the love and support of my family. This dissertation is dedicated to my late husband, Michael F. Stevens, who was my chief supporter throughout my undergraduate and part of my post-graduate education. Without his love and encouragement I would not be where I am today. This dissertation is also dedicated to my husband, James E. Sparks; my parents, James and Sarah Nichols; and my three children, Kaycee Lynn Stevens, Joshua Cole Stevens, and Cathryn Mechelle Stevens. I love you all very dearly!

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ABSTRACT

Hearing loss in dogs and cats following dental or ear procedures performed under anesthesia has recently been reported. The most likely causes for this acute-onset deafness were considered to be mechanical or vascular. Jaw manipulation as a possible cause of acute-onset deafness in the dog was investigated in the current study. Structures adjacent to the temporomandibular joint were of interest because changes in jaw orientation could disrupt vessels and nerves in this area. Current descriptions of the anatomy of the vasculature supplying the canine ear are either incomplete or inconsistent. Another considered cause was a jaw-ear connection via a ligamentous remnant of Meckel's cartilage. A ligament from the jaw to the ear, while recognized in humans, has not been described in the dog. The goal of this research was to provide more accurate anatomical descriptions of relevant canine juxta-articular structures to support future physiological studies.

The occurrence of hearing loss in dogs and cats following procedures performed under anesthesia was documented. Occurrence was low, with only 62 identified cases between 2002 and 2009, but the true occurrence may be greater. No relationship was observed between hearing impairment following these procedures and breed, gender, size of dogs, or anesthetic drug used; however, older animals may be more vulnerable.

New anatomical variations of three arteries are described in this study. (1) The rostral tympanic artery is a branch of the mandibular ramus and is accommodated by a small foramen located within a depression medial to the temporomandibular joint. (2) A rostral auditory tube branch of the caudal deep temporal artery was identified. (3) The origin of the caudal auricular artery occurred opposite the lingual artery in 25% of dissected specimens, contrary to published descriptions. The rostral tympanic artery and the rostral auditory tube branch may be susceptible to damage during jaw manipulation due to their locations. Variability of the caudal auricular artery can create problems during surgical procedures.

The tympanomandibular ligament, a remnant of Meckel's cartilage and equivalent to the human sphenomandibular ligament, was established as a jaw-ear connection in the dog. Tension on this ligament did not produce malleus movement.

CHAPTER 1 INTRODUCTION

Awareness of deafness and concern about its effects on quality of life in dogs and cats has increased in recent years. Hearing loss can be either hereditary or acquired, where acquired deafness is not genetic and often has a later onset. Causes of acquired hearing loss are often unknown, but have been reported to include ototoxicity, noise trauma, otitis, presbycusis, and anesthesia (Strain, 1996, 1999, 2011); the loss can be acute in onset or develop slowly. Hearing loss can also be categorized based on which region of the auditory system is compromised. Sensorineural hearing loss results from irreversible changes to the organ of Corti, which is a component of the inner ear, or retrocochlear structures; conductive hearing loss results when sound transmission is blocked due to obstructions in the outer and/or middle ear. Conductive hearing loss is often reversible. Although there are several documented reasons for acute onset-deafness, the causes are often unknown (National Institute on Deafness and Other Communication Disorders, 2010).

Anecdotal cases of acute-onset deafness in dogs and cats have been reported following dental procedures performed under anesthesia. These observations raised several questions about prevalence and possible etiologies:

- Have veterinarians noticed hearing changes in dogs and cats following dental procedures that were done under anesthesia?
- Could hearing loss have resulted in these cases from occlusion of the blood supply to the ear?
- Is there a jaw-ear connection that could affect structures of the ear and lead to conductive hearing loss subsequent to manipulation of the jaw, such as occurs during dental procedures?

To address these questions, information was needed to determine the extent of the problem and factors associated with the procedures and the anesthesia. Anatomical studies of relevant structures of the ear were necessary where descriptions in the current literature are incomplete or inconsistent. A better understanding of the blood supply and other anatomical structures that could be affected during jaw manipulation will provide the foundation for future physiological studies to verify the basis or bases for acute-onset deafness following dental procedures done under anesthesia.

An overview and literature review of the anatomy of the canine ear is presented in Chapter 2. This chapter discusses the anatomy, development, blood supply and innervation of the external, middle and inner ears. The literature review of the ear is followed by a discussion of the bony landmarks of the canine skull. These landmarks will be referenced throughout this dissertation. They were used for determining dissection approaches to the structures of the inner and middle ears and for orientation during the dissection studies.

The first goal of this research was to document the occurrence of hearing loss in dogs and cats following procedures done under anesthesia. Information obtained from pet owners between the years 2005 and 2009 was evaluated, as were data from cases of anesthesia-associated deafness obtained from postings on the Veterinary Information Network (VIN) web site between the years 2002 and 2009. A survey was sent to alumni of the Louisiana State University School of Veterinary Medicine and to members of the American Veterinary Dental College (AVDC) to assess the occurrence of deafness cases and potentially relevant details. These data are presented and discussed in Chapter 3.

Findings from studies of the blood supply to auditory structures are discussed in Chapter 4. Micro-dissection of injected vasculature was the main method used to study the blood supply to the inner and middle ears. Other methods included corrosion casting and computed tomography. The

focus of this study was to confirm the labyrinthine artery as the main blood supply to the inner ear and to identify branches of the maxillary artery that supply middle ear structures. By confirming the location and course of the labyrinthine artery, hearing loss due to the occlusion of this vessel subsequent to jaw manipulation could be ruled out. The maxillary artery was the main focus of this study due to its close relationship to the temporomandibular joint (TMJ). Arteries potentially affected by jaw manipulation would most likely be derivatives of the maxillary artery; therefore the branches that originated from this vessel near the jaw articulation were dissected to determine if their distribution was to ear structures. Branches of the maxillary artery supplying structures of the canine middle ear have been cited in anatomy texts (Evans, 1993; Ghoshal, 1975), but have not been thoroughly described. The rostral tympanic artery has been shown to be an important source of blood supply to the middle ear in the cat (Davis and Story, 1943); however, it is described as an inconstant branch of the canine maxillary artery (Evans, 1993; Ghoshal, 1975). One of the main focuses of this study was to determine the consistency of the rostral tympanic artery as well as its origin and distribution in the dog.

A jaw-ear connection was established through studies of ligamentous structures that connect the jaw and ear, reported in Chapter 5. Most of the findings from the anatomical studies reported were obtained using micro-dissection and histology. Initial studies were done in a human cadaver to assess the location and course of ligamentous structures known to connect the ear with the jaw. Prenatal canine specimen dissections were performed to identify the developmental connection between the ear and jaw made by Meckel's cartilage, a derivative of the embryonic first pharyngeal arch. The human and prenatal studies were followed by dissections of newborn, one-month-old, two-month-old and adult canine heads to determine if a ligamentous remnant of Meckel's cartilage was maintained and present in mature specimens. The human ligaments have been reported as capable of inducing movement of the malleus. A study was done to determine if tension on the newly identified

ligament in the dog would produce movement of the ear ossicles. Tension was placed at the juxta-articular (mandibular) end of the ligament, as this would be the region of the ligament that would be most susceptible to tension during jaw manipulation during dental procedures.

In all dissection studies, the chorda tympani nerve was an important landmark structure. The location of this nerve relative to the tympanic annulus, and the specific foramen and canal through which the nerve passes in its course to the middle ear is described in Chapter 6. In addition, a unique vascular foramen described in Chapter 6 was identified during blood supply studies. Both of these foramina, previously not described, are immediately adjacent to the TMJ. According to Mikheev and Tsybul'kin (1988), when the human mandibular head (condyle) is shifted backward or medially, the chorda tympani nerve is not affected; however, pathological processes occurring around the temporomandibular joint may involve the anterior (rostral) tympanic artery. Pressure on the anterior (rostral) tympanic artery may cause disturbance to the blood supply of the middle ear and could have a negative impact on sound perception (Anagnostopoulou, 2008).

Findings from these anatomical studies will enable future research to identify the cause or causes of acute-onset deafness in dogs and cats following dental procedures performed under anesthesia.

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CHAPTER 2

ANATOMY OF THE EAR*

The ear is the sensory organ for hearing, balance and equilibrium. This organ can be divided into the outer, middle, and inner ear; all of the inner ear structures are contained within the osseous labyrinth, a series of excavations within the temporal bone of the skull. The three components of the ear provide the ability for terrestrial mammals to transform sound pressure waves, i.e. longitudinal waves, into electrical energy. Pressure waves are transformed into mechanical energy in the middle ear, then into pressure waves in the fluid-filled inner ear and into electrical energy in the organ of Corti. This electrical energy is then processed and interpreted by the central nervous system. Sound waves are captured and transmitted to the middle ear by the structures of the external ear. The components of the external ear include the auricle, or pinna, and external auditory meatus, or ear canal, which receive sound waves and guide those to the eardrum, or tympanic membrane. The tympanic membrane separates the external ear from the middle ear. Components of the middle ear include the tympanic cavity and the three auditory ossicles (malleus, incus and stapes) and their associated ligaments and muscles. Components of the inner ear include the organ of hearing, the cochlea, and the organs of equilibrium, the semicircular canal ducts, the utricle and the saccule.

Anatomy of the External Ear

The external ear is comprised of the auricle (pinna) and external auditory meatus (ear canal). The auricle of the ear is shaped like a funnel in many mammals and its wall contains an elastic auricular cartilage as well as a hyaline annular cartilage. Both cartilages maintain the species-specific shape of the external ear and facilitate movement of the pinna by the auricular muscles. However a small portion of the external auditory passageway exists within the temporal bone of the skull (Fig. 2.1). In

* Stevens-Sparks, C.K. (2011) Anatomy of the ear. In: Strain, G.M. *Deafness in Dogs and Cats*. CAB International, Wallingford, UK, pp. 1-22. Reprinted with permission.

most domestic species, the cartilaginous external ear canal comprises two parts – a vertical and a horizontal part (Fig. 2.2). The vertical part extends ventromedially from the external orifice of

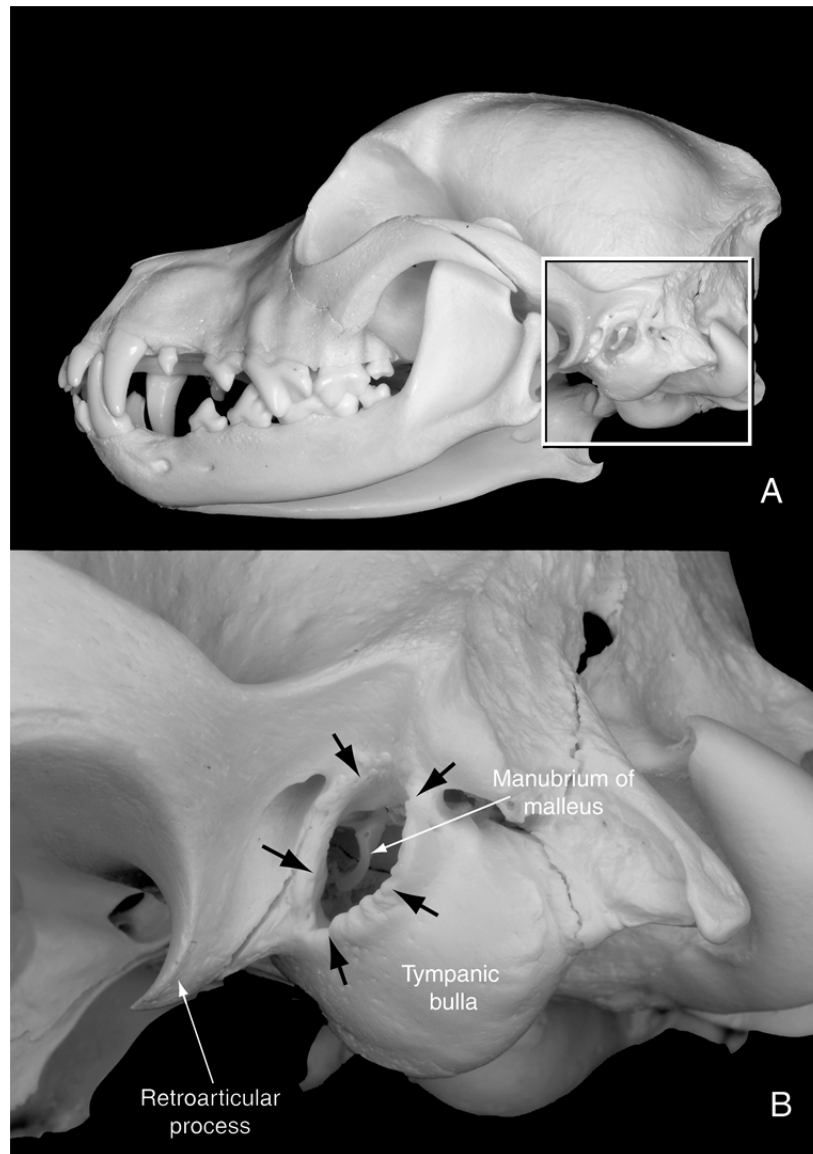


Figure 2.1. (A) Ventrolateral aspect of the canine skull showing the osseous external auditory meatus and tympanic bulla. (B) Enlarged view of the tympanic region (boxed area in A). Margins of the ossified external acoustic meatus are indicated by the black arrows (images courtesy of Dr. Daniel J. Hillmann).

the ear and is continuous with the horizontal part, which originates where the vertical part makes a sharp medial bend. The horizontal part extends to the bony external auditory meatus of the temporal bone. The epithelial lining of the ear canal is continuous on the external surface of the tympanic

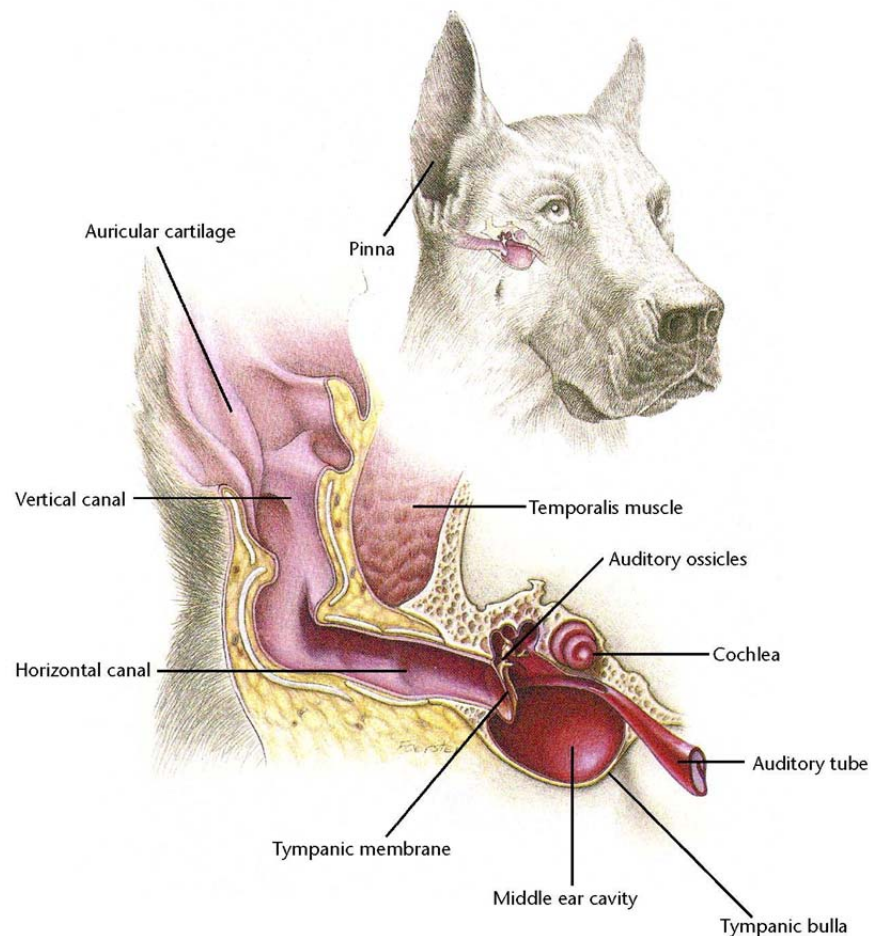


Figure 2.2. The two components of the external ear canal in domestic species are the vertical and horizontal canals. The horizontal canal originates where the vertical canal makes a sharp medial bend and terminates at the tympanic membrane (reproduced with permission from Hill's Pet Nutrition, Inc.).

membrane, which is suspended in the bony external auditory meatus. The auricular cartilage and annular cartilages form the auricle and the majority of the ear canal (Fig. 2.3). The auricular cartilage is shaped like an asymmetrical funnel, with its proximal end forming a canal. The free distal end of

the cartilage forms the pinna, which serves as a funnel for capturing and concentrating air vibrations or sound waves (Fig. 2.4).

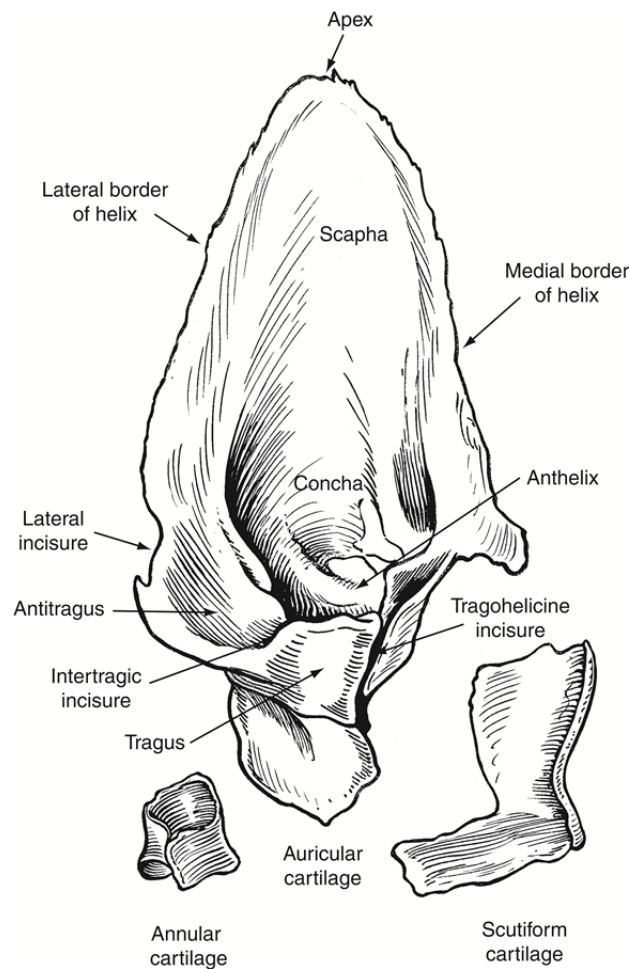


Figure 2.3. The two main cartilages of the ear are the auricular and annular cartilages. The scutiform cartilage serves as an attachment surface for the auricular muscles. The visible portion of the auricular cartilage has several regions that provide important surgical landmarks (image from MediClip software, Williams & Wilkins Co., Baltimore, MD).

The auricle, or pinna, is the visible portion of the outer ear that varies species specifically in shape and differs in the fibroelastic cartilage collagen/elastin composition among breeds, resulting in either an erect (small v-shaped), a semi-erect (lobated), or a non-erect (lop-ear) appearance. The auricle is necessary in sound wave collection and sound localization and guides these pressure waves

into the external acoustic meatus. The size and shape of the pinna determines which sound frequencies are collected optimally and also may reduce noise. For example, there appears to be a

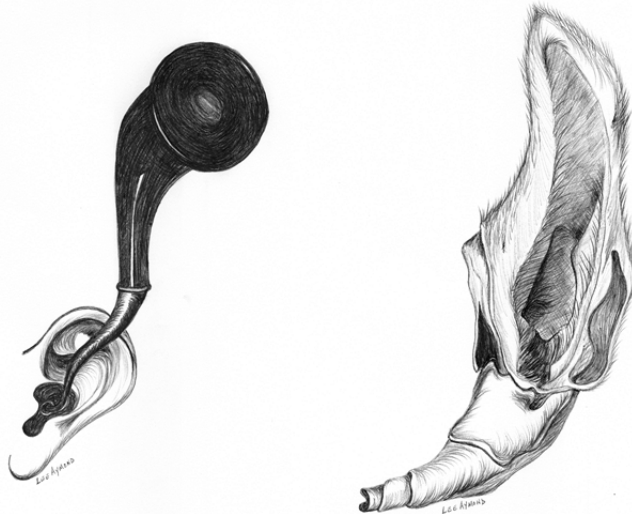


Figure 2.4. The shape of the canine external ear cartilages can be likened to a vintage ear trumpet, a device used by humans for sound wave collection and amplification. The size and shape of the ear pinna determines which sound frequencies are collected optimally (drawing by Lee Aymond).

correlation between increased auricle size and the detection of low-frequency (long-wavelength) sounds (i.e. longitudinal waves in gas/air), allowing the animal to detect sounds from greater distances (Nummela, 2008). The external surface of the auricle is slightly convex and covered by hairy skin, while the internal surface is concave and covered by hairy skin distally. Modified hairy skin with glands that produce earwax (cerumen) is found proximally and extends into the external ear canal. The orientation of the auricle is controlled by skeletal muscles, thus allowing the “funnel” to be oriented toward the source of sound. Unlike humans, who must turn their head toward the sound source, many mammals can move the auricle of each ear, even independently. The extensive movement of the auricle is permitted by the attachment of a complex set of auricular muscles (Fig. 2.5). These auricular muscles are often considered in groups, rather than individually – there is a rostral auricular group of muscles and a caudal group. The rostral auricular muscles rotate the ear medially and narrow the concave aspect of the erect auricle. The primary function of the caudal

auricular muscles is to raise the ear, but this group of muscles also rotates the ear (Heine, 2004). A flat, boot-shaped, sesamoid cartilage is located just rostral and medial to the base of the auricle. This is the scutiform cartilage, which serves as an attachment for many of the auricular muscles to the auricle (Fig. 2.3). This cartilage aids in the redirection of the pull of some of these muscles (Dyce *et al.*, 2010). In addition, a ventral muscle of the auricle pulls the pinna downwards.

The visible portion of the external ear has several regions that provide important surgical landmarks, namely the helix, scapha, anthelix, tragus and antitragus (Fig. 2.3). The helix is the rim of the auricle, whose free margins face rostrally and caudally in lobated and lop-eared breeds. In cats and dogs with erect, v-shaped auricles, the margins of the helix face medially and

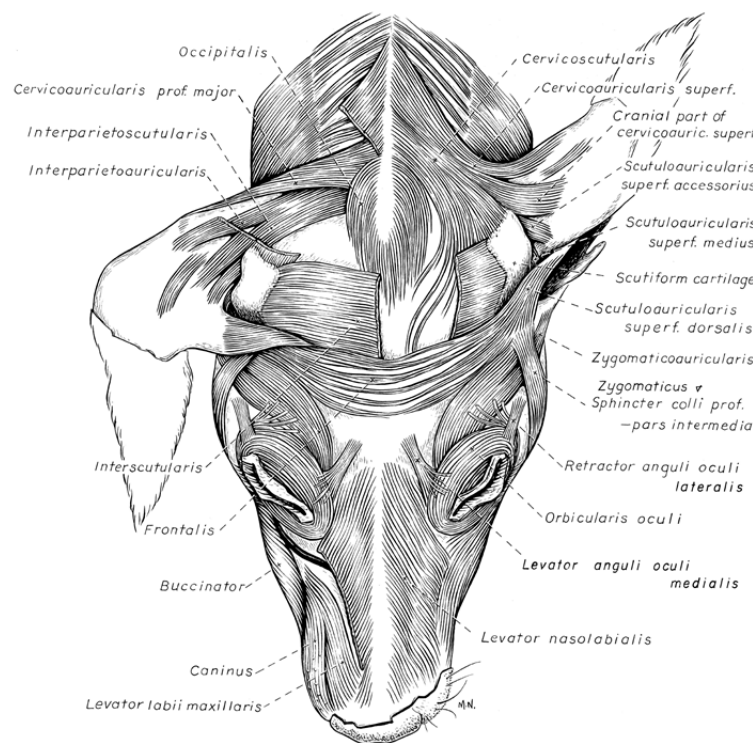


Figure 2.5. Dorsal view of the cephalic and cervical muscles of the canine. Several muscles attach to the ear by way of the scutiform cartilage. The muscles of the ear are often considered in two groups: the rostral auricular muscles and the caudal auricular muscles (from Hermanson and Evans, 1993; reproduced with permission).

laterally. Dorsally, the rostral and caudal borders of the helix meet at the apex. The scapha is the triangular portion of the auricle that is bounded by the apex dorsally, the rostral and caudal margins of the helix rostrally and caudally and the anthelix ventrally. The anthelix is a transverse ridge located on the concave surface of the auricular cartilage just distal and medial to the beginning of the ear canal. Opposite to the anthelix, the lateral border of the ear canal is formed by the quadrangular-shaped tragus. A deepened groove, the tragohelicine incisure, separates the tragus from the helix rostrally. The intertragic incisure separates the caudal portion of the tragus from the antitragus. The antitragus forms the caudolateral border of the external ear canal and extends to the lateral incisure. When covered with skin, a cutaneous marginal pouch is formed at this incisure.

The expanded opening of the ear canal is called the concha. The constricted, tubular vertical ear canal continues the concha ventromedially. The vertical ear canal is continued by the horizontal ear canal where the canal makes a sharp, medial bend. Depending on their shape and dimensions, the concha and ear canal will have resonant properties that will selectively amplify certain sound frequencies before they reach the tympanic membrane and middle ear (Rosowski, 1994; Hetherington, 2008). The skin covering of the auricle extends into the ear canal and has all of the characteristics of hairy skin, including sebaceous glands and hair follicles. Ceruminous glands, which are modified sweat glands, produce a waxy material called cerumen (ear wax), and are found only within the ear canal. The secretion of ear wax traps small particles and prevents those from gaining access to the innermost depths of the external ear canal. The most common tumors of the external ear canal arise from the ceruminous glands (ceruminous gland adenoma or adenocarcinoma), although proliferation of other cell types may also result in tumors (squamous cell carcinoma, basal cell tumor and mast cell tumor) (Fossum, 2007). Most canine ceruminous gland tumors are benign, but these tumors are more aggressive in cats and are usually malignant (White-Weithers, 2005; Fossum, 2007;). The annular cartilage connects the cartilaginous meatus to the bony external acoustic meatus. The external meatus ends at the tympanic membrane, which represents the barrier between the

external and middle ear. This membrane not only functions in the transfer of sound waves to the middle ear, but also serves a protective function against pathogen invasion of the middle ear from the external environment. However, aggressive pathogenic infections can cross the tympanic membrane and gain access to the middle ear (Fossum, 2007).

Anatomy of the Middle Ear

The middle ear is located in the air-filled cavity of the temporal bone, whose only direct contact to the external environment occurs by way of the auditory (eustachian or pharyngotympanic) tube. The middle ear is necessary for amplification of sound pressure waves to be transmitted to the inner ear. Additionally, the middle ear muscles, the tensor tympani muscle and the stapedius muscle can limit sound amplification by limiting the movement of the ossicles in order to prevent damage to the inner ear. Without the middle ear as an intermediate, many of the pressure waves being transmitted in air would be reflected at the air/fluid interface at the fluid-filled inner ear due to differences in acoustic impedance within these different media (Nummela and Thewissen, 2008).

The origin of the middle ear is demarcated by the medial surface of the tympanic membrane. The tympanic membrane has two parts: the pars tensa and the pars flaccida. The pars tensa is the tense region of the membrane that is readily visible through an otoscope. The outermost margins of the membrane are attached to an internal ring of bone, the tympanic annulus, located just inside the margin of the bony external auditory meatus. The external surface of the pars tensa is drawn into a slightly concave profile due to the medial and central attachment of the manubrium of the malleus. The pars tensa is comprised of three layers: an outer epithelial layer, an inner epithelial (mucosal) layer and a middle layer of fibrous connective tissue. The pars flaccida is a small, triangular portion of the tympanic membrane located dorsal to the lateral process of the malleus. Medial to the tympanic membrane is the cavity of the middle ear, also referred to as the tympanic cavity, in the tympanic part of the temporal bone. The middle ear cavity has three regions, the dorsal epitympanum (epitympanic recess), the intermediate mesotympanum (tympanic cavity proper) and the ventral

hypotympanum. This hypotympanum is enlarged in dogs and cats as the tympanic bulla (Fig. 2.1). The majority of the tympanic cavity is lined by simple squamous epithelium, which is continuous with the internal layer of the tympanic membrane.

The tympanic cavity houses a chain of three small bones, the ear ossicles, with associated ligaments and muscles (Fig. 2.6). The malleus is the lateral ossicle, named for its resemblance to a mallet; the incus is the middle ossicle, named for its resemblance to an anvil; and the stapes is the medial ossicle, named for its resemblance to a stirrup. The head and neck of the malleus, the incus and the stapes are located in the dorsalmost region of the tympanic cavity, the epitympanic recess. The three ossicles are articulated to each other and to the wall of the tympanic cavity by joints and ligaments. The joints between the ossicles are synovial (diarthrotic) joints. The handle, or manubrium, of the malleus extends ventrally from the neck of the malleus and attaches to the medial surface of the tympanic membrane. If the tympanic membrane is displaced by sound vibrations, the malleus will be moved, and this results in the movement of the incus, which then moves the stapes. The footplate, or base, of the stapes rests on a connective tissue membrane, which is suspended on the edge of the oval window. The base of the stapes is articulated at the oval window by a syndesmosis with the cartilage that covers the edge of the oval window (Getty et al., 1956). This flexible membrane of the oval window serves as a barrier between the cavity of the middle ear and the fluid-filled cavity of the inner ear. The oval window membrane is significantly smaller than the tympanic membrane. Ratios in size of oval window to tympanic membrane range from about 1:10 to 1:60 in vertebrates (Hetherington, 2008). This difference in size allows for amplification of sound, as energy is collected over a relatively large tympanic membrane and converged onto a relatively small oval window. When the ear ossicles are moved, the footplate of the stapes reverberates the membrane covering the oval window, thus displacing the fluids contained within the inner ear.

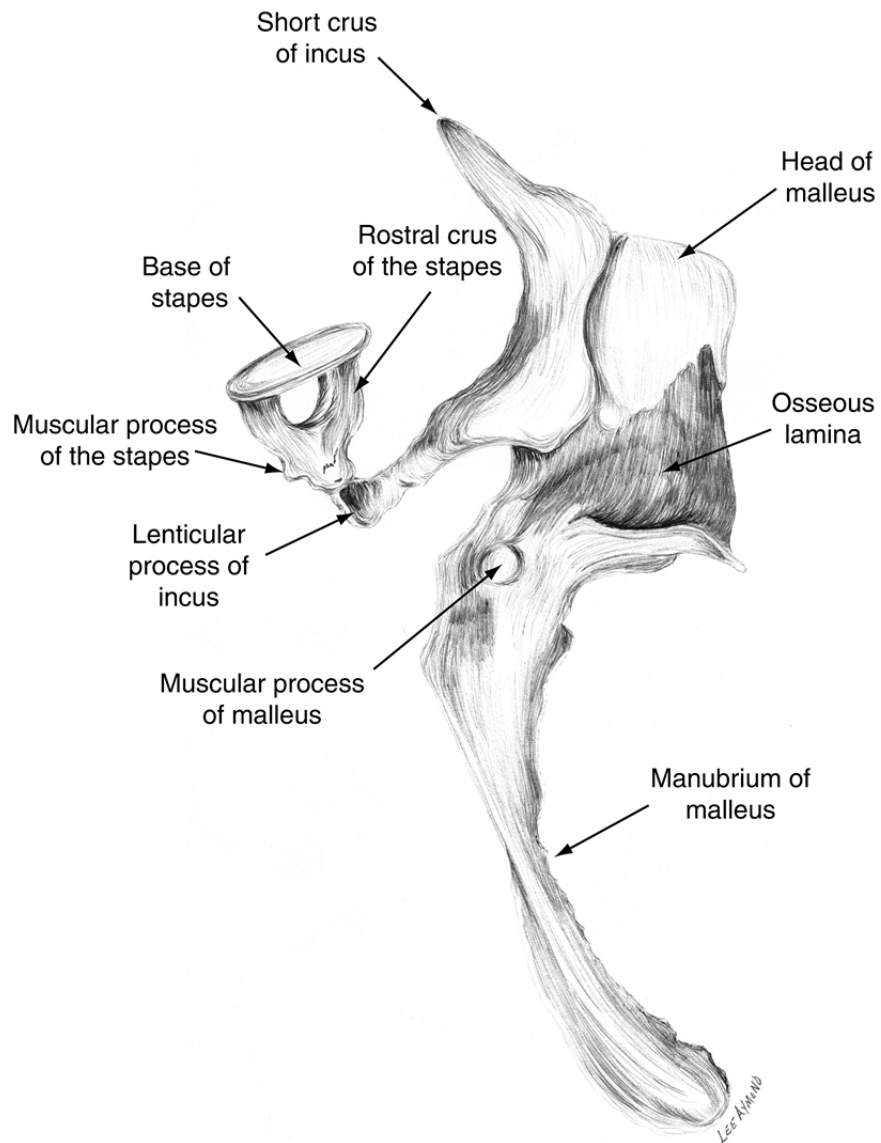


Figure 2.6. Medial view of the isolated, articulated middle ear ossicles of the left canine ear (drawing by Lee Aymond).

The malleus has three main parts, the head, the neck and the manubrium (handle) (Fig. 2.6). Three distinct processes project from the neck. The muscular process extends medially from the base of the neck and serves as an attachment for the tendon of the tensor tympani muscle. The rostral process of the malleus is located at a 90° angle to the muscular process and serves as the attachment site of the rostral malleolar ligament, which anchors the malleus to the rostral wall of the tympanic

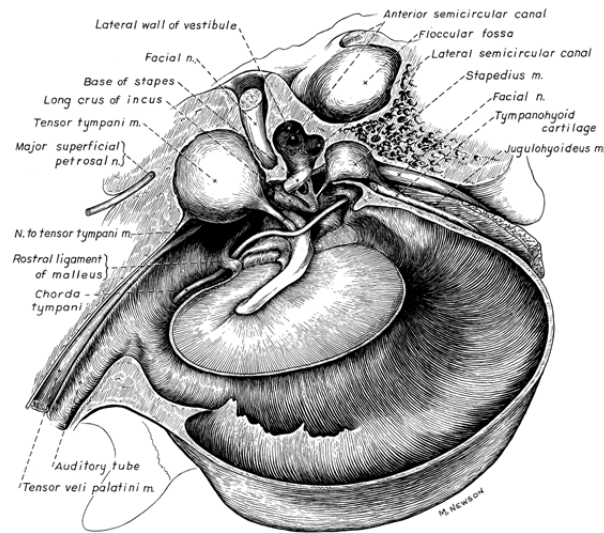


Figure 2.7. Medial view of the right canine middle ear showing the *in situ* auditory ossicles and their muscles. The cochlea and promontory have been removed (from Evans, 1993a; reproduced with permission).

cavity, close to the petrotympanic fissure (Fig. 2.7). It is through this fissure that the chorda tympani nerve passes on its course to unite with the lingual nerve (Cranial Nerve V). A thin lamina of bone (osseous lamina) extends between the head and rostral process of the malleus (Fig. 2.6). The lateral process of the malleus extends laterally and is located slightly ventral and at about a 180° angle relative to the muscular process. The lateral process is the attachment site for the lateral ligament of the malleus, which connects the malleus to the tympanic notch, located at the caudodorsal margin of the tympanic annulus. The dorsal ligament of the malleus attaches the head of the malleus to the roof of the epitympanic recess.

The incus is in the middle of the chain of ear ossicles and its structure is compared to that of a premolar tooth with two roots. The body of the incus articulates with the head of the malleus. The crura, or limbs, of the incus are asymmetrical, forming a short and an elongated crus. The short crus is directed caudodorsally and is anchored within a fossa caudal to the epitympanic recess (fossa incudis) by the caudal ligament of the incus. The long crus is directed caudoventrally and has a

medially directed lenticular process at its distal end, which articulates with the stapes. The dorsal ligament of the incus attaches its body to the roof of the epitympanic recess.

The head of the stapes articulates with the distal end of the lenticular process of the incus. The head is continued by a neck, which has a muscular process for the attachment of the tendon of the stapedius muscle (Fig. 2.7). Rostral and caudal crura extend from the neck and attach to the base, or footplate, of the stapes (Fig. 2.6). The stapes lies in a horizontal plane and its footplate projects medially toward the oval window, which is located on the dorsolateral surface of the bony promontory of the petrous part of the temporal bone (Fig. 2.8). A syndesmosis forms the articulation of the annular ligament of the footplate of the stapes with the cartilage that lines the edge of the oval window.

Atmospheric (air) pressure on each side of the tympanic membrane must be equalized in order to maintain proper tension for optimal vibration of the membrane. Atmospheric pressure is equalized by the fibrocartilaginous auditory (eustachian) tube, which connects the nasopharynx to the tympanic cavity. The left and right auditory tubes extend dorsocaudolaterally from their pharyngeal openings in the lateral wall of the pharynx to the rostradorsal region of the mesotympanum. The auditory tube passes through a bony canal, the musculotubal canal of the tympanic part of the temporal bone, on its course to the cavity of the middle ear. The mucosal lining in the region surrounding the entry of the auditory tube into the middle ear cavity contains many mucous glands covered by a ciliated epithelium. The ducts of these glands open into the tympanic cavity. Polyps, i.e. benign proliferations of the mucosa, that form in this region can block the auditory tube opening, thereby preventing proper drainage and pressure equalization of the middle ear cavity. Running along the lateral wall of the auditory tube is the elongated tendon of the tensor veli palatini muscle and a branch of CN (cranial nerve) V, which innervates the tensor tympani muscle.

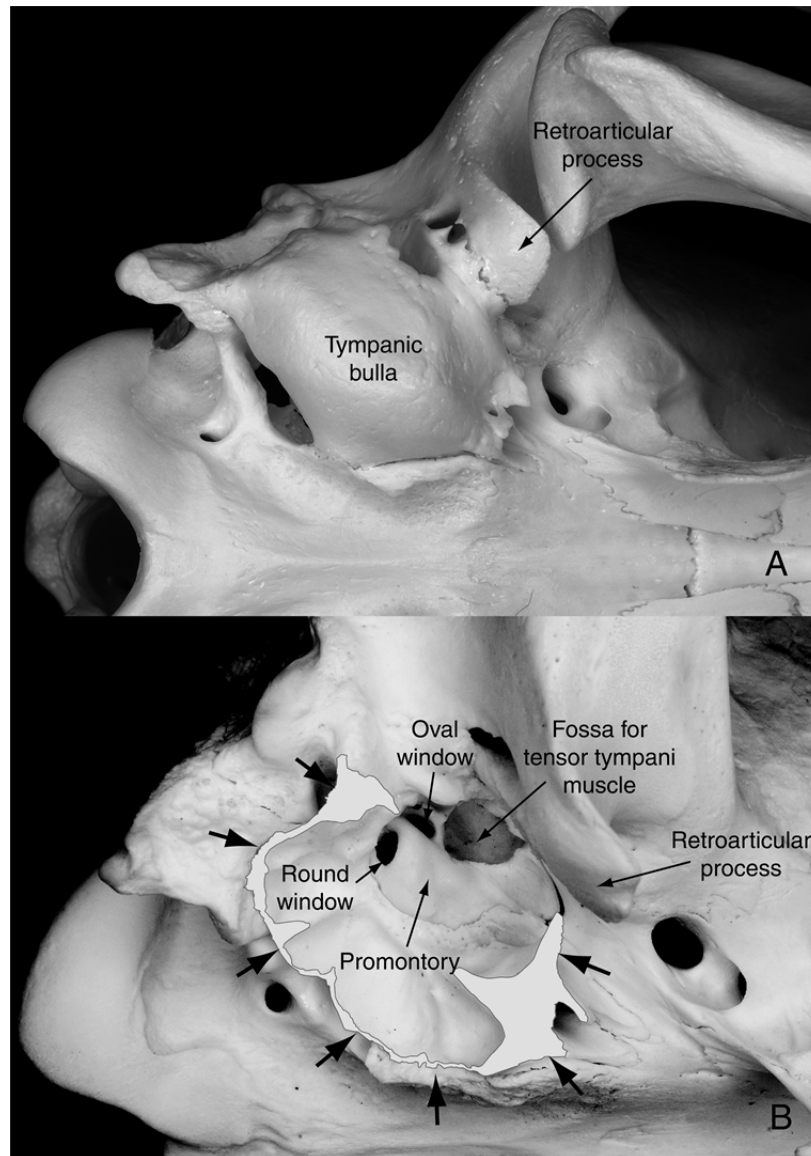


Figure 2.8. (A) Ventral view of the intact canine right tympanic bulla. (B) Ventrolateral view of the canine middle ear cavity with the ventral part of the tympanic bulla removed, showing the promontory of the petrous temporal bone with affiliated oval and round windows. Black arrows indicate the cut margin of the bulla (images courtesy of Dr. Daniel J. Hillmann).

Anatomy of the Inner Ear

The structures of the inner ear are protected by the petrous part of the temporal bone. The internal architecture of the inner ear can be divided into a bony, or osseous, labyrinth and a membranous labyrinth. The osseous labyrinth is an excavation in the petrous temporal bone, which is filled with perilymph fluid. The osseous labyrinth includes the vestibule, semicircular canals and the

cochlea. The membranous labyrinth of the inner ear is contained within the osseous labyrinth and is comprised of a continuous network of endolymph (a viscous fluid similar to intracellular fluid)-filled sacs and tubes. The membranous labyrinth includes the utricle, saccule, cochlear duct and semicircular ducts. The membranous labyrinth represents the structures necessary for hearing and equilibrium.

The bony promontory is the ventral expansion of the petrous part of the temporal bone and protrudes into the dorsomedial region of the tympanic cavity proper (Fig. 2.8). The promontory houses the cochlea and the vestibule and is located medial to the flaccid portion of the tympanic membrane (Getty *et al.*, 1956). The round window is an opening from the cavity of the middle ear into the cochlea that is located at the caudal end of the promontory and is covered by the membrane of the round window. The oval window, which is closed by the annular ligament attached to the footplate of the stapes, is located on the dorsolateral surface of the bony promontory and leads into the vestibule of the osseous labyrinth. The membranes that close these two windows separate the middle ear cavity from the cavity of the inner ear.

The vestibule of the inner ear can be thought of as the entryway, or foyer, of the osseous labyrinth of the inner ear. A series of canals extend from this foyer. The canal that runs medially from the central vestibule to the interior of the caudal cranial fossa of the cranial cavity is the vestibular aqueduct. The cochlea is medial and ventral to the vestibule and is directed rostroventrally and slightly lateral (Fig. 2.9). The cochlear canal is spiral in shape and is reminiscent of a snail shell, from which it derives its name (Fig. 2.10). The central core of the cochlea is supported by a hollow, bony prominence, the modiolus. A thin, hollow, continuous shelf of bone spirals around the modiolus from its base to its apex. This bony spiral lamina extends outward like a shelf from the modiolus toward, but does not completely reach, the walls of the cochlear canal. The cochlear aqueduct is a canal that leads ventrally from the perilymphatic space surrounding the cochlea to the meninges that surround the brain. This canal connects the perilymphatic space, filled with perilymphatic fluid, to

the subarachnoid space, which contains cerebrospinal fluid (CSF). The perilymphatic fluid of the osseous labyrinth is absorbed into the CSF by way of this canal. Perilymphatic fluid is a filtrate produced by the periosteal blood vessels of the osseous labyrinth and has the same ionic concentration as extracellular, or interstitial, fluid (Banks, 1993). Caudal and slightly dorsal to the vestibule are three separate canals that tunnel through the petrous bone. These canals are semicircular and are located dorsally, laterally and caudally. Each semicircular canal is located in a plane approximately perpendicular to the other two. The crura are the proximal ends of each canal that communicate with the vestibule. One crus of each canal is dilated, or ampullated, where it joins with the vestibule. The non-ampullated ends of the caudal and dorsal canals form a common crus before joining caudally with the vestibule.

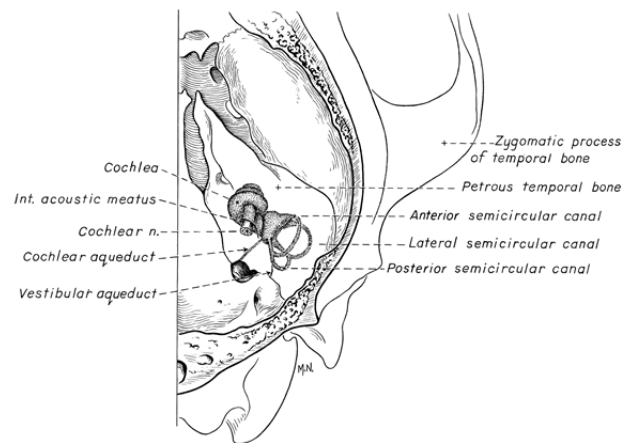


Figure 2.9. Dorsal view of the canine cranial fossa showing the petrous temporal bone and position of the membranous labyrinth of the inner ear (from Evans, 1993a; reproduced with permission).

Contained within the bony vestibule are two membranous sacs, the saccule and utricle. The saccule is located ventrally within the vestibule and the utricle is located dorsally within the vestibule (Fig. 2.11). From the utricle arise three semicircular ducts, which extend into the canals of the osseous labyrinth. Endolymph is displaced within these ducts when the head is rotated. Because the

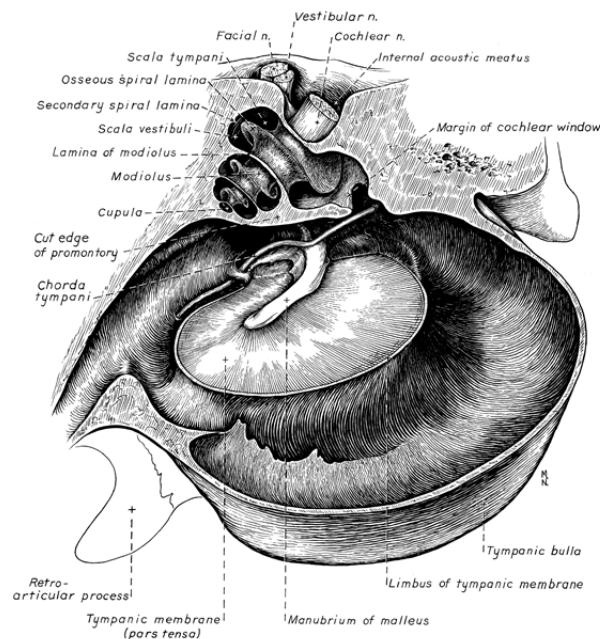


Figure 2.10. Medial view of the right canine middle ear. The promontory has been sectioned to show the cochlea (from Evans, 1993a; reproduced with permission.)

three ducts are oriented in different planes, circulation of fluid within the ducts is determined by the direction of head movement. This allows for spatial orientation, and thus aids in the maintenance of balance following positional changes of the head. The sensory cells in the epithelium of the wall of the semicircular ducts are located within specialized sensory structures of the ampullae termed cristae ampullares. Two other receptor areas, the maculae, monitor the position of the head with respect to gravity. These are represented by circumscribed areas of sensory cells within the epithelial walls of the saccule (macula sacculi) and utricle (macula utriculi). The maculae respond mainly to changes in linear acceleration, rather than rotational movements of the head. Afferent information from the maculae and cristae ampullares is transported within the vestibular portion of the vestibulocochlear nerve (CN VIII). Two ducts that extend medially from the saccule and utricle unite and form the endolymphatic duct. The endolymphatic duct runs within the vestibular aqueduct and terminates as

the expanded endolymphatic sac on the caudal, internal surface of the petrous part of the temporal bone, where it is in contact with the dura mater. Studies suggest that the endolymphatic duct and endolymphatic sac perform both absorptive and secretory (Wackym *et al.*, 1987a; Rask-Andersen *et al.*, 1991; Schuknecht, 1993; Yeo *et al.*, 1995;), as well as phagocytic (Fukuzawa *et al.*, 1991) and immunodefensive functions (Wackym *et al.*, 1987b).

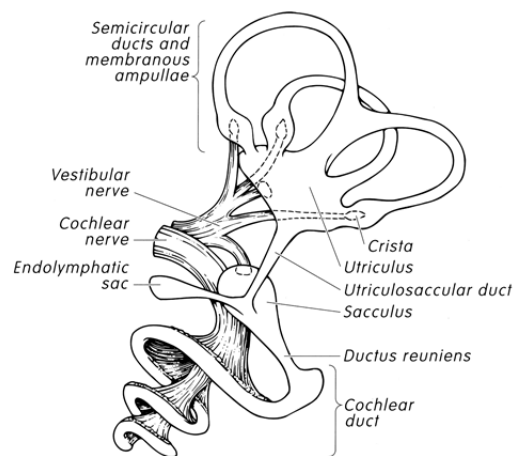


Figure 2.11. The isolated membranous labyrinth of the canine inner ear (from Evans, 1993a; reproduced with permission.)

The cochlear duct is connected ventrally to the saccule via the ductus reuniens (Fig. 2.11). The cochlear duct spirals within the cochlea, due to its attachment to the spiral lamina on one side and the cochlear cavity wall on the other. The duct has a wedge shape in cross-sections, with the apex of the wedge attached to the osseous lamina of the modiolus and its base forming the spiral ligament where it fuses with the periosteum lining the cochlear cavity wall. This peripheral wall of the cochlear duct is termed the stria vascularis, and is comprised of a bistratified layer of cuboidal or columnar cells which are underlain by a highly vascularized connective tissue, the spiral ligament (Banks, 1993). Endolymph is produced by the stria vascularis, circulates within the membranous

labyrinth and finally drains into the venous sinuses of the dura mater via the endolymphatic duct (Banks, 1993). Together with the osseous spiral lamina, the cochlear duct divides the cochlear space into upper and lower chambers. The upper spiral chamber is the scala vestibuli and begins at the vestibule, close to the oval window; the lower spiral chamber is the scala tympani and ends at the round window. These two chambers communicate at the helicotrema, which is located at the apex of the cochlea, rostral to the tip of the osseous modiolus. The endolymph-filled chamber of the blind-ending cochlear duct, which is wedged between the upper and lower perilymph-filled chambers, is the scala media. Reissner's membrane (vestibular membrane) separates the cochlear duct from the scala vestibuli, while the basilar membrane separates the cochlear duct from the scala tympani. The limbus occurs where these two membranes approximate each other at the spiral lamina of the modiolus.

The organ of Corti, which is the organ of hearing, is comprised of groupings of specialized sensory cells called hair cells, which are embedded within supporting cells, all of which rest on the basilar membrane. These sensory cells serve as mechanoreceptors that convert mechanical energy into electrical energy, or nerve potentials. The apical surface of the hair cells contains hair-like projections called stereocilia (modified cilia, non-motile), which project into the lumen of the cochlear duct. The stereocilia of these hair cells descend in height across the apical surface of the cell and the tips of the most elongated stereocilia are embedded within the overlying tectorial membrane. This membrane is a gelatinous structure that is secreted by specialized epithelial cells (interdental cells) located at the limbus of the spiral lamina. When activated by mechanical stimulation, deflections of the apical stereocilia result in the release of neurotransmitters from the bases of the hair cells onto afferent neuron receptive endings of bipolar cells. Bundles of afferent fibers direct impulses medially within the hollow, bony spiral lamina toward the bodies of bipolar nerve cells. The cell bodies of these bipolar neurons are located within spiral ganglia, which are located in the modiolus at the origin of the spiral lamina. Afferent axons extend from the spiral ganglia and collect

centrally within the hollow modiolus, thus forming the cochlear portion of the vestibulocochlear nerve (CN VIII).

Movement of the stapes at the oval window results in the displacement of perilymphatic fluid as a transverse wave in the scala vestibuli toward the helicotrema, where this wave is continued in the perilymphatic fluid of the scala tympani. The round window is a membrane-covered opening at the caudal end of the promontory that provides a relief valve function so that when the perilymphatic fluid is compressed by inward deflection of the oval window, the membrane of the round window bulges outward. As the wave in the perilymphatic fluid circulates through the scala vestibuli and scala tympani, Reissner's membrane and the basilar membrane are deflected, resulting in stimulation of the embedded hair cells with subsequent firing of afferent cochlear neurons. The width of the bony spiral lamina decreases from the base of the modiolus to the apex, and so the width of the basilar membrane, which extends from the bony spiral lamina to the cochlear wall, increases from base to apex. Different portions of the sensory epithelium of the organ of Corti respond maximally to sound waves of specific frequencies, with low-frequency (long-wavelength) detection being greatest at the cochlear apex and high-frequency (short-wavelength) detection being maximal at the cochlear base. An extended length of the cochlear duct increases the range of detectable audible frequencies. In dogs, there are 3.25 cochlear turns and the organ of Corti is sensitive to a frequency range of 67 – 45,000 Hz, compared with a range of 64 – 23,000 Hz in humans who have 2.75 cochlear coils (deLahunta and Glass, 2009; see Chapter 3 of Strain, 2012). The cat has 3.0 cochlear spirals (Brown, 1987; Heine, 2004), with a hearing frequency range of 45 – 64,000 Hz.

Development of the Ear

One distinguishing feature of the development of the head region of the vertebrate embryo (Moore and Persaud, 2003; McGeady *et al.*, 2006; Sinowatz, 2010) is the formation of the pharyngeal arches, pouches and grooves. Discrete aggregations are visible as a result of the migration of neural crest-derived mesenchymal cells and proliferation of mesoderm within the developing head

and neck region. These aggregations give rise to six pharyngeal arches, of which four are visible in most mammalian embryos. Externally, the arches are lined by ectoderm; internally, by endoderm. The external invaginations between subsequent arches lined by ectoderm are called pharyngeal grooves. The internal outpouchings of the primitive foregut between subsequent arches lined by endoderm are called pharyngeal pouches. Local circumscribed proliferation of mesodermally and neural crest-derived tissue between the ectoderm and endoderm results in pharyngeal arch restructuring. Structures of the external and middle ear are derived from the first and second pharyngeal arches and the first pharyngeal groove and pouch.

External and Middle Ear

Mesenchymal tissue from the first and second pharyngeal arches proliferates and surrounds the first pharyngeal groove, forming the auricle and external auditory meatus. The auricle and ear canal are thus lined by ectodermally derived epithelium. The tympanic membrane is formed when the recess of the first pharyngeal groove and the first pharyngeal pouch excavate deeper into the mesoderm between the first and second pharyngeal arches to a face of apposition. The lateral, or external, epithelium of the tympanic membrane is derived from the deepest recess of the first pharyngeal groove ectoderm; the medial, or internal, epithelium of the tympanic membrane is derived from the deepest recess of the first pharyngeal pouch endoderm. Between these two layers is a thin layer of mesoderm. The first pharyngeal pouch maintains its connection to the pharynx and gives rise to the auditory tube and middle ear cavity. The ossicles of the middle ear are formed by neural crest-derived mesenchyme and their affiliated muscles are formed from the mesoderm of the first and second pharyngeal arches. The malleus, incus and tensor tympani muscle are derivatives of the first pharyngeal arch. The stapes and stapedius muscle is derived from tissues of the second pharyngeal arch. The cranial nerve that supplies structures of the first pharyngeal arch is the trigeminal nerve (CN V); therefore branches of the trigeminal nerve supply the auricle, ear canal, malleus, incus and tensor tympani muscle. The cranial nerve that supplies structures of the second

pharyngeal arch is the facial nerve (CN VII), and therefore branches of the facial nerve supply caudal parts of the auricle, ear canal, stapes and stapedius muscle. Nerves of the cervical plexus, which is formed by the ventral branches of cervical spinal nerves, also supply auricular structures derived from the second pharyngeal arch.

Inner Ear

The development of the inner ear begins with the induction of the otic placodes, which are bilateral ectodermal thickenings located lateral to the caudal part of the rhombencephalon. These ectodermal placodes invaginate to form the otic pits, which maintain their connection for a time with the surface ectoderm. Eventually, the connection with the surface ectoderm is lost and the result is the formation of the otic vesicles. The cavity of the otic vesicle fills with endolymph, which is produced by the ectodermal epithelial cells and has a composition similar to that of intracellular fluid. Proliferation of the ectodermal cells of the otic vesicle results in the elongation of the otic vesicle and the initial differentiation into two distinct regions: the dorsally expanded utricle and the ventrally located saccule. A series of outpocketings from the utricle and saccule establishes the primordia of the semicircular and cochlear ducts. The cochlear diverticulum evaginates from the ventral portion of the saccule. As the cochlea elongates it constricts and coils, forming the cochlear duct. The connection between the cochlea and the saccule is maintained by the narrow ductus reuniens.

Blood Supply of the Ear

External Ear

The main blood supply to the external ear is derived from branches of the external carotid artery, the caudal auricular artery, and the superficial temporal artery (see Evans, 1993b, Fig 11.14, p. 606). The caudal auricular artery supplies the auricle, caudal auricular muscles and the ear canal. The rostral auricular branch of the superficial temporal artery supplies the rostral auricular muscles. The caudal auricular artery arises from the external carotid artery at the base of the ear canal, deep to

the parotid salivary gland at the caudal border of the masseter muscle. It courses caudally and dorsally at the base of the ear, initially deep and then superficial to the caudal auricular muscles, and sends branches distally toward the apex of the auricle. The branches of the caudal auricular artery include the lateral, intermediate and medial auricular branches to the convex external surface of the auricle and the deep auricular artery that supplies the cutaneous portions of the external auditory canal. The lateral, intermediate and medial auricular branches of the caudal auricular artery provide perforating branches through numerous foramina in the auricular cartilage to supply the concave surface of the auricle. It is these blood vessels that may be affected during head shaking and fighting, resulting in the formation of aural hematomas (Fossum, 2007). Branches of the intermediate auricular branch also supply the caudal auricular muscles. The superficial temporal artery arises and ascends dorsally from the external carotid artery, immediately rostral to the auricle. One of its branches, the rostral auricular branch, extends caudally to supply the rostral auricular muscles (Evans, 1993a; Heine, 2004).

Middle Ear

The canine middle ear blood supply has not been thoroughly described and there are some ambiguities in its description among various authors. In 1943, Davis and Story provided a thorough description of the feline middle ear blood supply through investigating the carotid circulation in this species. The following description of the middle ear vasculature will therefore be based on the feline, with canine comparisons discussed where applicable.

The middle ear receives its arterial supply from branches of the ascending pharyngeal, occipital and maxillary arteries. The ascending pharyngeal artery provides a Eustachian branch (ramus eustachii) that supplies the auditory tube and accompanies it through the musculotubal canal of the temporal bone into the lateral chamber of the bulla. The vessel then ramifies over the surface of the promontory, where it anastomoses with the rostral and caudal tympanic arteries. The caudal (inferior) tympanic artery, a branch of the occipital artery, enters the skull at the foramen lacerum,

where it immediately branches. The most posterior of the two branches derives several twigs that ramify either within the mucosa covering the promontory or over the posterior part of the dorsal surface of the bulla. According to Evans (1993a), the condyloid artery of the canine is synonymous with the caudal (inferior) tympanic artery described by Davis and Story (1943). The condyloid artery enters the petro-occipital fissure and passes through the petrobasilar canal, accompanied by the accessory nerve, where it dissipates in the dura at the ventral end of the pyramid of the medulla oblongata. In its course it supplies twigs to the middle and inner ear. Ghoshal (1975) and Schummer *et al.* (1981) state that the canine caudal tympanic artery (a. tympanica caudalis) is a branch of the occipital artery-derived caudal meningeal artery. In the cat, the branches of the maxillary artery that supply the middle ear are the deep auricular (a. auricularis profunda) and rostral (anterior) tympanic (a. tympanica anterior or a. tympanica rostralis). In the dog, the deep auricular artery is not a direct branch of the maxillary artery, but is a branch of the caudal auricular artery, which is a branch of the external carotid artery. This deep auricular artery supplies the periosteum of the malleus manubrium as well as the tympanic membrane (Cole, 2009). The rostral (anterior) tympanic artery is a constant branch of the feline maxillary artery and is an important supply to the middle ear. However, in the dog, the rostral tympanic artery is described as an inconstant vessel that branches from the maxillary artery rostral and medial to the retroarticular process of the temporal bone (Ghoshal, 1975; Evans, 1993a). When present, this vessel extends caudally from its origin and enters the middle ear cavity via a small foramen medial to the temporomandibular joint, near the petrotympanic fissure. In the dog, the caudal auricular artery, a branch of the external carotid artery, provides middle ear vascularization (Schummer *et al.*, 1981). A stylomastoid branch of the caudal auricular artery enters the stylomastoid foramen and courses parallel to the facial nerve within the facial canal. This vessel gives off neural, mucosal and muscular branches to the middle ear cavity. Neural branches supply the facial nerve, while mucosal branches supply the tympanic membrane and the epithelial lining of the tympanic cavity (Maher, 1988). Cole (2009) describes a more specific branch of the stylomastoid

artery, the caudal (posterior) tympanic branch, which supplies the fibrous propria (intermediate layer) of the tympanic membrane. The middle meningeal artery arises from the maxillary artery, distal to the rostral tympanic artery. It arises just prior to the course of the maxillary artery through the alar canal. Branches of the middle meningeal artery supply the tensor tympani muscle and structures within the epitympanum.

Inner Ear

The main blood supply to the inner ear in dogs and cats is provided by the vertebral-basilar system (Shambaugh, 1923; Bernstein and Silverstein, 1966). The left and right vertebral arteries unite at the ventral midline at the level of the caudal part of the brainstem to form the basilar artery. The basilar artery provides bilateral branches that supply structures of the cerebellum and pons in its rostral ascent toward its confluence at the arterial circle. Caudal to the pons, the basilar artery gives off bilateral labyrinthine arteries, which course laterally toward the internal acoustic meatus of the petrous temporal bone. The labyrinthine artery can be found between the roots of CN VII and CN VIII before these nerves enter the bony labyrinth at the internal acoustic meatus. The labyrinthine artery supplies terminal branches to the membranous labyrinth of the inner ear.

Innervation of the Ear

External Ear

The external ear is supplied by both cranial and cervical spinal nerves (Evans and Kitchell, 1993). The auricular muscles are supplied by motor branches of CN VII (see Evans and Kitchell, 1993, Fig. 12.19, p. 965). Muscles of the rostroauricular group are innervated by the rostral auricular nerve, a branch of the auriculopalpebral branch of the facial nerve that is located rostral to the ear base. Muscles of the caudal auricular group are innervated by caudal auricular branches that emerge from the trunk of the facial nerve caudal to the ear base. Sensory innervation of the central and caudal concave auricle and vertical ear canal is provided by sensory branches of the facial nerve: the middle internal auricular nerve, the caudal internal auricular nerve and the lateral internal auricular

nerve. The rostral part of the auricle is supplied sensory innervation by the mandibular branch of the trigeminal nerve (CN V). The medial horizontal ear canal is supplied by the auriculotemporal branch of the mandibular nerve of CN V. Cervical innervation of the cutaneous convex surface of the auricle is provided by the great auricular nerve, one of the two branches of the ventral branch of the second cervical nerve (C2).

Middle Ear

Innervation of the mucosal lining of the tympanic cavity is provided by the tympanic plexus; its main source comes from the tympanic branch of the glossopharyngeal nerve (CN IX). The plexus ramifies over the surface of the promontory and supplies innervation to the mucosal linings of the auditory tube and tympanic cavity, including the tympanic membrane and the oval and round window membranes. The innervation of the tensor tympani muscle is provided by a branch of the mandibular nerve of CN V. This branch is closely affiliated with the long tendon of the tensor veli palatini muscle, which lies on the lateral wall of the auditory tube. As the facial nerve passes through the facial canal, it gives off a muscular branch and an autonomic branch. The muscular, or stapedial, branch is given off where the facial canal opens into the cavity of the middle ear near the vestibular (oval) window and supplies the stapedius muscle. The chorda tympani, a branch of CN VII, passes through the middle ear cavity. Although direct innervation to middle ear structures is not provided by the chorda tympani, this nerve is usually described due to its prominent course through the middle ear cavity. The chorda tympani can be seen passing ventrostrally through the tympanic cavity proper, medial to the neck of the malleus, on its course from CN VII to the petrotympanic fissure, which lies just ventral to the attachment of the rostral ligament of the malleus. After emerging from the petrotympanic fissure, it will join the lingual branch of the mandibular nerve to provide sensory innervation to the rostral part of the tongue.

Inner Ear

Innervation of the inner ear was for many years thought to be purely afferent. However, recent evidence has shown that the outer hair cells of the cochlea also have an efferent innervation (Ryugo, 1992). Sensory afferent innervation of the inner ear is provided by CN VIII, which has a vestibular and a cochlear branch. The vestibular branch is formed by the bundle of axonal processes arising from bipolar cell bodies within vestibular ganglia. The dendritic processes of the vestibular bipolar cells are distributed in the sensory epithelium of the cristae ampullares of the semicircular ducts and the maculae of the utricle and saccule. The cochlear branch is formed by a bundle of axonal processes of the spiral ganglia. Both nerve branches unite within the bony vestibule and emerge as the vestibulocochlear nerve from the internal acoustic meatus.

(End of reproduction of Stevens-Sparks 2011)

Skull Structures with Relevance to the Ear

Studies of the relevant bony skull elements, ear structures and adnexa were performed to provide orientation and identification of important reference landmarks in the micro-dissection studies of the blood supply and ligaments of the ear in the chapters that follow. These structures are well-understood and described in numerous anatomy references (Dyce *et al.*, 2010; Evans and deLahunta, 2010; Evans, 1993a) and provided here as background for the reader.

The petrous temporal bone, which houses structures of the inner ear, has several important landmark regions. It is broad caudally and narrows to a pointed apex that is directed medially at its rostral end (Fig. 2.12). The heightened dorsal ridge along the length of the petrous bone is called the petrosal crest. There are three concavities in the petrous bone that are located medial to the petrosal crest. The most caudal and dorsal of these is a fossa that houses the flocculonodular lobe of the cerebellum. Ventral and slightly rostral to the fossa of the flocculonodular lobe is the internal acoustic meatus, through which the vestibulocochlear and facial nerves and the labyrinthine artery pass; the mouth of the meatus is often referred to as the porous acousticus. The most rostral of these

three concavities is a foramen located near the apex of the petrous bone that accommodates the passage of the trigeminal nerve (CN V), which branches into mandibular and maxillary branches as it leaves the foramen at the ventral aspect of the petrous bone. The mandibular branch of CN V passes through the foramen ovale, which is located lateral to the apex of the petrous bone; the maxillary branch of CN V passes through the foramen rotundum, which is rostral and medial to the foramen ovale. The mandibular branch emerges from the foramen ovale medial to the TMJ and immediately gives rise to three branches. From caudal to rostral, these branches are the mylohyoid, inferior alveolar and lingual nerves. These branches are used as important landmarks for orientation throughout this study.

Most of the specimens used throughout this study were prepared for dissection by reducing the heads to manageable blocks of the tympanic region that contained the ear structures, the temporomandibular joint (TMJ) and the proximal mandible. Several blocks were used for preliminary anatomical studies of the ear (Figs. 2.13 – 2.15). Before any dissection, certain structures were identified in the medial view (Fig. 2.13A). The dorsum sellae, a dorsal bony extension of the basisphenoid bone, is the caudal extent of the hypophyseal fossa which houses the pituitary gland. The pons of the metencephalon is located caudal to the dorsum sellae. Cranial nerves V, VII and VIII originate from the lateral brainstem immediately caudal to the pons. The pharyngeal opening of the auditory tube is located ventral to the dorsum sellae, within the nasopharynx. Removal of the brain within this region of study (Fig. 2.13B) reveals several important landmarks: the petrosal crest, the internal acoustic meatus which is occupied by cranial nerves VII and VIII, the flocculonodular lobe fossa and the middle cranial fossa. Access to the cochlea is gained by removing the area of the petrous bone that is located rostral and medial to the internal acoustic meatus and cranial nerves VII and VIII (Fig. 2.13C). The tensor tympani muscle is located ventral to the petrosal crest, rostral to the fossa of the flocculonodular lobe and lateral to the cochlea.

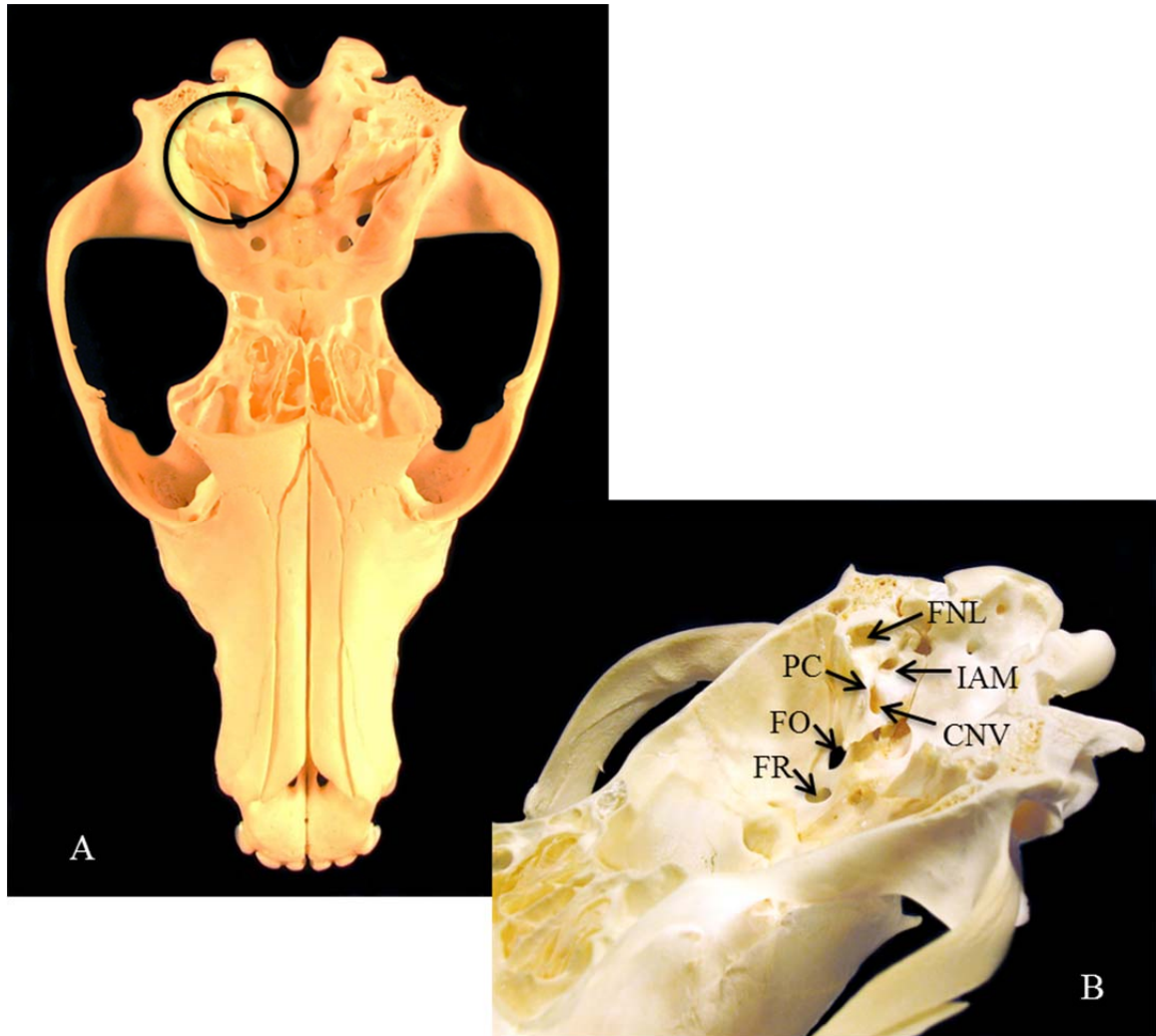


Figure 2.12. (A) Dorsal view of the floor of the cranial cavity. The petrous temporal bone (circle) is broad caudally and narrows to a pointed apex that is directed medially at its rostral end. (B) Medial view of the right cranial cavity, rotated from (A). The petrous temporal bone has several important landmark regions: the foramen of the trigeminal nerve (CN V), the fossa for the flocculonodular lobe (FNL), the foramen ovale (FO), the foramen rotundum (FR), the internal acoustic meatus (IAM), and the petrosal crest (PC).

To see the tympanic annulus and manubrium of the malleus, the ventral and medial portions of the tympanic bulla must be removed and the specimen examined in ventro-medial orientation (Fig. 2.14). The opening of the auditory tube into the middle ear cavity is seen rostral to the tensor tympani muscle (Figs. 2.13 and 2.15). The auditory tube extends from its opening in the nasopharynx, extends caudolaterally through the bony musculotubal canal, and opens into the middle ear cavity just rostral

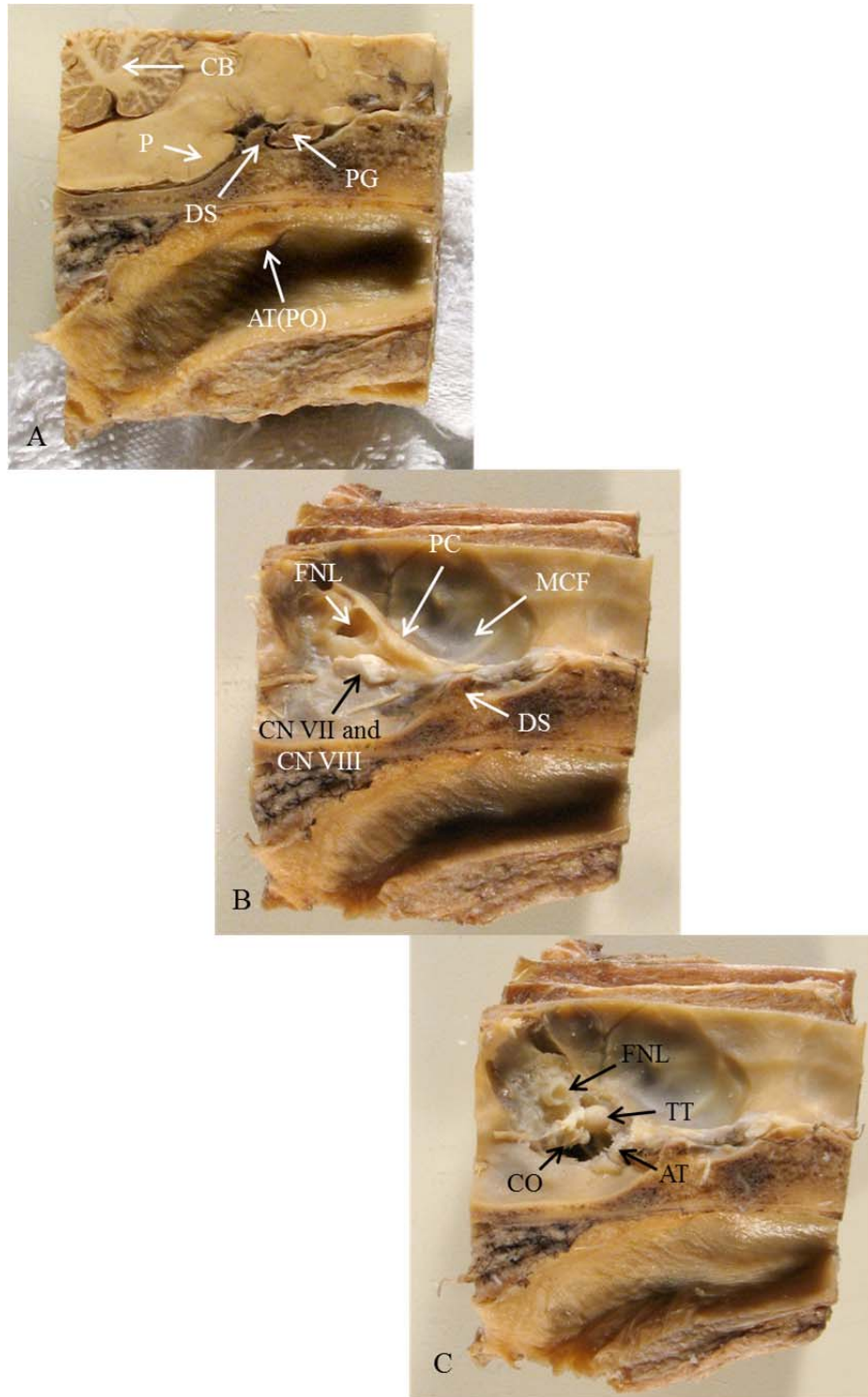


Figure 2.13. (A) Medial view of an intact block of the tympanic region. (B) Medial view showing landmarks within the cranial cavity after removal of the brain. (C) Medial view of dissected block showing structures of the inner and middle ears. For approximate scale, refer to Figs. 2.14 and 2.15. AT = auditory tube, AT(PO) = pharyngeal opening of the auditory tube, CB = cerebellum, CO = cochlea, DS = dorsum sellae, FNL = fossa of the flocculonodular lobe, MCF = middle cranial fossa, P = pons, PC = petrosal crest, PG = pituitary gland, TT = tensor tympani muscle.

to the tensor tympani muscle (Figs. 2.13, 2.15 and 2.16). The musculotubal canal, the canal through which the auditory tube extends, passes dorsal to the muscular process of the tympanic bone (Figs. 2.16 and 2.17). The tendon of the tensor veli palatini muscle lies along the lateral wall of the auditory tube (Fig. 2.15). This muscle originates from the cartilaginous wall of the auditory tube and inserts on the hamulus of the pterygoid bone and the soft palate and aids in the dilation of the pharyngeal orifice of the auditory tube (Evans and Kitchell, 1993).

Subsequent chapters will describe vascular and ligamentous ear structures in the dog in greater detail, building on the anatomy presented in this chapter.

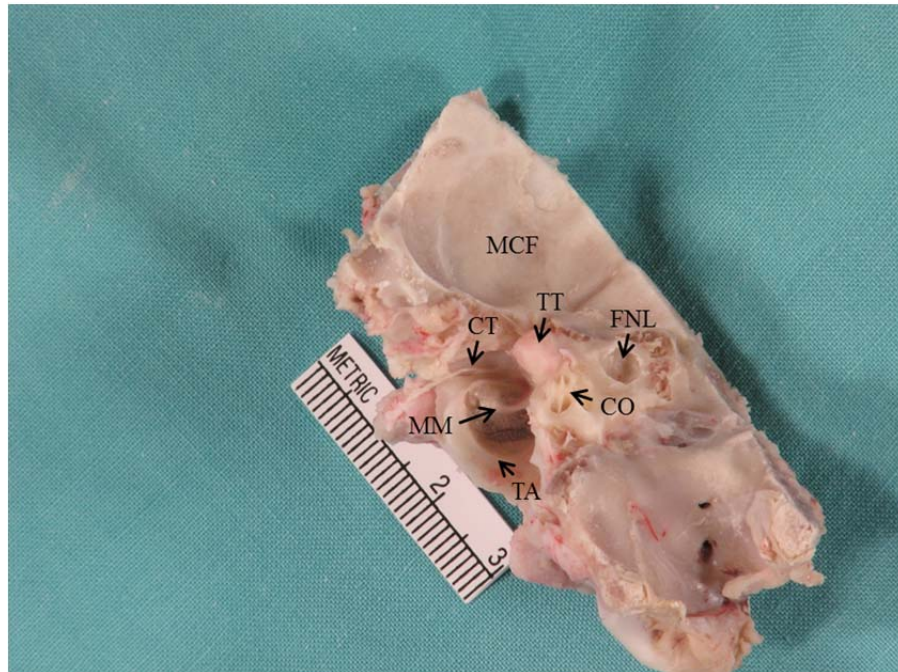


Figure 2.14. Ventro-medial view of a dissected block of the temporal region showing structures of the inner and middle ear. CO = cochlea, CT = chorda tympani nerve, FNL = fossa of the flocculonodular lobe, MCF = middle cranial fossa, MM = manubrium of the malleus, TA = tympanic annulus, TT = tensor tympani muscle.

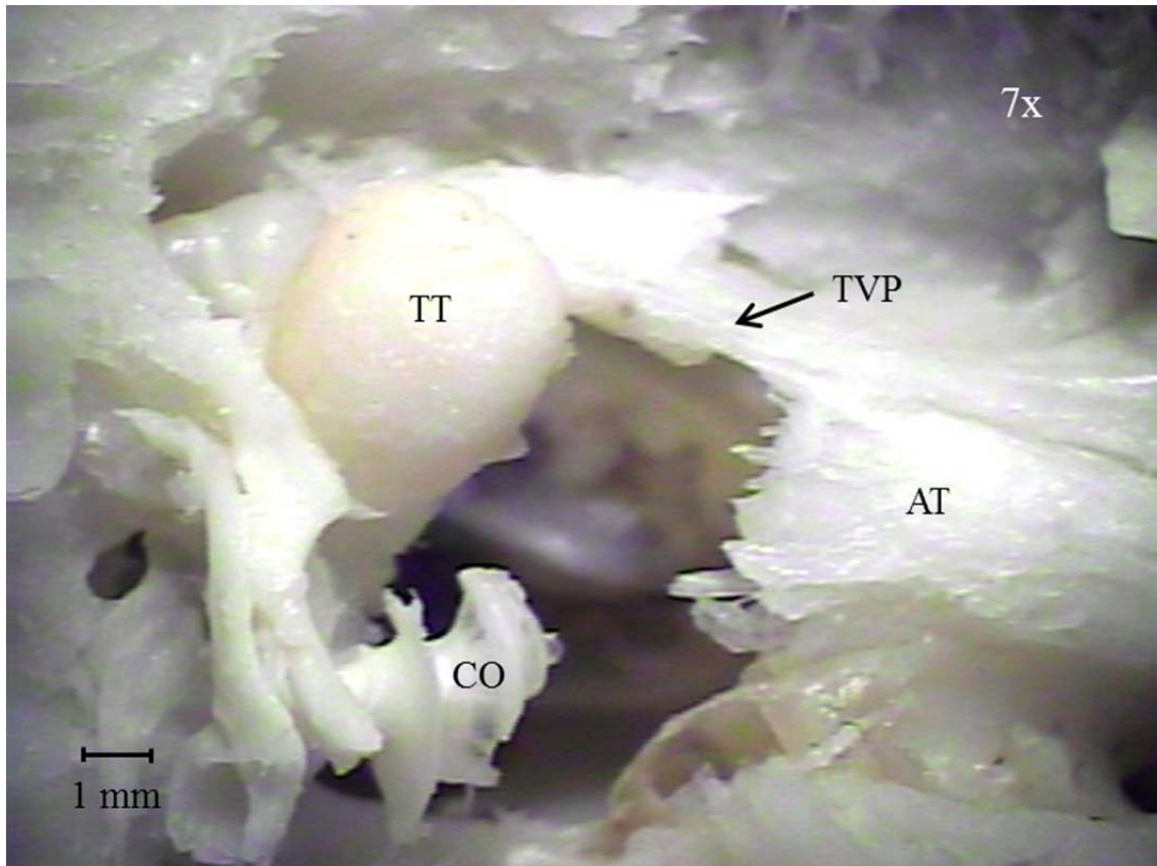


Figure 2.15. Magnified medial view of the dissected inner and middle ear structures. AT = auditory tube, CO = cochlea, TT = tensor tympani muscle, TVP = tendon of the tensor veli palatini muscle.

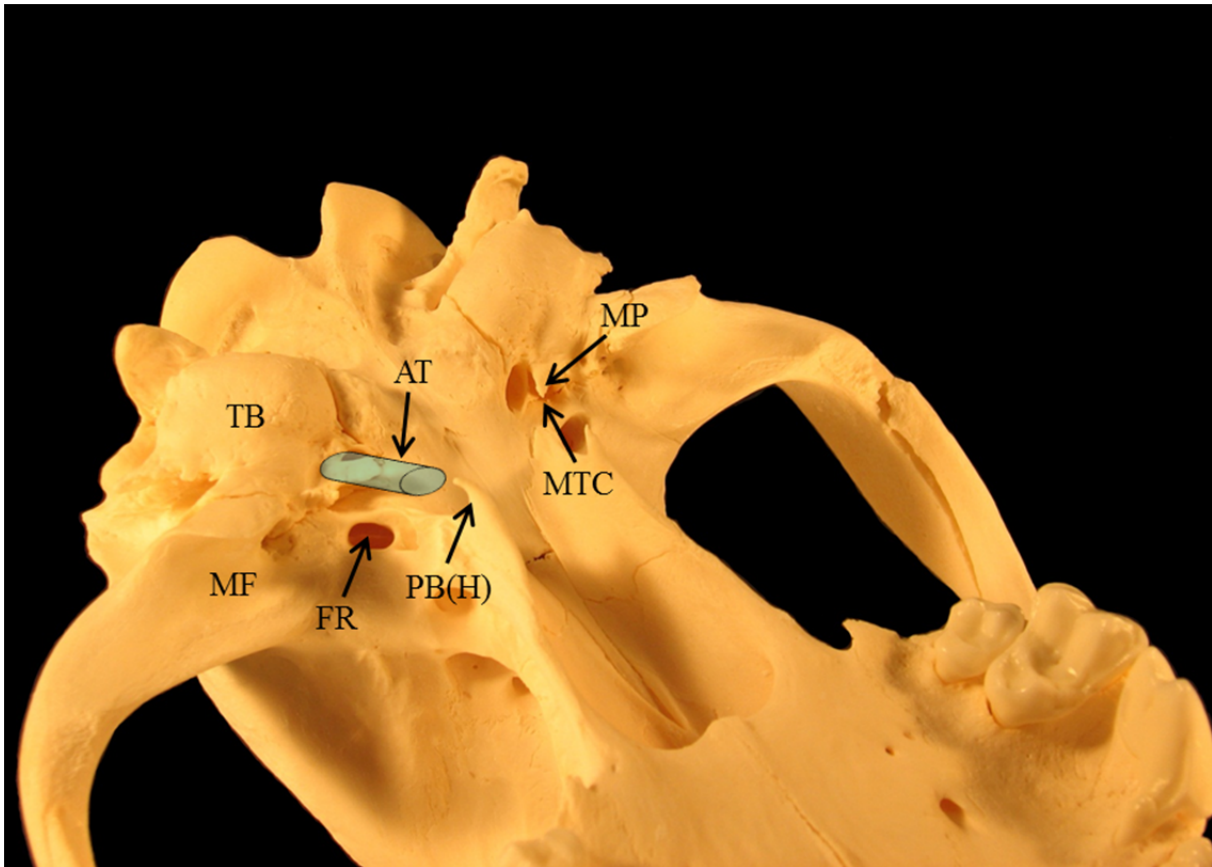


Figure 2.16. Ventral oblique view of the canine skull with the course of the auditory tube (AT) demonstrated by a cylinder drawn where it would appear in an un-macerated specimen. FR = foramen rotundum, MF = mandibular fossa, MP = muscular process of the tympanic temporal bone, MTC = opening of the musculotubal canal, PB(H) = hamulus of the pterygoid bone, TB = tympanic bulla.

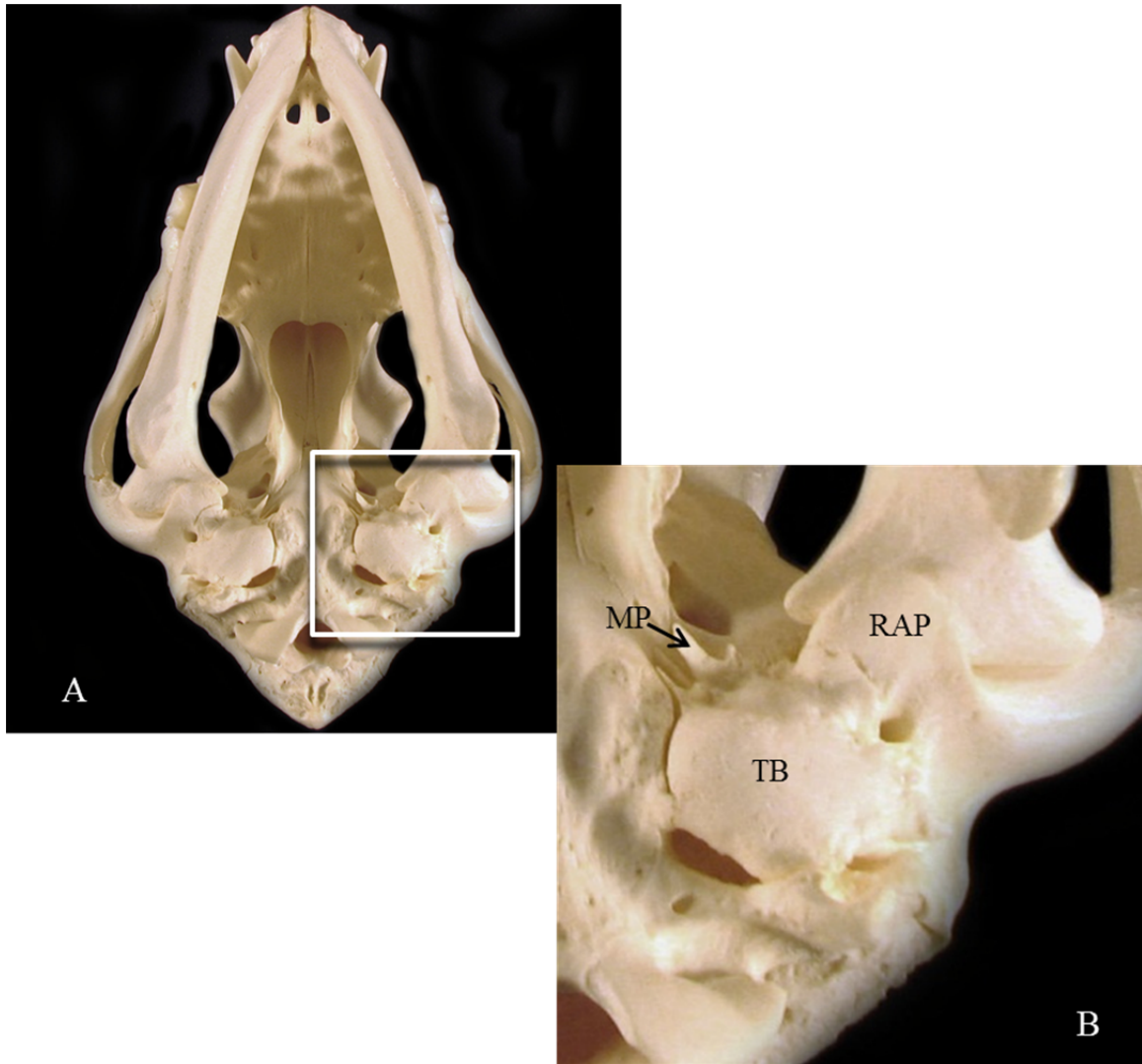


Figure 2.17. (A) Ventral view of the canine skull. (B) Enlarged boxed region in (A). The muscletubal canal is dorsal to the muscular process (MP) of the tympanic temporal bone. RAP = retroarticular process, TB = tympanic bulla.

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CHAPTER 3

POST-ANESTHESIA DEAFNESS IN DOGS AND CATS FOLLOWING DENTAL AND EAR CLEANING PROCEDURES*

Introduction

Deafness in dogs and cats can be hereditary or acquired; causes of acquired hearing loss include drug toxicity, noise trauma, infection, aging, middle ear effusion, excess cerumen production, and physical trauma (Strain 1996, 1999). The causes of acquired deafness are frequently unknown. A correlation between dental and/or ear cleaning procedures under anesthesia and the acute onset of deafness in dogs and cats may exist, based on personal communications with pet owners (Strain GM, unpublished observations), but clear prevalence data are unavailable and causes have not been established in these cases. Pet owners have reported bilateral deafness to be present upon anesthesia recovery from such procedures and the hearing loss was nearly always permanent. It is difficult to establish whether the reported hearing loss is sensorineural or conductive without the brainstem auditory evoked response (BAER) test, which can distinguish between sensorineural hearing loss (using an air-conducted auditory stimulus) and conductive hearing loss (using a bone stimulator) (Strain et al, 1993; Wilson & Mills, 2005). Reports of hearing loss following dental and/or ear cleaning are uncommon, however, so follow-up of these cases with BAER testing has not occurred. Causes of both sensorineural and conductive hearing loss must therefore be considered as possible explanations for post-anesthetic deafness.

The goal of the present study was to document the occurrence of anesthesia-associated deafness with data from three different sources: (1) email and phone communications directed to the author of an established dog and cat deafness web page, (2) case descriptions posted by

* Stevens-Sparks CK, Strain GM. (2010) Post-anesthesia deafness in dogs and cats following dental and ear cleaning procedures. *Veterinary Anaesthesia and Analgesia* 37, 347-351. Reprinted with permission. Supplemental tables not included in the publication (A.1 – A.11) are located in Appendix A.

members on an established veterinary information web page, and (3) a survey of general practice and dental specialist veterinarians. These data would then provide a foundation for mechanistic studies.

Materials and Methods

Information from personal communications to one author (GMS) was compiled for the following variables: dog or cat breed (Table A.1), age, gender (Table A.1), performed procedure (Tables A.2 and A.3), hearing effects, vestibular effects, and recovery of auditory or vestibular function based on owner assessment. Since all reports were received from pet owners, anesthetic information was not available. Case follow up varied from days to several months. Identification that subjects were deaf was made by the owner and/or their veterinarian, without BAER testing.

Queries posted to message boards on the Veterinary Information Network (VIN) website (<http://www.vin.com>) were searched under the topic key words ‘deafness and anesthesia’. Individual case discussions were identified for the period of 2002 to 2009. Information was compiled for dog or cat breed (Table A.1), age, gender (Table A.1), performed procedure (Tables A.2 and A.3), hearing effects, vestibular effects, recovery of auditory or vestibular function, and anesthetic administered (Table A. 4).

A survey was sent to two groups of veterinarians to assess experiences of anesthesia-associated deafness following dental procedures (Table A.6). An email listing of the Louisiana State University (LSU) School of Veterinary Medicine alumni was obtained and the survey was sent as an attachment to 1091 email addresses. A second set of surveys was mailed to members of the American Veterinary Dental College (AVDC), along with a self-addressed, stamped return envelope. The survey asked whether any animals had been deaf upon recovery from a dental procedure in their clinic; if yes, how many times and what species and breed, and the head type of the affected animal.

Results

Between the years 2005 and 2009, there were 24 reported cases (email and phone communications) of deafness following anesthesia (19 dogs, five cats) reported to one author (GMS)

(Fig. A.1). Of these 24 cases, 21 described animals that were deaf, or had a significant decrease in hearing, following dental cleaning, tooth extraction and/or ear cleaning procedures. Of these 21 cases, seven (four dogs, three cats) described deafness following ear cleaning only, or in conjunction with another non-dental procedure (i.e., aural tumor removal, dew claw removal). One dog was reported to be discharged with a neomycin-containing ointment following ear cleaning. Two cats recovered hearing after several days, while the third cat progressed to a comatose state. There were three reports of vestibular signs accompanying the hearing loss (one dog, two cats). All three animals were reported to have recovered vestibular function, but not hearing. The remaining three cases were an anterior cruciate ligament repair, an MRI, and an unspecified procedure; in the latter case hearing recovered.

Thirty-eight discussions of cases involving post-anesthetic hearing loss (23 dogs, 15 cats) were posted on the VIN network between the years 2002 and 2009. Of these 38 cases, 33 occurred following dental treatment, tooth extraction, and/or ear cleaning, with nine (six dogs, three cats) reporting deafness following ear cleaning only, or in combination with a procedure other than dental (i.e., ovariohysterectomy, neuter). The remaining five cases were three spays (two dogs, one cat), a circumanal adenoma excision, and one unspecified procedure. Four of the nine ear cleaning only cases (all dogs) were discharged with ear ointment containing either gentamicin or neomycin following an ear cleaning procedure. None of these nine animals were reported to have recovered hearing. There were nine reports of vestibular signs in combination with hearing loss (two dogs, seven cats). All nine animals were reported to have recovered vestibular function, but not hearing. Twenty-six out of 42 reported dog deafness cases were dental-related and 13 out of 20 reported cat deafness cases were dental-related (personal communication and VIN data combined); however, there was no apparent relationship between a specific dental procedure performed under anesthesia and hearing impairment.

The types of anesthetic/sedative drugs used, many in combination, were cited in 28 out of 38 VIN postings; these included neuroleptics (4), opioids (12), benzodiazepines (10), α_2 agonists (3), dissociatives (15), thiopental (1), propofol (4), and inhalants (23) (Table A.4). There was no apparent relationship between anesthetic drug and hearing impairment.

There was no apparent relationship between dog breed or dog breed size and hearing impairment following dental and/or ear cleaning procedures. Affected breeds included Bichon Frise, Boston Terrier, Brussels Griffon, Cairn Terrier, Chihuahua, Chow Chow, Cocker Spaniel (3), Collie, Dachshund, German Shepherd (6), Golden Retriever, Jack Russell Terrier, Labrador Retriever (2), Lhasa Apso, Maltese (2), mixed breed (8), Pit Bull Terrier, Poodle (4), Rat Terrier, Schnauzer, Scottish Terrier, Sheltie (4), and Staffordshire Terrier (2) (Table A.1). Reported cat breeds included Abyssinian, Himalayan, Siamese (2), and domestic short hair (8). No cat breed association could be drawn due to the small numbers of subjects per named breed. Gender did not appear to be a factor in reported cases of deafness in dogs or cats. Of the 42 canine cases, 35 reported the gender (21 male, 14 female) (Table A.1); of the 20 feline cases, 18 reported the gender (nine male, nine female).

Thirty-seven dog cases provided age information (10.0 ± 3.8 years, mean \pm SD), and 16 cat cases provided age information (7.4 ± 3.4 years). Dogs are considered geriatric based on a combination of age and typical breed body weight established from the age when dogs typically begin to have age-related diseases (Goldston, 1989): small dogs (0 – 9 kg) at 11 ± 2 years, medium dogs (10 – 23 kg) at 11 ± 2 years, large dogs (24 – 41 kg) at 9 ± 1 years, and giant dogs (>41 kg) at 7 ± 2 years (Table A.5). Cats are considered geriatric at 12 ± 2 years regardless of size. There did appear to be a trend in age and occurrence of post-anesthetic, post-procedural hearing loss in both dogs and cats. Using the above classifications, 76% (28 of 37) of dogs with reported post-anesthesia hearing loss were geriatric (Fig. 1, top, ages 9 – 14). Forty-four percent (7 of 16) of cats with reported post-anesthesia hearing loss were geriatric (Fig. 1, bottom, ages 10 – 11).

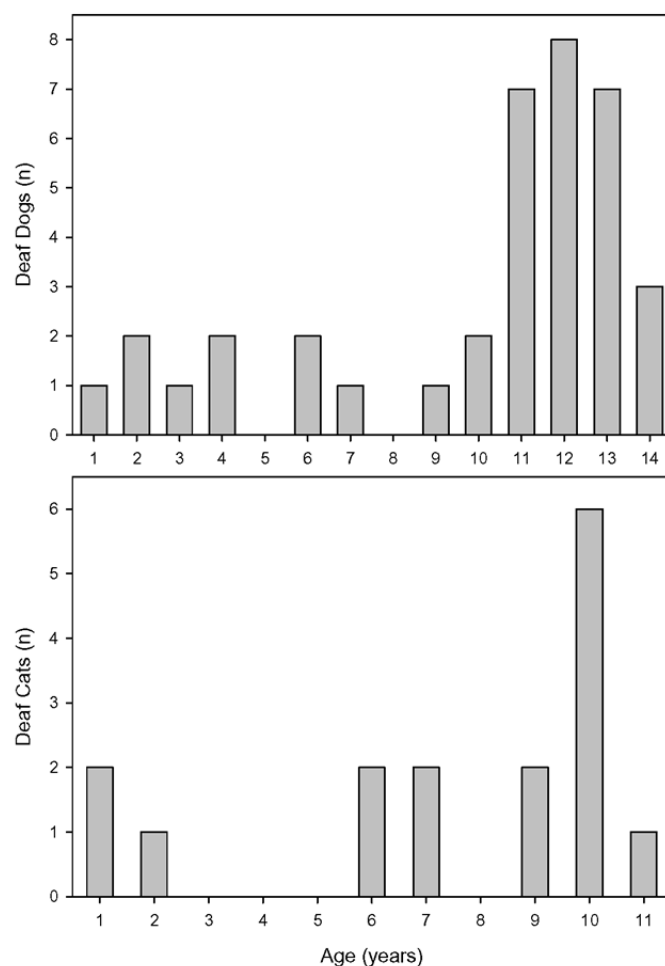


Figure 3.1. Numbers of dogs (top) and cats (bottom) reported with deafness following anesthesia as a function of age, based on email and VIN data. Twenty-eight of 37 deaf dogs (9-14 years) and 7 of 16 deaf cats (10-11 years) were considered to be geriatric.

Fifty-seven out of 1091 LSU alumni contacts (5.2%) completed and returned surveys, excluding responses stating that the survey did not apply. Of the 84 survey mailings sent to members of the AVDC, 44 completed surveys were returned (52.4%). Data obtained from the survey (see Table A.6) is summarized in Tables A.7 – A.11. Three out of 44 AVDC members (6.8%) reported having had animals (all dogs) that were deaf upon recovery from a dental procedure in their clinic. Each respondent reported this to be a one-time occurrence. Only one out of 57 LSU veterinary alumni (1.8%) reported that deafness had occurred following dental procedures performed in their

clinic (two dog cases). All five dogs affected were of the mesocephalic head type. Ten AVDC members (22.7%) reported having heard of cases of deafness in animals following routine dental procedures, whereas only one LSU alumnus reported the same.

Discussion

A link between dental procedures under general anesthesia and bilateral hearing loss in the dog and cat has not been previously documented to the authors' knowledge. Although the reported occurrence of anesthesia-related deafness is uncommon, there were 39 cases of bilateral hearing loss subsequent to a dental procedure (dogs and cats), either alone or in combination with another procedure, reported over the past seven years (May 2002 through March, 2009, personal communications and VIN combined) (Tables A.2 and A.3). Five additional cases of deafness following a dental procedure were documented by the current survey, but with limited detail being provided.

Cases of bilateral hearing loss were also reported in animals that had undergone other non-dental procedures while under anesthesia. There were 16 reported cases of hearing loss following ear cleaning (dogs and cats), or ear cleaning in combination with another non-dental procedure, and six cases of hearing loss following a non-dental, non-ear procedure under anesthesia (Tables A.2 and A.3). The procedure was not specified for one reported deafness case.

To our knowledge, none of the reported cases of hearing loss following a dental procedure resulted in a later recovery of hearing; however, two cases of hearing loss recovery following ear cleaning were reported in cats, possibly reflecting a mechanism of conduction deafness due to edema or fluid in the ear canal or middle ear. Five animals were discharged with ear ointment containing either gentamicin or neomycin following an ear procedure. Gentamicin- and neomycin-based ear ointments are known to cause hearing loss in both dogs and cats (Strain, 1996). Ototoxicity cannot be excluded as a cause of the deafness in these cases, but aminoglycoside ototoxicity usually requires prolonged exposure which did not appear to be the case with these animals. Because unilateral

deafness is not behaviorally obvious without BAER testing, the numbers of affected animals is potentially much larger than the levels reported here since unilateral deafness in dogs or cats usually goes unnoticed by owners.

No relationship was observed between hearing impairment following dental or ear cleaning procedures and species (dog, cat), breed, gender, size of dogs, or anesthetic drug use. There did appear to be a relationship between age and hearing loss in both dogs and cats. Older animals appeared more susceptible to deafness following anesthesia during dental procedures. It is possible that these older animals had undetected preexisting hearing impairment before anesthesia from causes such as ageing (presbycusis), otitis, noise trauma, or ototoxicity. Since it is more typical for dogs and cats to first undergo dental procedures in their adult and later years, this apparent age effect may also reflect a sampling bias in the data. A larger sample size from a population of animals, whose age and health was known, would be required to clarify this question. Nevertheless, older dogs and cats may be at greater risk for this deafness.

The blood supply to the cochlea is the labyrinthine artery, which derives from the vertebral artery via the middle cerebellar artery and the basilar artery (Anderson & Anderson, 1994). Review of this anatomy in relation to jaw structures did not indicate any apparent mechanism by which jaw articulation might mechanically compromise the cochlear blood supply, but there might be individual variation in the location of these vessels that could be affected by jaw opening. Shunting of blood flow from peripheral tissues during systemic hypoxia is known to occur as a mechanism to protect the brain and heart; however, if hypoxia of this extent had been responsible for the deafness, it is quite likely that other signs of the hypoxia would have been observed by the practitioner, yet none were reported.

In conclusion, deafness following anesthesia for dental and ear cleaning procedures is described in dogs and cats. Prevalence was low, but may be greater than indicated by the present study. Most subjects did not experience recovery of auditory function, but vestibular dysfunction

resolved. Older animals may be more subject to this outcome, but animals of all ages can be affected. Further studies will be required to identify the mechanism or mechanisms behind this condition, which may include anoxia or mechanical disruption.

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CHAPTER 4

THE BLOOD SUPPLY OF THE CANINE EAR: NEW FINDINGS OF VASCULAR BRANCHING PATTERNS

Introduction

A correlation between dental procedures performed under anesthesia and the acute onset of deafness in dogs and cats has been identified recently (Stevens-Sparks and Strain, 2010). One possible explanation is compromised blood flow to ear structures. A potential link exists between arterial occlusion and manipulation of the temporomandibular joint (TMJ)/mandible and/or use of anesthesia. Disturbance of the vascular supply to the inner ear can lead to cochlear ischemia, resulting in sudden sensorineural deafness. Disruption of the blood supply to the middle ear could affect such structures as the tympanic membrane, ear ossicles, muscles of the middle ear, and the auditory tube, resulting in conduction deafness. It is well established that the labyrinthine artery provides circulation to the inner ear; occlusion of this vessel in the dog results in acute-onset deafness (Alford, 1965). Branches of the maxillary artery supplying structures of the canine middle ear have been cited in anatomy texts (Evans, 1993; Ghoshal, 1975), but have not been thoroughly described. The rostral tympanic artery has been shown to be an important source of blood supply to the middle ear in the cat (Davis and Story, 1943); however, it is described as an inconstant branch of the canine maxillary artery (Evans, 1993; Ghoshal, 1975). One of the main focuses of this study was to determine the consistency of the rostral tympanic artery as well as its origin and distribution in the dog. Additionally, the vascularization of the inner ear by way of the labyrinthine artery was confirmed. During the course of this study, variations in arterial branching patterns of branches originating from the external carotid and maxillary arteries were observed.

The main blood supply to the inner ear in dogs and cats is provided by the vertebral-basilar arterial system (Shambaugh, 1923; Bernstein and Silverstein, 1966). The basilar artery extends rostrally from an arterial anastomosis located at the level of the caudal part of the brainstem (Fig.

4.1). This arterial anastomosis (circular in shape) is formed when the left and right vertebral arteries unite with the ventral spinal artery at the ventral midline, just before the brainstem emerges from the foramen magnum of the occipital bone to become continuous with the spinal cord. The basilar artery provides bilateral branches that supply structures of the cerebellum and pons in its rostral ascent toward its confluence at the arterial circle with the internal carotid artery. This more rostral arterial circle is located at the base of the diencephalon and encircles the pituitary gland.

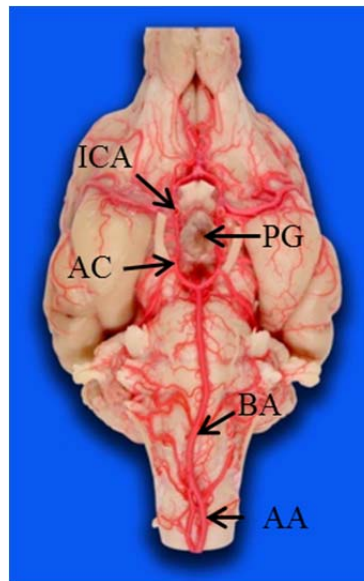


Figure 4.1. Ventral view of the latex-injected vasculature of a canine brain. The basilar artery (BA) extends rostrally from an arterial anastomosis (AA) located at the level of the caudal part of the brainstem and contributes to the arterial circle (AC) that surrounds the pituitary gland (PG). ICA = internal carotid artery.

According to the *Nomina Anatomica Veterinaria* (2005), the labyrinthine artery (*A. labyrinthi*) is a direct branch of the basilar artery. This vessel extends rostrolaterally from the basilar artery and gives off small branches at the internal acoustic meatus that supply structures of the membranous labyrinth. Several authors do not believe that the labyrinthine artery is a direct branch of the basilar artery, but rather describe the labyrinthine artery as the specific branch that supplies the membranous labyrinth. Anderson and Kubicek (1971) discuss the canine labyrinthine artery as a branch of the anterior inferior cerebellar artery (AICA): “It appears logical that the term labyrinthine

should be reserved for the artery or arteries coursing to the membranous labyrinth and the term anterior inferior cerebellar be used for the parent artery which originates from the basilar .” Although other authors agree with Anderson and Kubicek (1971) that the term labyrinthine artery should be reserved specifically for the vessel, or vessels, supplying the membranous labyrinth, there is a discrepancy concerning the nomenclature of the parent artery across vertebrates. Some describe the canine labyrinthine artery as a branch of the middle cerebellar artery (Anderson & Anderson, 1994; Ghoshal, 1975); while the human parent vessel of the labyrinthine artery is the anterior inferior cerebellar artery (Sunderland, 1945); and the labyrinthine artery is a branch of the anterior cerebellar artery in the cat (Bernstein & Silverstein, 1966).

Shambaugh (1923) describes the labyrinthine artery in both the human and the dog as entering the labyrinth through the internal acoustic meatus, along with CN VII and CN VIII. Shambaugh never states specifically that the labyrinthine artery supplies the cochlea, but rather gives a thorough description of supply to the vestibule and semicircular canals. There was difficulty confirming the functional blood supply of the cochlea due to a great deal of variation in the nomenclature. Anderson and Kubicek (1971) noted that “The AICA in the dog has been referred to as the acoustic, internal auditory, labyrinthine and anterior cerebellar artery.” Using this expanded list of terms to search the literature returned Alford *et al.* (1965) that discusses the effects of microembolism of the internal auditory artery using dogs as a study model. The internal auditory artery, as described in this paper, is synonymous with the labyrinthine artery and is a direct branch of the basilar artery. It is concluded that microscopic intravascular occlusions of the terminal branches of the internal auditory artery (a.k.a. labyrinthine artery) produced physiologic and histopathologic alterations in the cochlear and vestibular systems in the canine. Alford *et al.* (1965) confirmed that the functional blood supply to the canine cochlea has been described, although controversy remains concerning the labyrinthine artery as being a direct branch of the basilar artery. The current author

accepts the NAV description of the labyrinthine artery as a direct branch of the basilar artery and agrees that this vessel represents the functional blood supply to the canine inner ear, as demonstrated by Alford *et al.* (1965).

The canine middle ear blood supply has not been thoroughly described and there are some ambiguities in its description among authors and between species. The most thorough description of middle ear blood supply, in domesticated animals, was provided by Davis and Story (1943) while investigating the carotid circulation in cats. According to these authors the middle ear of the cat receives its arterial supply from branches of the ascending pharyngeal, occipital and maxillary arteries. The ascending pharyngeal and occipital arteries are the first two branches of the external carotid artery and the maxillary artery is one of the two terminal branches of the external carotid artery. The ascending pharyngeal artery provides a Eustachian branch (*Ramus eustachii*) that supplies the auditory tube and accompanies it through the musculotubal canal of the temporal bone into the lateral chamber of the bulla. The vessel then ramifies over the surface of the promontory, where it anastomoses with the rostral and caudal tympanic arteries. The caudal (inferior) tympanic artery, a branch of the occipital artery, enters the skull at the foramen lacerum, where it immediately branches. The most caudal of the two branches gives off several small arteries that either ramify within the mucosa covering the promontory or ramify over the caudal part of the dorsal surface of the bulla. According to Evans (1993), the condyloid artery of the canine is synonymous with the caudal (inferior) tympanic artery described by Davis and Story (1943). The condyloid artery enters the petro-occipital fissure and passes through the petrobasilar canal, accompanied by the accessory nerve, where it dissipates in the dura at the ventral end of the pyramid of the medulla oblongata. In its course it supplies small branches to the middle and inner ear. Ghoshal (1975) and Schummer *et al.* (1981) state that the canine caudal tympanic artery (*a. tympanica caudalis*) is a branch of the occipital artery-derived caudal meningeal artery. In the cat, the deep auricular (*a. auricularis*

profunda) and rostral (anterior) tympanic (*a. tympanica anterior* or *a. tympanica rostralis*) arteries are described as direct branches of the maxillary artery. In the dog, the deep auricular artery is not a direct branch of the maxillary artery, but is a branch of the caudal auricular artery, and the rostral auricular artery is often described as an inconstant branch. According to Cole (2009), the canine deep auricular artery supplies the periosteum of the malleus manubrium, as well as, the tympanic membrane. In addition to the deep auricular branch, the canine caudal auricular artery supplies a stylomastoid branch that enters the stylomastoid foramen and courses parallel to the facial nerve within the facial canal (Schummer *et al.*, 1981). This vessel gives off neural, mucosal and muscular branches to the middle ear cavity. Neural branches supply the facial nerve, while mucosal branches supply the tympanic membrane and the epithelial lining of the tympanic cavity (Maher, 1988). Cole (2009) describes a more specific branch of the canine stylomastoid artery, the caudal (posterior) tympanic branch, which supplies the fibrous propria (intermediate layer) of the tympanic membrane. The rostral (anterior) tympanic artery is a constant branch of the feline maxillary artery and is an important supply to the middle ear. However, in the dog, the rostral tympanic artery is described as an inconstant vessel that branches from the maxillary artery rostral and medial to the retroarticular process of the temporal bone and opposite the caudal deep temporal artery (Ghoshal, 1975; Evans, 1993). According to Evans (1993), this vessel may branch from the origin of the caudal deep temporal artery, as an alternative to being a direct branch of the maxillary artery. When present, this vessel extends caudally from its origin and enters the middle ear cavity via a small foramen medial to the temporomandibular joint, near the petrotympanic fissure. It is this vessel that is of specific interest to us due to its close relationship to the temporomandibular joint. The middle meningeal artery arises from the maxillary artery, distal to the rostral tympanic artery. It arises just prior to the course of the maxillary artery through the alar canal. Branches of the middle meningeal artery supply the tensor tympani muscle and structures within the epitympanum.

A review of the blood supply to the canine inner and middle ear must precede any experiments to determine a link between vascular occlusion and deafness onset. The vasculature that approximates, or comes near, the temporomandibular joint (TMJ) is of specific interest in this study due to the fact that jaw manipulation is one of the variables that must be considered relative to dental procedures that may result in deafness. In a review of the literature on canine blood supply, the only vessel that is located in the vicinity of the TMJ, which has been described as having a role in middle ear vascularization, is the rostral tympanic artery. There is not much information on this vessel in the dog; other than its description as an inconstant branch of the maxillary artery that branches opposite the caudal deep temporal artery (Ghoshal, 1975; Evans, 1993). The goals of this study are to confirm the labyrinthine artery as the main blood supply to the canine inner ear and to identify juxta-articular branches of the maxillary artery as sources of middle ear blood supply.

Materials and Methods

Dissection Studies

Embalmed specimens dissected for this study (n=15) included: latex-injected adult canine ears (n=5), latex-injected and decalcified adult canine ears (n=5), Microfil-injected and decalcified adult canine ear (n=1), India ink-injected adult canine ears (n=2), and India ink-injected two-month-old puppy ears (n=2). Most specimens were prepared for dissection by reducing the heads to manageable blocks of the tympanic region that contained the ear structures, the temporomandibular joint (TMJ) and the proximal mandible (see Chapter 2, Fig. 2.13). Specimens were decalcified by submerging in either Cal-Ex (Fisher Scientific, Pittsburgh, PA) (n=2) for a minimum of 14 days, or RDO rapid decalcifying agent (Apex Engineering Products, Aurora, IL) (n=4) for 9 days. Following decalcification, acid in the decalcifying agent was neutralized by placing the blocks into a saturated solution of sodium bicarbonate and agitated. The blocks were maintained in a fresh solution of

saturated sodium bicarbonate overnight. The blocks were then flushed under running water for several hours and then transferred to a 4% formaldehyde solution for storage.

Most specimens were mongrel dog heads that were obtained from one of five sources.

- Six specimens were obtained from dogs purchased from Sergeant's Wholesale Biological (Bakersfield, CA).
- One dog, an eleven-year-old Schnauzer with cancer, was donated for this study following euthanasia.
- The two-month-old puppy (CRL = 48) was a Labrador mix obtained from the East Baton Rouge Animal Control facility following euthanasia.
- Two dogs were obtained from the hound colony at the LSU School of Veterinary Medicine.
- Two dogs were obtained following a terminal surgery lab.

The study was approved by the LSU Institutional Animal Care and Use Committee. The specimens purchased from Sergeant's Wholesale Biological were pre-embalmed and latex-injected dogs purchased for dissection by first year veterinary students. The donated Schnauzer and the two-month-old puppy were embalmed within 45 minutes of euthanasia. The common carotid arteries were located and cannulated (#10 cannula, adult; #12 cannula, puppy) toward the head (cranially), and the caudal portion of the arteries was ligated. The adult (donated Schnauzer) head was perfused with approximately 4 L of 10% buffered neutral formalin and the puppy head was perfused with approximately 3 L of a 4% formaldehyde solution at a constant pressure (4 psi). The puppy head was removed following perfusion and stored in a formaldehyde solution for later dissection. The adult head was injected simultaneously in both cannulated carotid arteries with approximately 20 cc of India ink (10 cc per side) before removing the head from the body. The head was submerged in a formaldehyde soak solution overnight. The skull cap and brain of the adult head were carefully reflected to observe the contents of the internal acoustic meatus. The puppy head was later injected

with ink using a 14 gauge needle in the left common carotid artery stump. Approximately 30 cc of ink was injected while the specimen was submerged under water. The head was then immersed in a formaldehyde solution overnight and later cut with a band saw into two blocks that contained the ear structures. Both blocks were stored in a formaldehyde solution for later dissection.

The two dogs (approximately 16 kg each) obtained from the LSU SVM hound colony were heparinized (200U/kg, IV) thirty minutes prior to anesthesia administration (1mg/kg acepromazine (IM), 1 mg/kg small animal xylazine (IV), 12 mg/kg ketamine (IV)). The left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated caudally. The heads were perfused at constant pressure (4 psi) with a 0.9% saline solution (9 L) until fluids from the opened cranial vena cava ran clear. The heads were removed with cannulae still in place and submerged in an ice bath for 2.5 hours before freezing. The specimens were later thawed and prepared for perfusion and injection. One head was perfused at constant pressure (4 psi) with 10% buffered neutral formalin (BNF). Following perfusion, the head was placed neck down into a solution of 10% BNF and stored under refrigeration for 10 days. The vasculature was injected via the left and right common carotid arteries with 20 ml of 1% ammonium hydroxide to neutralize the acidity of the formaldehyde in preparation for latex injection. Two 35 cc syringes were filled with filtered red latex (Carolina Biological, Burlington, NC) and the latex was slowly injected into the common carotid arteries. Cheesecloth soaked in acetic acid was placed into the vertebral venous sinus and spinal central canal to minimize latex seepage. The head was then stored in a formaldehyde solution for later dissection. The unfixed vasculature of the second head was injected with Microfil (Flow Tech, Inc., Carver, MA), a radio-opaque material. Two 35 cc syringes were used to simultaneously inject the prepared media into both common carotid arteries. A total of 210 cc of Microfil was injected into the head vasculature. Following x-ray and CT scanning, the head was sectioned into blocks and

placed into 10% BNF for fixation. The block containing the right ear structures and proximal jaw was decalcified with Cal-Ex for three months prior to dissection.

The two dogs that were obtained following the terminal surgery lab were heparinized (200U/kg, IV) before euthanasia. The left and right common carotid arteries were cannulated (#10) cranially and tied off caudally before perfusion with a 1% saline solution (10 L/dog). The heads were then perfused with 4% formaldehyde. The heads were removed from the bodies, sagittally sectioned, and placed in a formaldehyde soak solution. Two of the halves were cut down into small blocks that contained the temporal region. The two temporal blocks were used for the injection of latex into the maxillary artery to target the filling of the small vessels that originated from the maxillary artery adjacent to the TMJ. The specimens were dissected using a medial approach to visualize the maxillary artery. The maxillary artery was cannulated distal to the origin of the facial artery and was ligated just before its entrance into the caudal alar foramen before injection with filtered red latex. One half head was used to cannulate and latex-inject the external carotid artery at its origin. In this specimen, the maxillary artery was ligated immediately proximal to the caudal alar foramen to maintain the pressure and enhance vascular filling of the maxillary artery and its branches in the region adjacent to the jaw articulation.

Injected specimens were dissected under a Stereomaster Digital Zoom dissecting microscope (Fisher Scientific, Pittsburgh, PA) so that minute branches of the maxillary artery could be identified and traced. The maxillary artery was approached medially following removal of the tongue, pharynx, and pterygoid muscles. Of special interest were the branches of the maxillary artery that were in close proximity to the temporomandibular joint. The inferior alveolar and caudal deep temporal arteries were used as landmark vessels to describe the origin and orientation of other small arterial branches in this region. A rotary drill tool with ball end miniature carbide bur attachment and bone rongeurs of various sizes were used to track the vessels through the tympanic bone in the calcified

specimens. In the decalcified specimens, dissecting forceps were used to scrape through and pick away portions of the tympanic bone to follow the course of the arteries. Photographs were taken to document these dissections using the Stereomaster Digital Zoom dissecting microscope.

Corrosion Casting Studies

A dog (approximately 16 kg) from the LSU SVM hound colony was anesthetized (1 mg/kg acepromazine (IM), 1 mg/kg small animal xylazine (IV), 12 mg/kg ketamine (IV)) and the left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated caudally. The head was perfused with a 0.9% saline solution (9 L) until fluids from the opened cranial vena cava ran clear. The head was then removed with cannulae still in place and submerged in an ice bath for 2.5 hours before freezing. The specimen was thawed for vascular injection with Batson's #17 (Polysciences, Inc., Warrington, PA). A freshly prepared Batson's #17 working solution consisted of 200 ml base, 24 ml catalyst, 24 drops of promoter and red color pigment (5 mg) for clear observation of the vessel casts. All of the prepared media was injected simultaneously into both common carotid arteries under a constant pressure (5 psi) while the specimen was submerged in an ice bath. Both jugular veins were ligated and paper towels were used to block seepage from the vertebral venous sinus, the transverse vertebral canals and the spinal central canal. The specimen was left in an ice water bath until complete polymerization had occurred (3 hrs.). The specimen was then transferred into a container and frozen in water. A band saw was used to cut the specimen while still embedded in ice to prevent tearing of the corrosion-casted vasculature. The head was sagittally sectioned and then two axial cuts were made in each half, one immediately caudal to the orbit and the other immediately caudal to the nuchal crest, to generate a block that contained the entire temporal bone. The two specimens were then macerated (decomposed) in water for one month. The specimens were next flushed in running water for two days. The left block was further macerated in a 1% potassium hydroxide solution for 2 days. The right block was further macerated in water for two weeks,

followed by maceration with 5% potassium hydroxide for 2 days. Photographs were taken to document these dissections using a Powershot S3IS digital camera (Canon USA, Lake Success, NY). Images were taken of the finer vasculature at higher magnification using the Stereomaster Digital Zoom dissecting microscope.

Imaging Studies

The Microfil-injected specimen was used for several imaging studies. The head underwent x-ray and computed tomography (CT) imaging at the LSU SVM Veterinary Teaching Hospital. Head orientation for x-rays was from dorsal-ventral, ventral-dorsal and lateral-lateral. The head was then sagittally sectioned and imaged by CT (GE Lightspeed 16-Slice CT, Waukesha, WI). The temporal region was imaged in 2 mm thick sections. The head was then sent to the Utah School of Medicine for higher resolution CT scans of the same regions at a 1 mm section thickness with 0.4 mm overlap (Somatome Sensation 64, Siemens Medical Solutions, Forchheim, Germany). The head was then cut into blocks of approximately 6.5 cm x 5 cm x 4 cm that contained the temporal region. The right block was put into Cal-Ex for decalcification and used for dissection. The entire left block was cut into 30 cubes (1 cm x 1 cm x 4 cm), with the long dimension being from dorsal to ventral. One block containing the tympanic annulus, malleus and tympanic membrane was imaged at the LSU Center for Advanced Microstructures and Devices (CAMD) facility using a Skyscan-1074 Portable x-ray microtomograph system (Micro Photonics, Inc., Kontich, Belgium) interfaced to a desktop computer for image acquisition. Maximum resolution was 0.037 mm section thickness. The scanner was limited to a 1 cm x 1 cm x 2 cm sample size, requiring that the block be scanned with the dorsal side facing up and then with the ventral side facing up to acquire images for the entire block. Scanned CT images were viewed and reconstructed in three dimensions using OsiriX imaging software (Pixmeo, Geneva, Switzerland).

Results

Dissection Studies

The complete course of the labyrinthine artery was difficult to visualize in gross dissection due to its small size (0.4 mm) and incomplete filling with injection media. Additionally, the small vessels that approximated the temporomandibular joint capsule were difficult to trace in their entirety, particularly when they penetrated and then coursed within the bone. The results of this study are therefore limited to the observed origins of these vessels and no definitive conclusions can be made concerning their complete distributions. It should be noted that if a blood vessel was observed in some, but not all specimens, it did not necessarily reflect a variable presence, but may have been due to variation in vascular filling between specimens. The specimen blocks that were point-injected with latex could not be utilized due to latex seepage into the surrounding tissues from vascular damage done during dissection to the target region of injection. The specimens that were injected with India ink were difficult to dissect for two reasons: the ink did not remain in the vessels because of its high fluidity, and the specimens could not be subjected to decalcification because submerging them in any media resulted in further loss of the ink.

The bilateral labyrinthine arteries were observed originating from the basilar artery on latex-injected (Fig. 4.2) and ink-injected (Fig. 4.3) brains. After originating from the basilar artery at the level of the mid-medulla, the labyrinthine artery coursed craniolaterally toward the roots of the facial (CN VII) and vestibulocochlear (CN VIII) nerves. The artery entered the internal acoustic meatus along with CN VII and CN VIII (Fig. 4.3B), but its distribution to the membranous labyrinth of the inner ear could not be confirmed due to loss of injected media.

The rostral tympanic artery, described by Evans (1993) as an inconstant vessel that branches from the maxillary artery opposite the caudal deep temporal artery, was not observed in any of the dissected specimens. However, a branch that originated from the caudal deep temporal artery was

clearly observed in 4 of 15 specimens. This small branch of the caudal deep temporal artery coursed dorsally, parallel to the maxillary artery and lingual nerve, and was crossed medially by the chorda tympani nerve (Fig. 4.4) and supplied the proximal part of the auditory tube (Fig. 4.5). In two specimens, this auditory tube branch contributed a very fine branch to the chorda tympani nerve (Figs. 4.6 and 4.7).

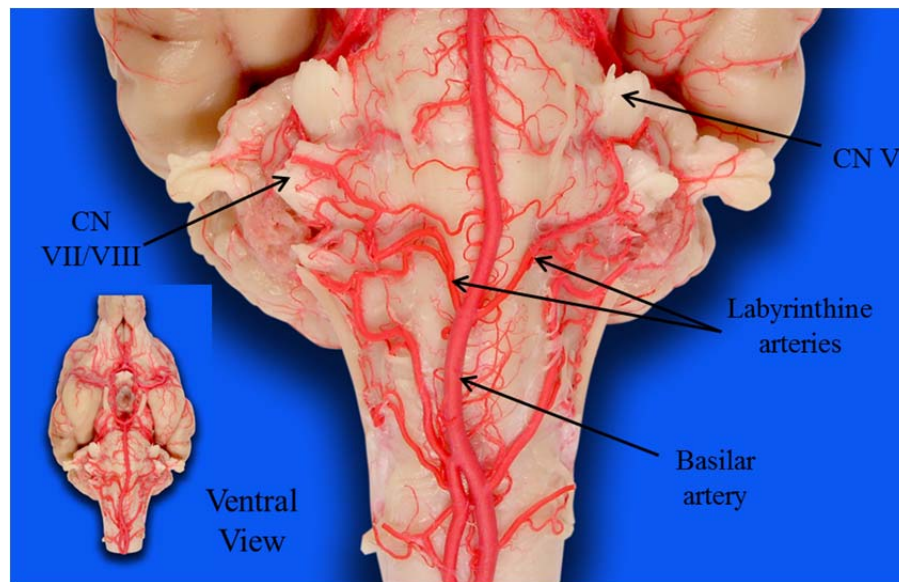


Figure 4.2. After originating from the basilar artery at the level of the mid-medulla, the labyrinthine artery coursed craniolaterally toward the roots of the facial (CN VII) and vestibulocochlear (CN VIII) nerves (prepared and photographed by Dr Daniel J. Hillmann).

The only other vessel approximating the TMJ, that also appeared to supply the middle ear, originated from a small branch of the maxillary artery. This vessel was observed in five latex-injected specimens, one ink-injected specimen, and the Microfil-injected specimen. The parent branch of this vessel originated from the maxillary artery, caudal to the retroarticular process, distal to the superficial temporal artery, and proximal to the inferior alveolar artery. The location of this vessel is similar in position to a vessel depicted by Evans and deLahunta (2010) as the mandibular ramus (Fig. 4.8). Three small branches were derived from this parent branch of the maxillary artery (Fig. 4.9). One of these branches extended dorsally toward the ventral aspect of the tympanic bulla

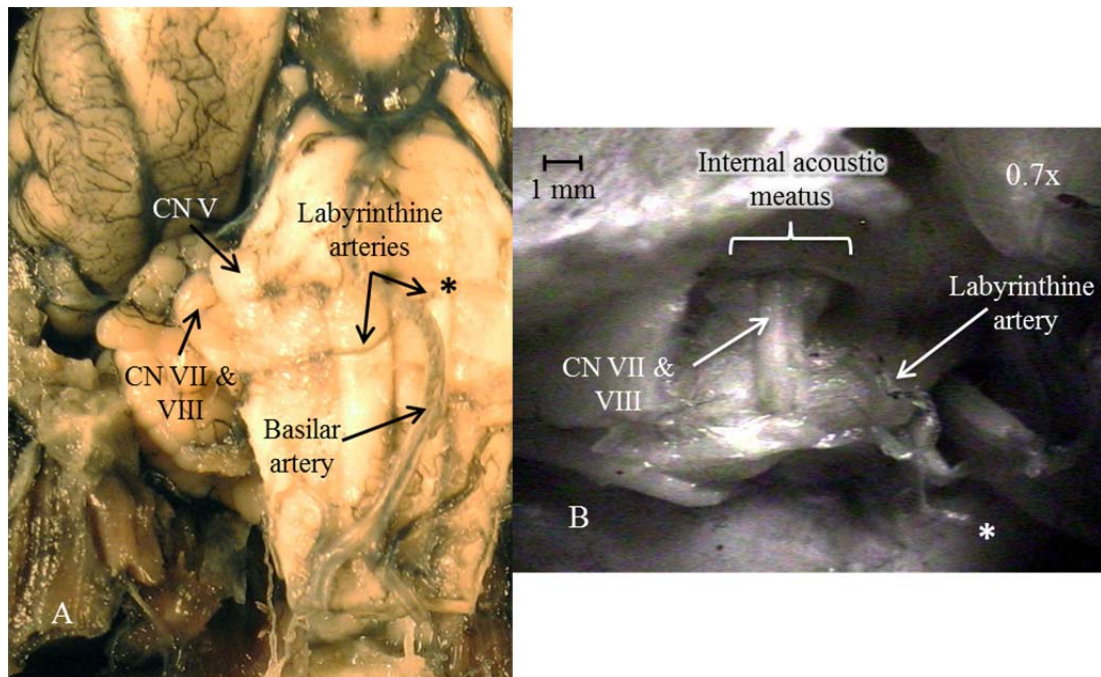


Figure 4.3. (A) The bilateral labyrinthine arteries originating from the basilar artery in an ink-injected specimen. The asterisks (A and B) demonstrate where the left labyrinthine artery was cut when the brain was removed. The black asterisk (A) represents the stump of the left labyrinthine artery that was maintained on the ventral brain surface and the white asterisk (B) shows the continuation of the left labyrinthine artery into the internal acoustic meatus along with CN VII and CN VIII (right panel).

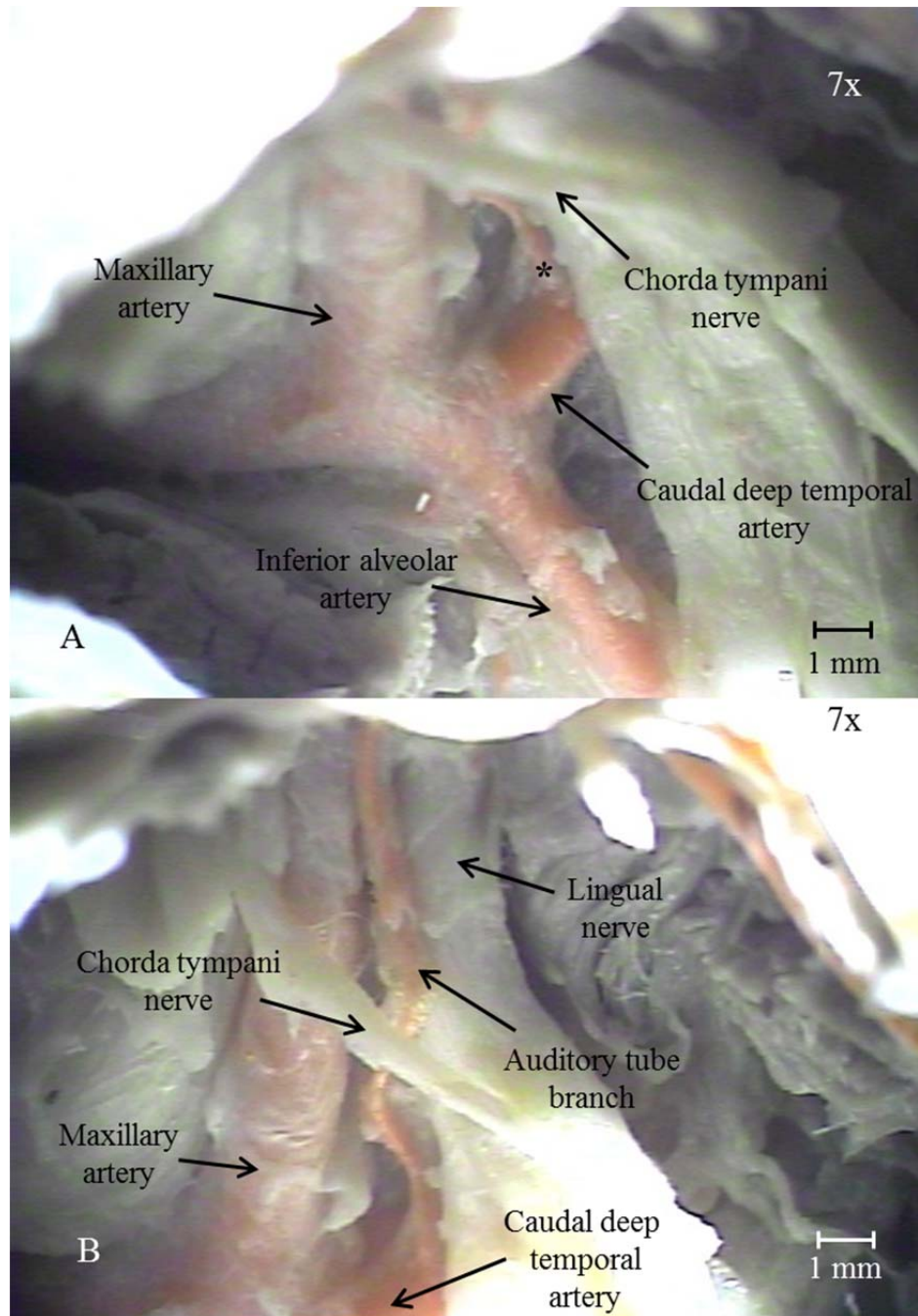


Figure 4.4. (A) A small arterial branch (Auditory tube branch) originated from the caudal deep temporal artery (asterisk). (B) This arterial branch coursed dorsally and parallel to the maxillary artery and lingual nerve and was crossed medially by the chorda tympani nerve. Left medial view.

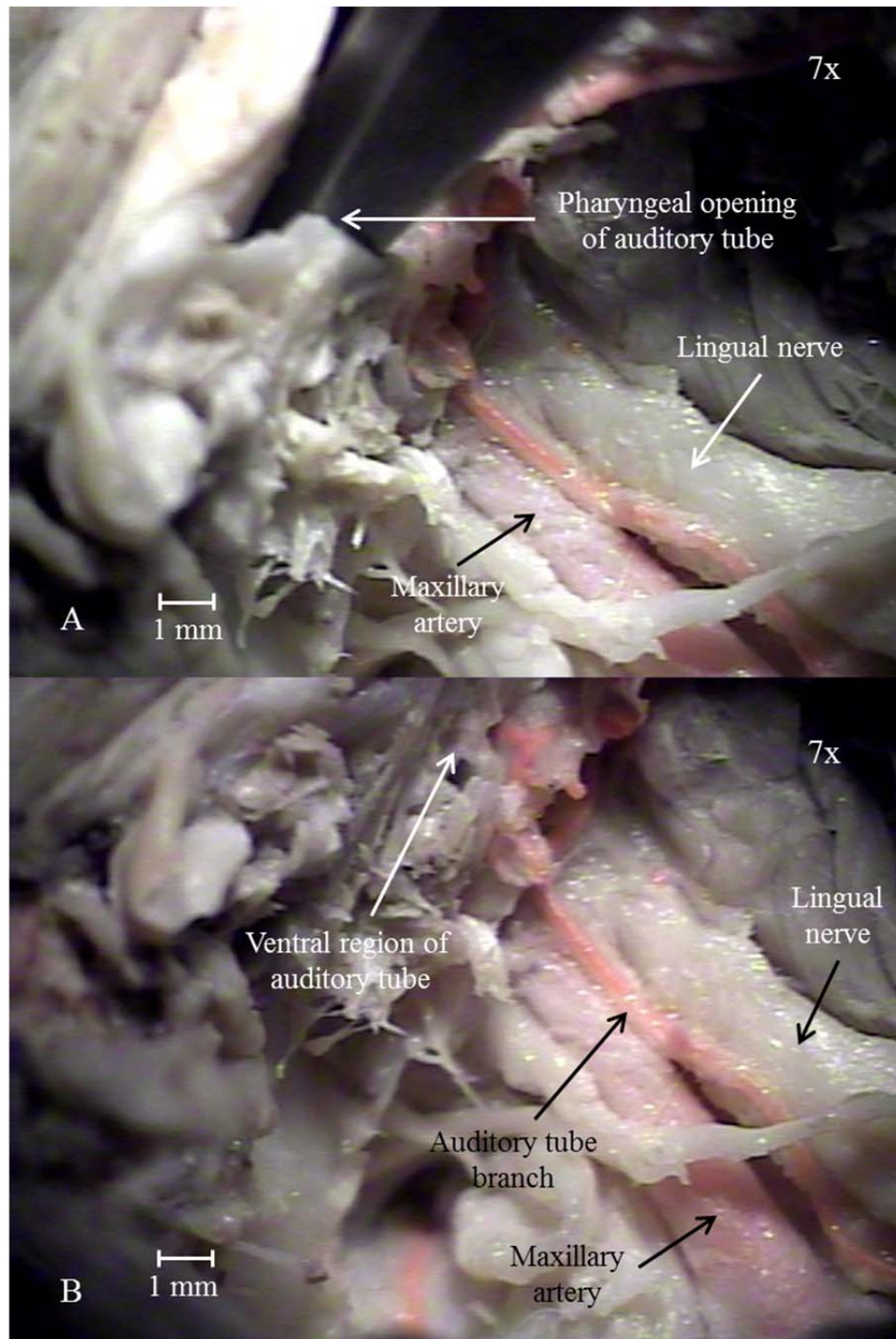


Figure 4.5. A small branch of the caudal deep temporal artery supplied the proximal portion of the auditory tube. (A) Forceps have been placed into the pharyngeal opening of the auditory tube. (B) The forceps were removed to demonstrate the blood supply to this region more clearly. Left medial view.

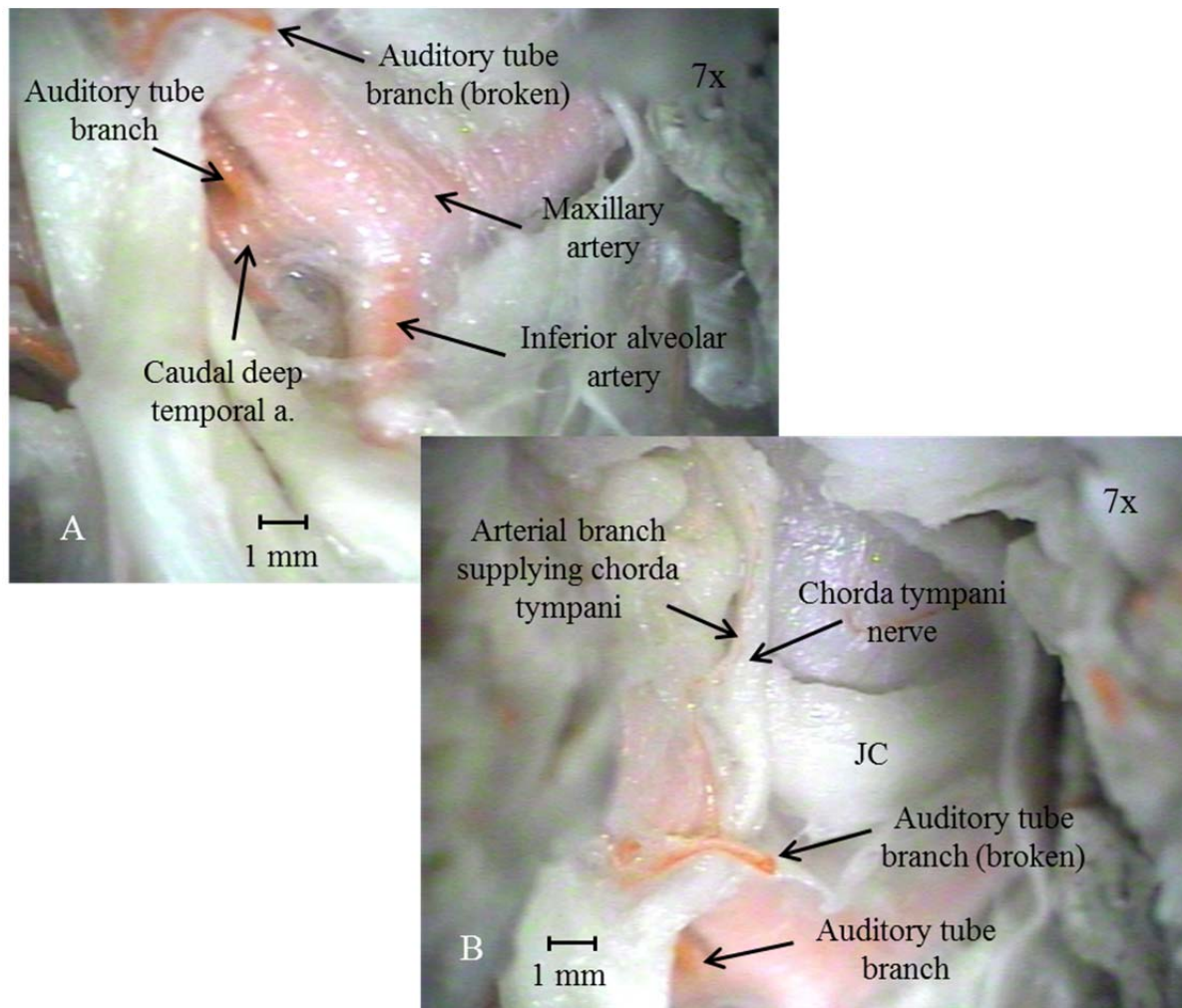


Figure 4.6. The auditory tube branch (A), a branch of the caudal deep temporal artery, contributed a fine branch to the chorda tympani nerve (B). JC = joint capsule of TMJ. Right medial view.

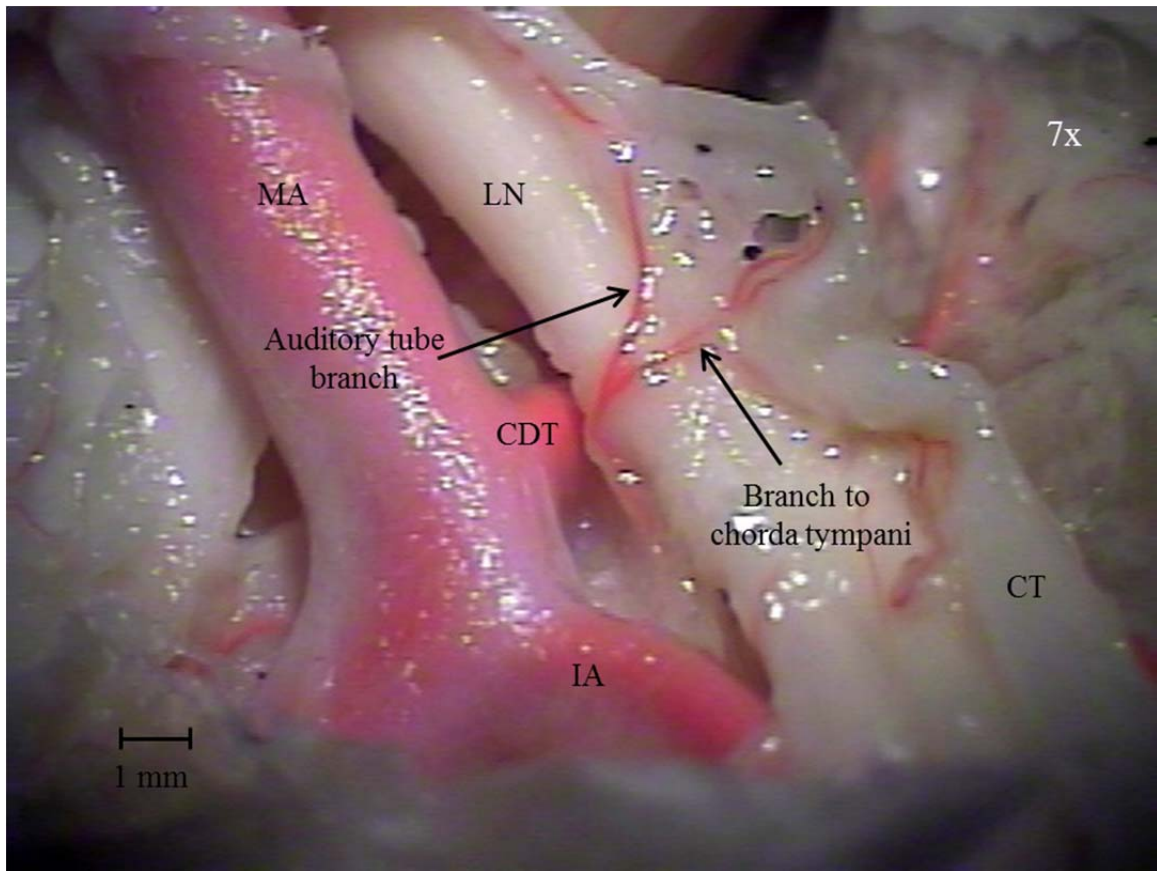
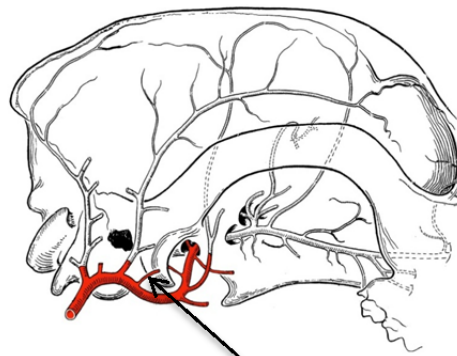
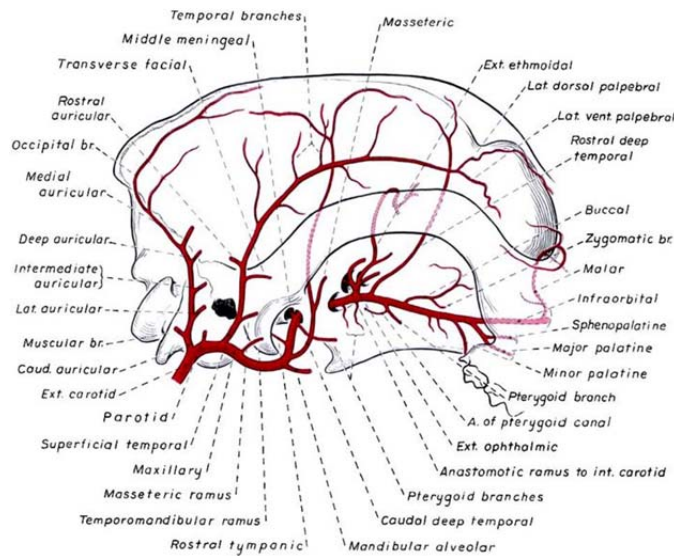


Figure 4.7. The auditory tube branch of the caudal deep temporal artery (CDT) contributed a branch to the chorda tympani nerve (CT). IA = inferior alveolar artery, LN = lingual nerve, MA = maxillary artery. Left medial view.

and bony portion of the external ear canal. A second branch extended rostrally, ventral to the retroarticular process, to supply the joint capsule. A third branch extended rostr dorsally and coursed between the retroarticular process and the tympanic bulla. After crossing arterial branch 3 medially, the chorda tympani extended parallel and caudal to this arterial structure. The complete distribution of these small vessels could not be determined due to the lack of vascular filling.

In five dissected specimens, branch 3 curved around the caudal aspect of the retroarticular process and extend medially toward a foramen located immediately medial to the mandibular fossa of the temporal bone (Fig. 4.10). The chorda tympani nerve entered a small foramen between the tympanic bulla and the retroarticular process in several specimens (n=25, inclusive of specimens from other

studies) (Fig. 4.11). The foramen for the chorda tympani was located medial and slightly caudal to the foramen that accommodated arterial branch 3.



Mandibular ramus

Figure 4.8. A vessel approximating the TMJ that coursed toward the middle ear was a derivative of a parent branch, which originated from the maxillary artery caudal to the retroarticular process, distal to the superficial temporal artery and proximal to the inferior alveolar artery. The location of the parent vessel is similar in location to a vessel depicted by Evans and deLahunta as the mandibular ramus. Right lateral view of a canine skull with depicted blood supply of the external carotid and maxillary arteries (from Evans, 1993; reproduced with permission).

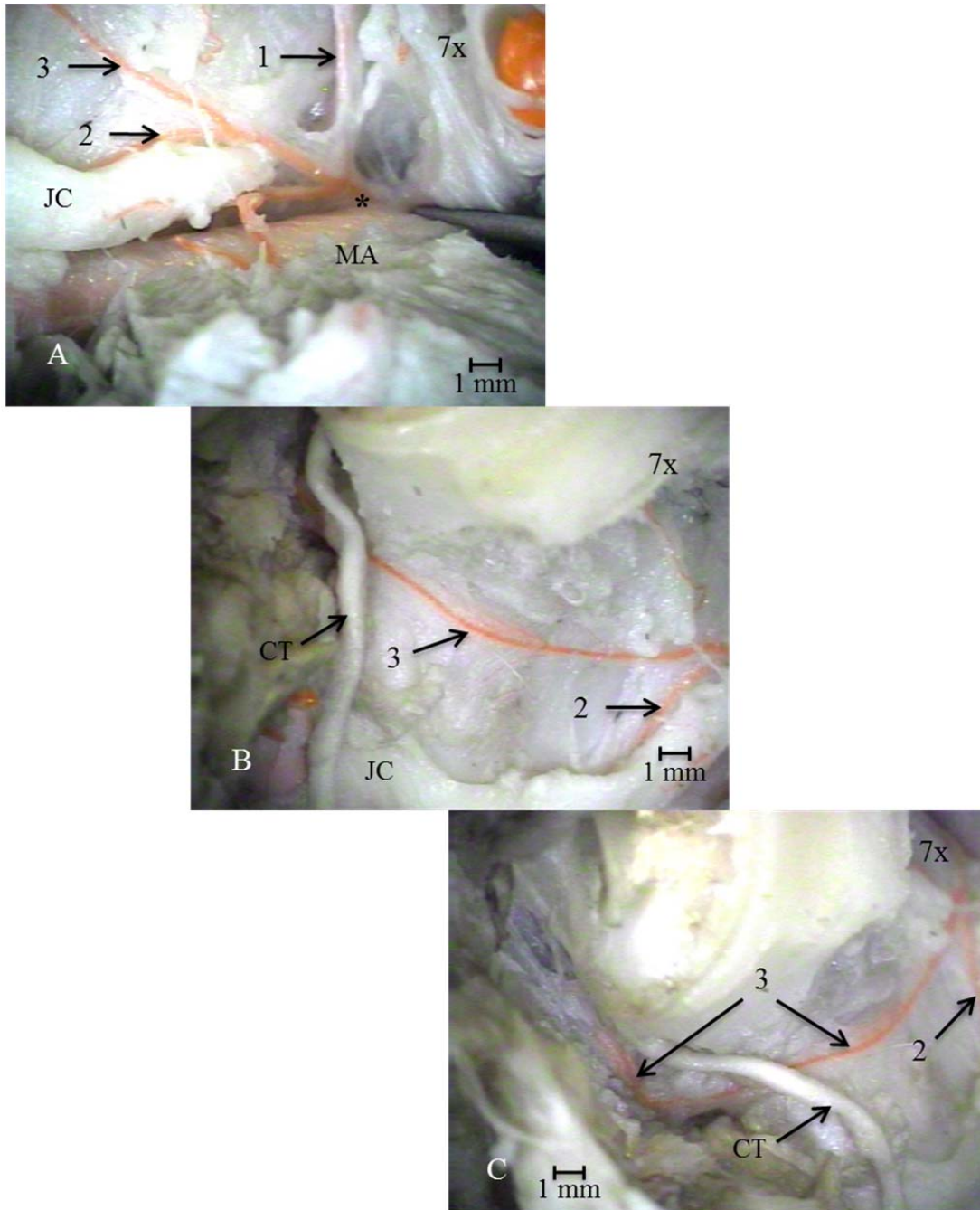


Figure 4.9. An artery branched from the maxillary artery (MA) caudal to the joint capsule (JC) of the TMJ. This parent branch (asterisk, A) gave rise to three small branches: One branch (1) extended dorsally toward and appeared to supply the ventral aspect of the tympanic bulla and bony external ear canal. A second branch (2) extended rostrally, ventral to the retroarticular process, to supply the joint capsule. A third branch (3) extended rostr dorsally and coursed between the retroarticular process and the tympanic bulla. After crossing this branch medially, the chorda tympani nerve (CT) extended parallel and caudal to this arterial structure. Right medial (A, B) and ventral (C) views.

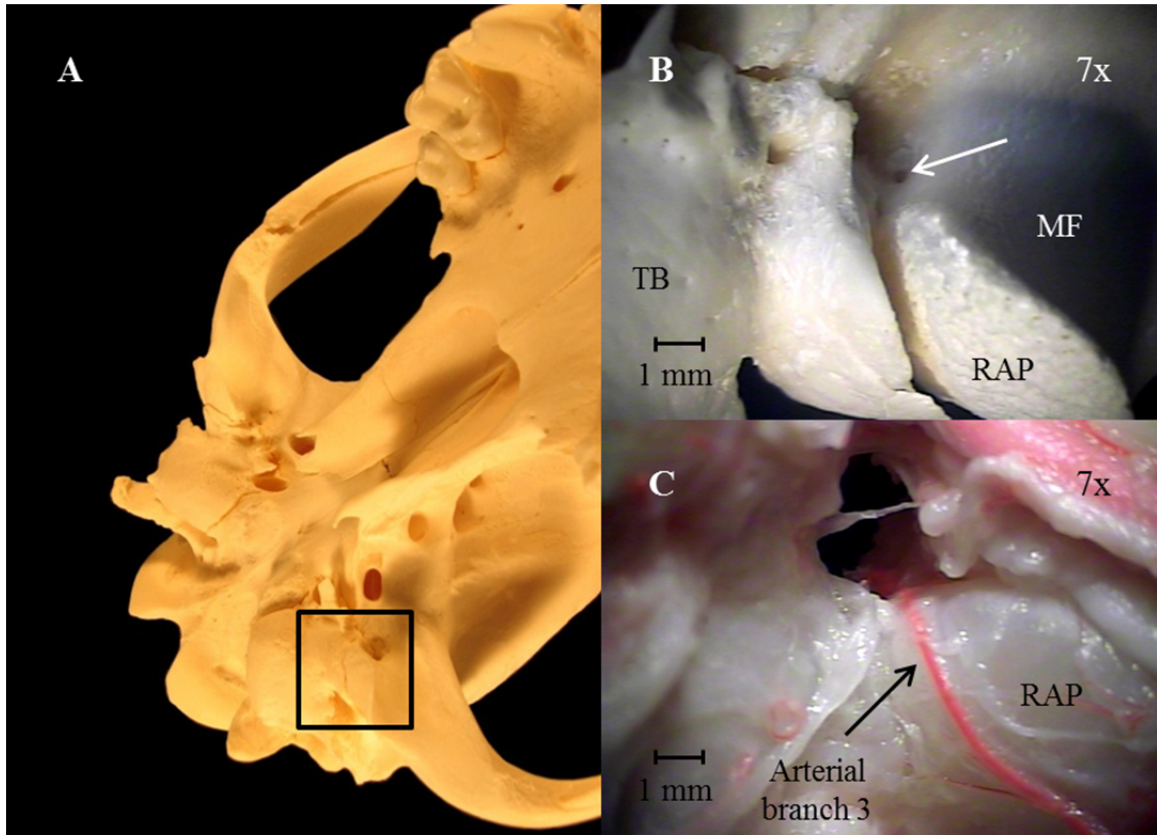


Figure 4.10. (A) Oblique lateral view of the ventral surface of the canine skull. (B) Magnified (7x) view of the region boxed in (A). Arterial branch 3 (C) curved around the caudal aspect of the retroarticular process (RAP) and entered a foramen (arrow, B) immediately medial to the mandibular fossa (MF) of the temporal bone. TB = tympanic bulla.

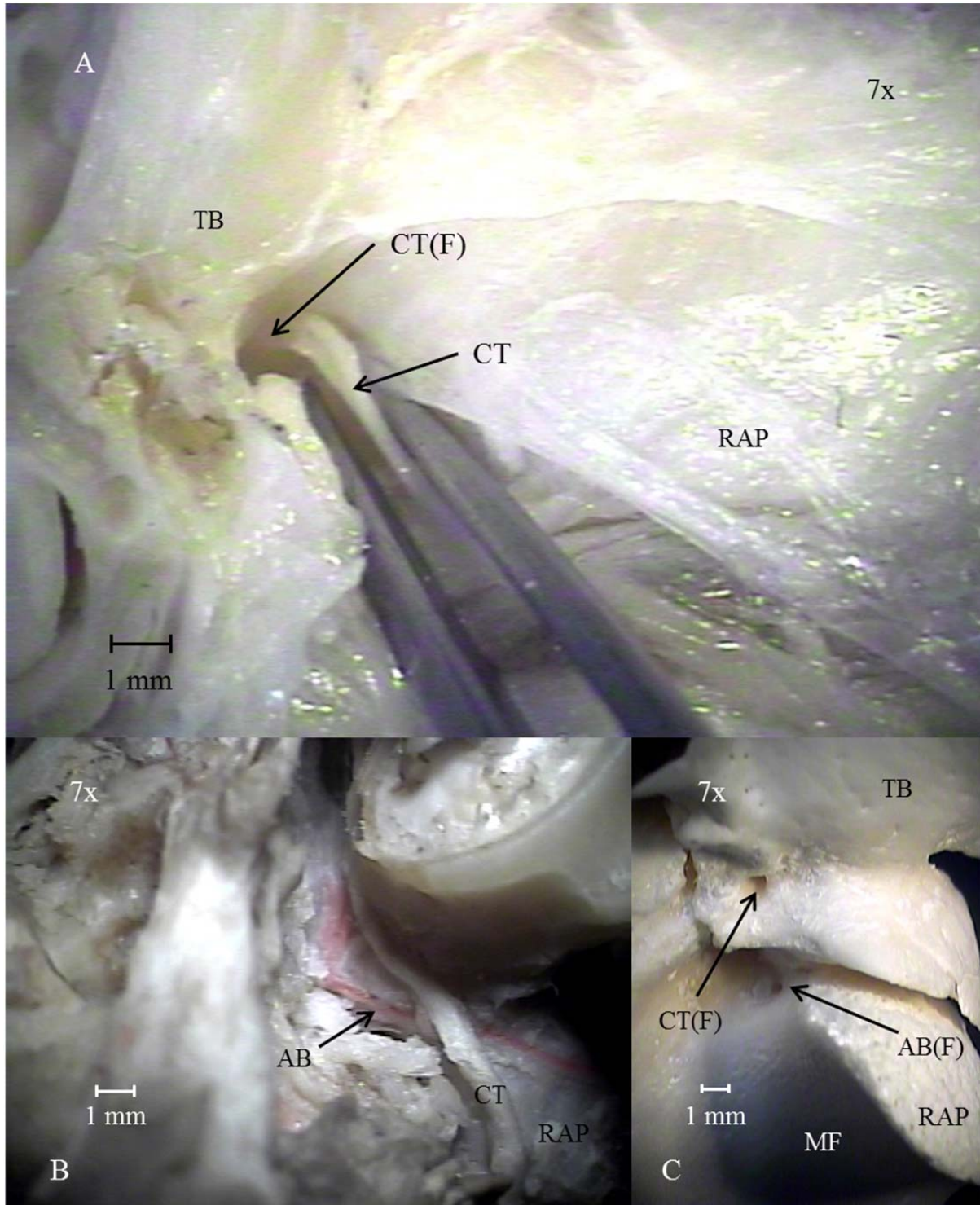


Figure 4.11. (A) The chorda tympani nerve (CT) entered a small foramen (CT(F)) between the tympanic bulla (TB) and the retroarticular process (RAP) in several specimens. (B) The chorda tympani nerve coursed caudal and medial to arterial branch 3 (AB). (C) Same image as 4.10B, rotated for proper orientation in relation to Fig. 4.11B. The foramen for the chorda tympani was located medial and slightly caudal to the foramen that accommodated arterial branch 3 (AB(F)) MF = mandibular fossa.

In the majority of the latex-injected specimens, all of the ink-injected specimens and the Microfil-injected specimen, the fine arterial branches, including arterial branch 3, lost injection medium shortly after it entered the foramen. In two of the latex-injected specimens, arterial branch 3 could be followed for a brief distance coursing caudally within a bony passage (canal or fissure) before it lost visibility due to loss of injection. In these two specimens, arterial branch 3 gave off fine branches that coursed caudoventrally toward the tympanic bulla (Fig. 4.12). Neither the tympanic membrane vasculature, nor the vasculature supplying the epithelial lining of the internal tympanic bulla were visible in any of the injected specimens, so it is not surprising that the terminal distribution of these microscopic vessels could not be determined.

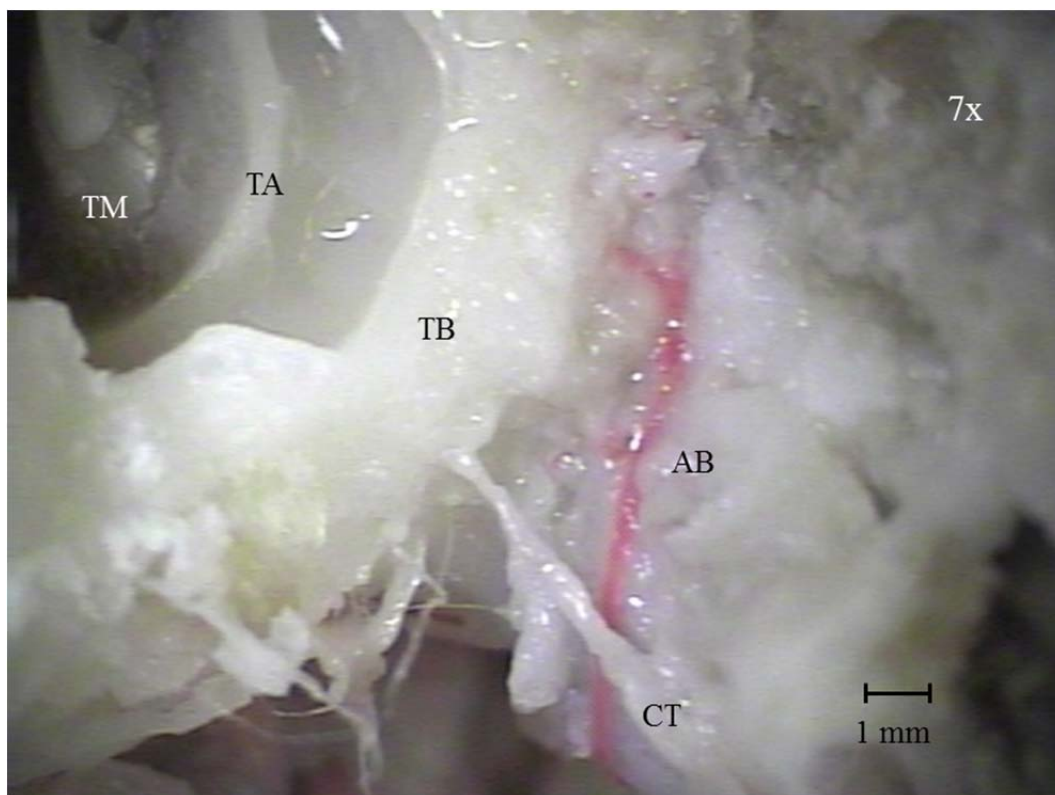


Figure 4.12. After its entry into the foramen, arterial branch 3 (AB) coursed caudally within a bony passage (canal or fissure) and gave off several fine branches that coursed caudoventrally toward the tympanic bulla. The canal containing the arterial branch has been opened in this specimen, CT = chorda tympani, TA = tympanic annulus, TB = tympanic bulla (cut edge of), TM = tympanic membrane. Left ventromedial view.

Corrosion Casting Studies

Results from the corrosion cast were inconsistent, with greater resolution possible from the right half than the left. However, the bony structures of the right half were not well maintained and it was difficult to visualize the relationships between the vessels and bony landmarks. The cranial floor was maintained in the right specimen well enough to note a small vessel that coursed craniomedially toward the internal acoustic meatus within the caudal cranial fossa (Fig. 4.13). The origin of this vessel could not be determined because it was obscured by the filling of the venous sinuses. The cast of this presumed labyrinthine artery was incomplete and was terminated near the internal acoustic meatus. The labyrinthine artery appeared to form a loop just inside the meatus, with the distal portion of the vessel coursing away from the meatus (Fig. 4.13B).

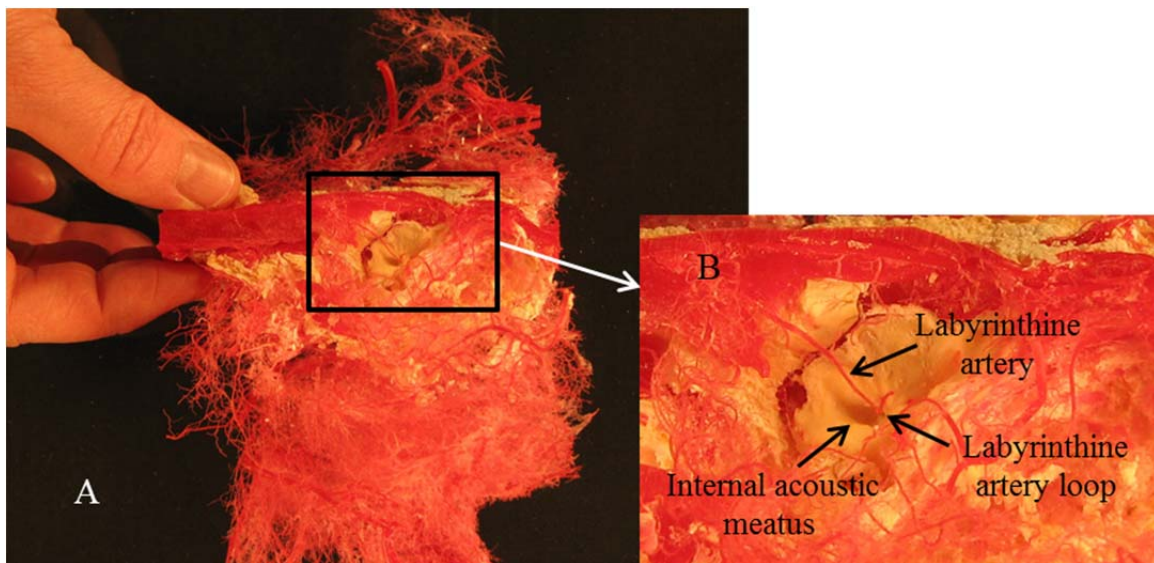


Figure 4.13. (A) Ventromedial view of a corrosion cast of the right temporal region of the canine skull. (B) Magnified view of boxed region in (A). A small vessel, presumed to be the labyrinthine artery, was observed coursing craniomedially toward the internal acoustic meatus within the caudal cranial fossa. The origin of this vessel could not be determined because it was obscured by the filling of the venous sinuses.

Branches of the maxillary artery were also assessed on the right corrosion cast specimen. A small arterial branch arose from the caudal deep temporal artery (Fig. 4.14). This vessel extended caudodorsally, and coursed parallel and rostral to the maxillary artery. The position of this vessel was

similar in position to the vessel observed in Fig. 4.4 that supplied the proximal auditory tube; however, the distribution of the corrosion cast vessel could not be determined due to the degradation of the surrounding tissues.

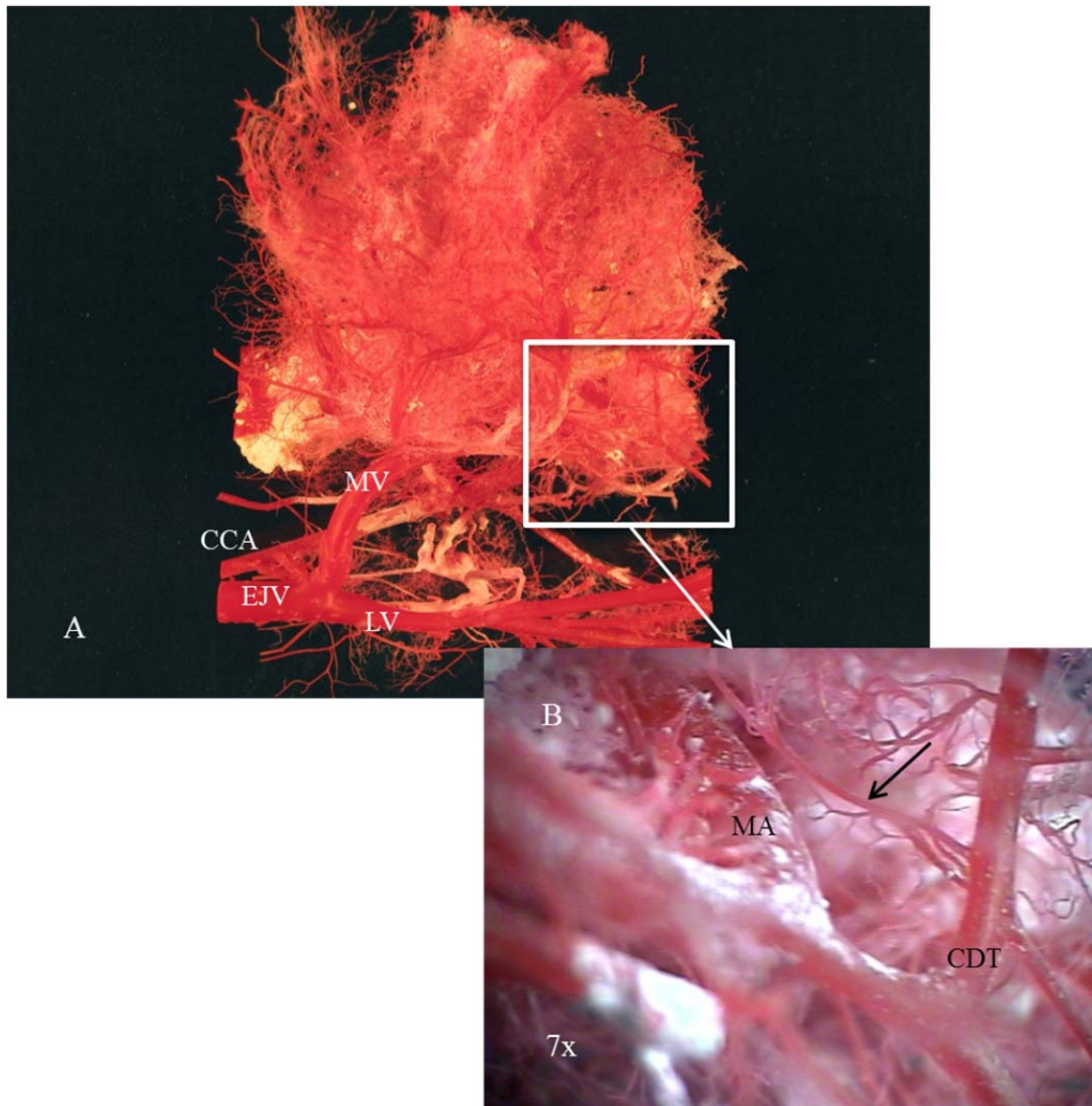


Figure 4.14. (A) Lateral view of a corrosion cast of the right temporal region of the canine skull. Only the larger vessels can be identified in this unmagnified view. (B) Magnified (7x) view of boxed region in (A). A small arterial branch (arrow, B) arose from the caudal deep temporal artery (CDT) and extended caudodorsally, and coursed parallel and rostral to the maxillary artery (MA). CCA = common carotid artery, EJV = external jugular vein, LV = lingual vein, MV = maxillary vein.

Vascular filling of the left Batson's-injected specimen was not as complete as the right; however, arterial branch 3, as described above (Figs. 4.9 – 4.12), was identifiable. The bone remained intact in this specimen, so the relationship of arterial branch 3 with the surrounding bony elements could be seen. This vessel coursed medially along the caudal aspect of the retroarticular process and gave off several branches to the region of the joint capsule (Fig. 4.15). The vessel then entered a small foramen that was located within a depression immediately medial to the mandibular fossa of the temporal bone (Fig. 4.16).

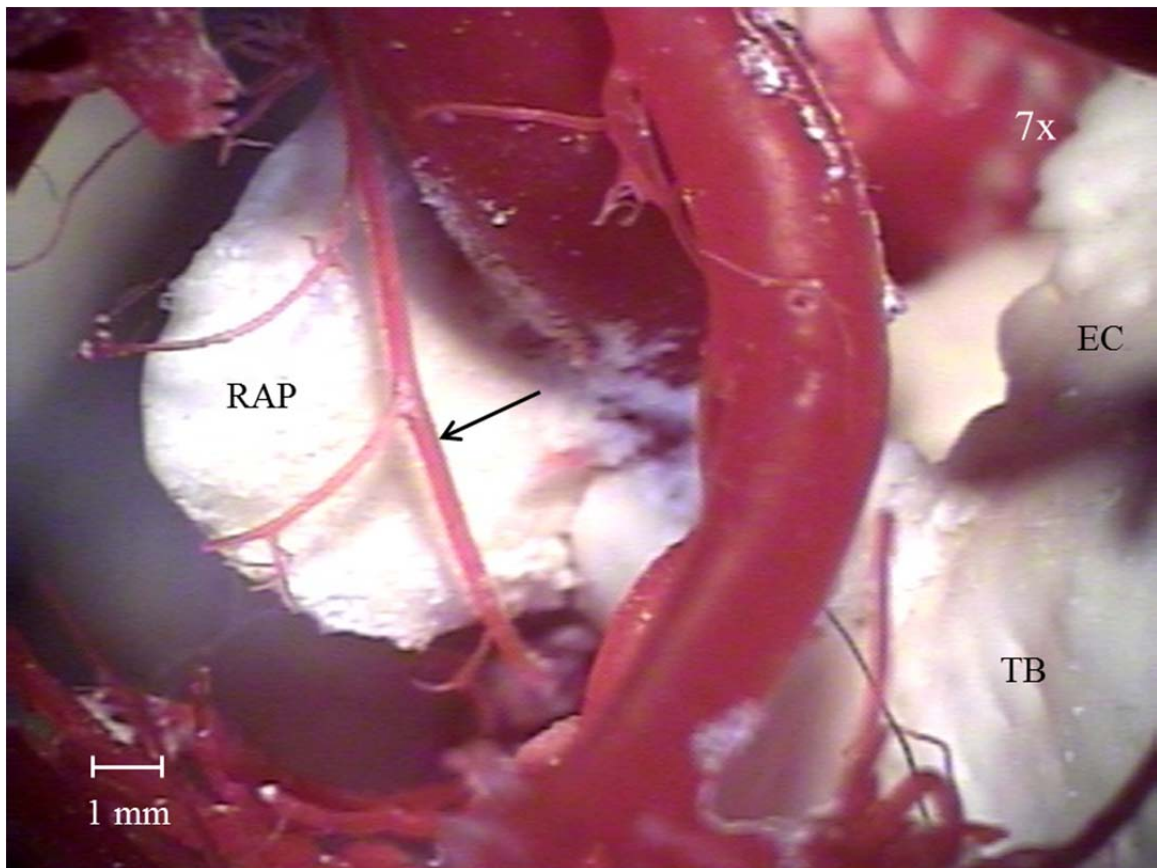


Figure 4.15. Arterial branch 3 in a corrosion cast of the left temporal region of the canine skull (arrow). This vessel coursed medially along the caudal aspect of the retroarticular process (RAP) and gave off several branches to the region of the joint capsule. EC = bony external ear canal, TB = tympanic bulla. Left ventral view.

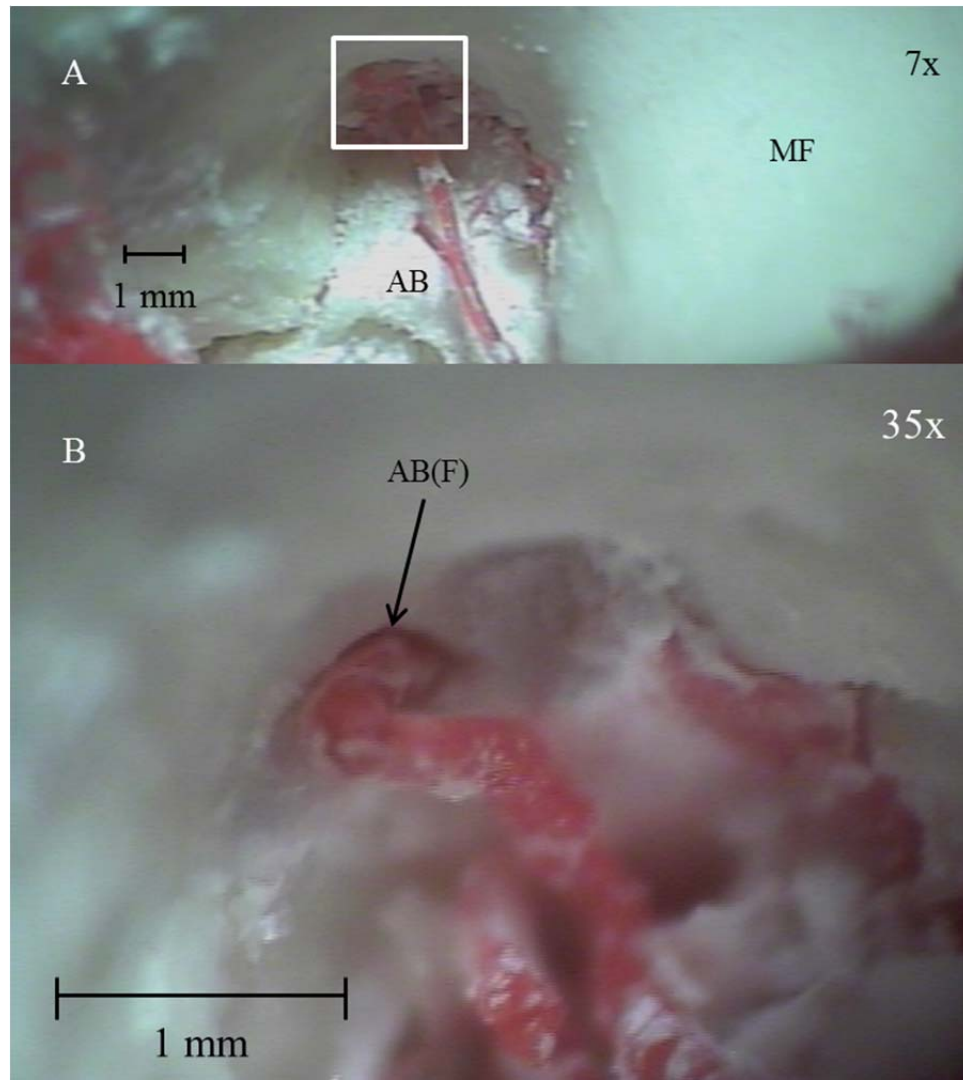


Figure 4.16. Arterial branch 3 (AB) extended into a depressed region immediately adjacent to the mandibular fossa (MF) and entered a small foramen (AB(F)). (B) Magnified (35x) view of boxed area in (A).

Imaging Studies

All x-ray and computed tomography (gross and micro) images were inconclusive in determining the fine vasculature of the inner and middle ears. The Microfil injection media successfully filled most arteries and veins, making it difficult to track any given vessel from the x-ray data, even at higher magnification, due to the superimposition of the diffuse vascular network (Fig. 4.17).

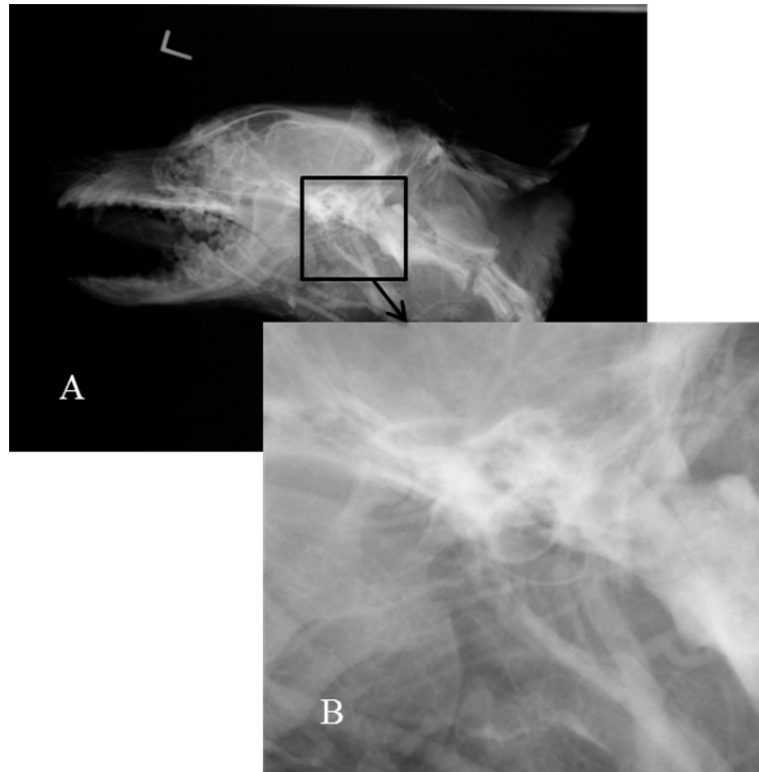


Figure 4.17. The Microfil injection media was successful in filling both arteries and veins. X-ray images were inconclusive, as it was difficult to track any given vessel, even at higher magnifications of the temporal region (B), due to the superimposition of the diffuse vascular network. Left lateral view.

After three-dimensional reconstruction of the micro-CT block, it was difficult to gain orientation due to the limited sample size accommodations of the scanner (1 cm x 1 cm x 2 cm) (Fig. 4.18). It was not possible to determine which vessels were arteries and which were veins without being able to trace these vessels back to a point of origin from a known anatomical landmark. A total of 60 scans, with dorsal side oriented superiorly followed by the ventral surface being oriented superiorly, would have been needed of the 30 prepared blocks to include the entire temporal region of interest with included major vessels for orientation. Each of these reconstructed blocks would then have to be superimposed exactly, without the benefit of a registration tool, in order to trace a small vessel from its origin to its distribution. The complexity of reconstruction was determined to be beyond the scope of this project.

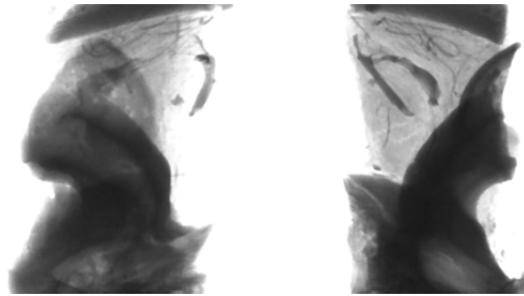


Figure 4.18. After 3D reconstruction of the micro-CT block, it was difficult to gain orientation due to the limited sample size accommodations of the scanner (1 cm x 1 cm x 2 cm). The above figure includes two opposing views from a block that contained the tympanic annulus and manubrium of the malleus.

Computed tomography scans of the entire temporal region were produced, but the limitations in resolution (1 mm section thickness with 0.4 mm overlap between sections) did not allow for the detection of vessels less than approximately 0.6 mm in diameter. The labyrinthine artery is known to accompany the facial (CN VII) and vestibulocochlear (CN VIII) nerves into the internal acoustic meatus. A small vascular bleb was noted in the canal of the meatus (Fig. 4.19), but the resolution of the scanner was not sufficient to reconstruct this vessel in its entirety.



Figure 4.19. A small vascular bleb was seen in the canal of the meatus following CT three-dimensional reconstruction, but the resolution of the scanner was not sufficient to follow this vessel in its entirety. Right dorsal view (image three-dimensional reconstruction by Kent Sanders, MD, University of Utah School of Medicine).

The smallest artery identified with confidence in the CT three-dimensional reconstruction was the occipital artery (Fig. 4.20). This vessel is approximately 0.8 mm in diameter. Although these reconstructions were not successful in identifying the fine vasculature supplying the inner and middle ears, a deviation from the well-established branching pattern of the caudal auricular artery was seen. In *Miller's Anatomy of the Dog* (Evans, 1993) the caudal auricular artery branches from the maxillary artery distal to where the lingual and facial arteries originate and just proximal to the origin of the superficial temporal artery (Fig. 4.8). In both the left and right sides of the CT three-dimensional reconstruction, the caudal auricular artery branched opposite the lingual artery, just distal to the origin of the occipital artery (Fig. 4.21). This variation was also noted in both the left and right halves of the corrosion casted specimens. This prompted a study of 56 dissected dog head halves used in teaching veterinary gross anatomy. These were further dissected and the presence of this variation determined. In 14 of the 56 specimens (25%), the origin of the caudal auricular artery occurred immediately distal to the origin of the ascending pharyngeal and occipital arteries and opposite the origin of the lingual artery. In the remaining 42 specimens, the branching pattern of the caudal auricular artery as previously described was observed (Evans, 1993; Ghoshal, 1975).

Discussion

Causes of deafness can be hereditary or acquired. The causes of acquired deafness are frequently unknown; however, several origins have been identified. For example, acquired hearing loss may result from drug toxicity, noise trauma, infection, aging, middle ear effusion, excess cerumen production, and physical trauma (Strain 1996, 1999, 2011). According to Lee and Baloh (2005), two common etiologies for sudden deafness are circulatory disturbance of the vertebrobasilar arterial system, and inflammation, which is most likely virus related. Vertebrobasilar ischemia (VBI) causes deafness because most of the auditory system, and specifically the inner ear, is supplied by

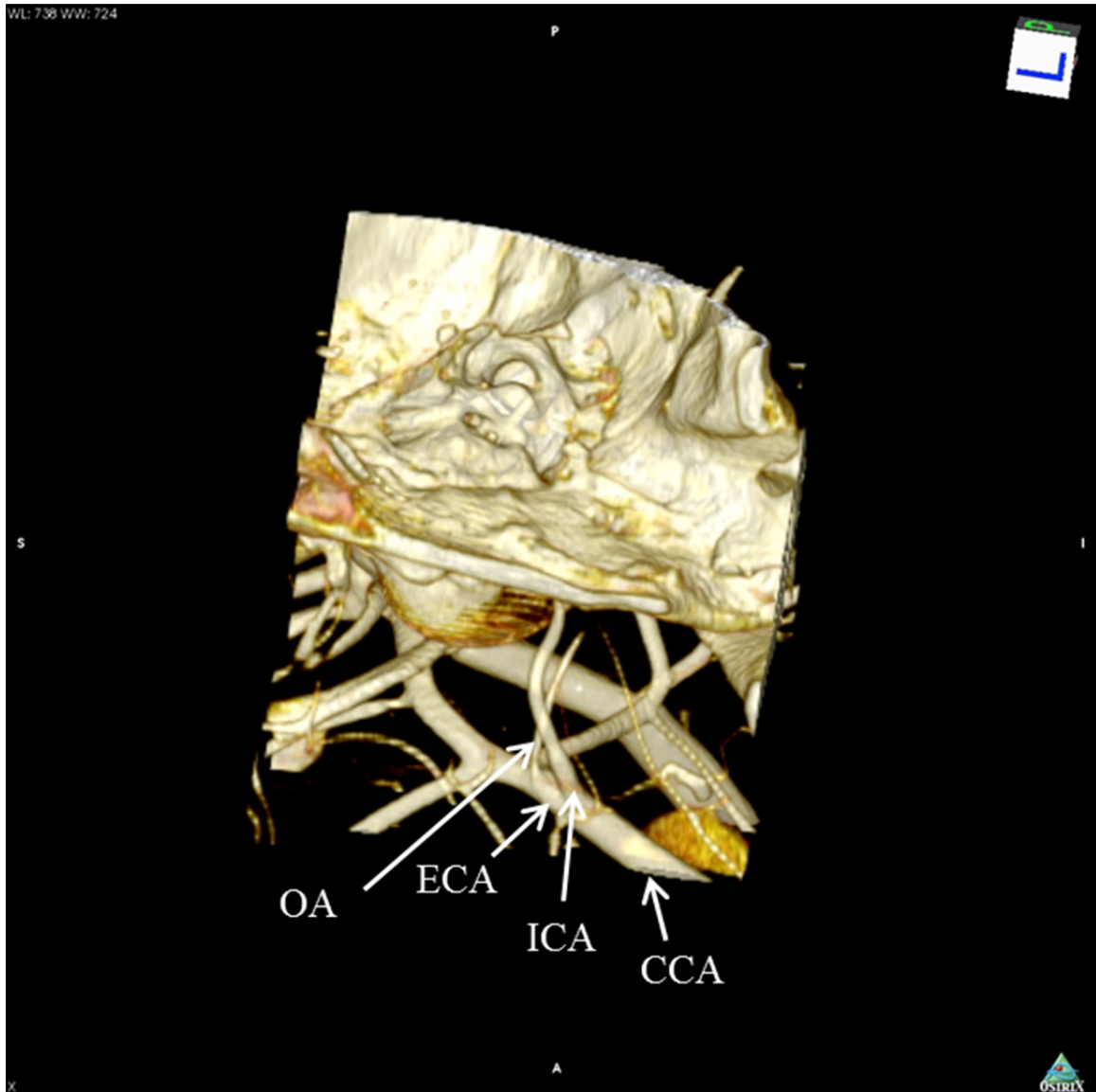


Figure 4.20. The smallest artery (~0.8 mm) confidently identified upon CT three-dimensional reconstruction was the occipital artery (OA). CCA = common carotid artery, ECA = external carotid artery, ICA = internal carotid artery. Left caudal view.

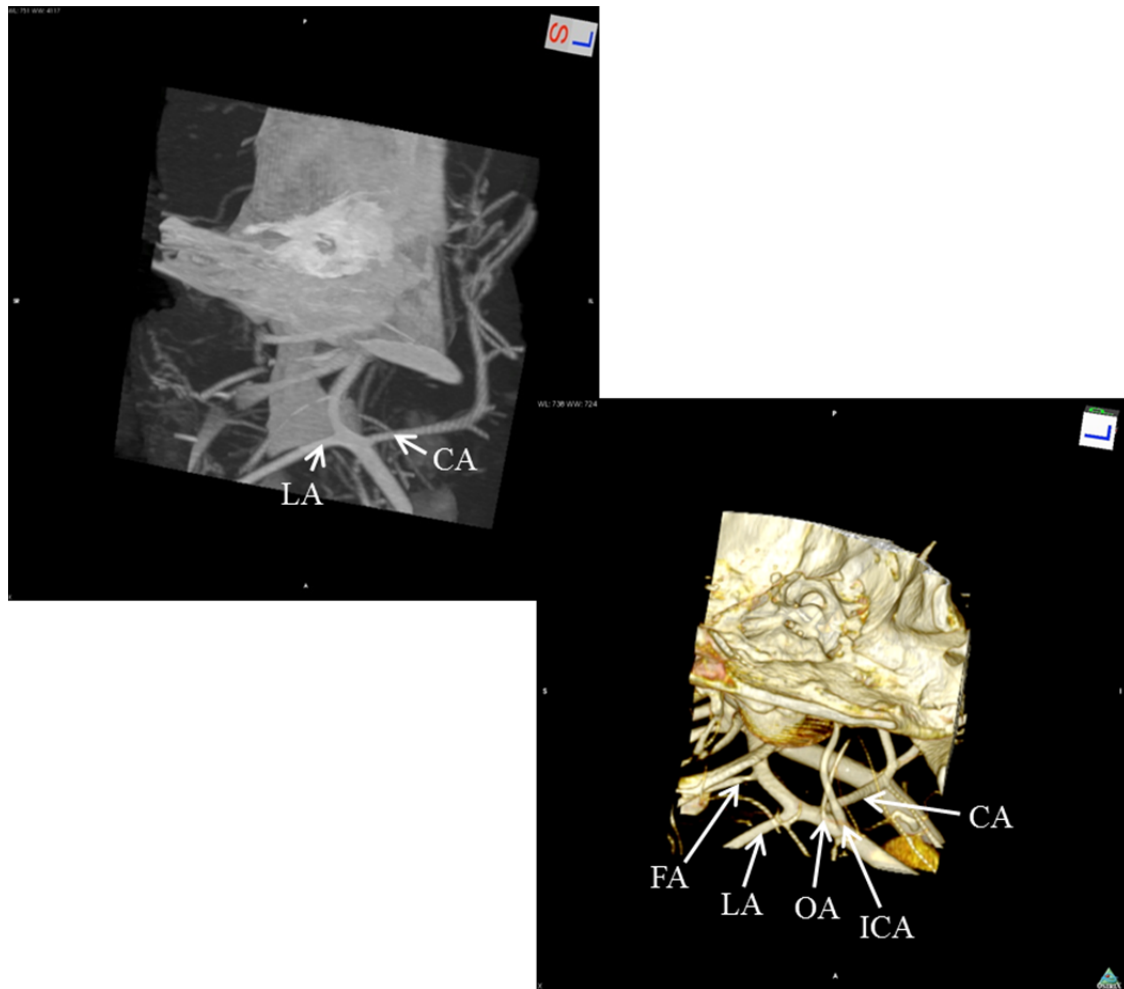


Figure 4.21. A deviation from the previously described branching pattern of the caudal auricular artery (CA) was seen on the CT three-dimensional reconstructed specimens. In these specimens, the caudal auricular artery branched opposite the lingual artery (LA), just distal to the origin of the occipital artery (OA). FA = facial artery, ICA = internal carotid artery. Right medial view.

branches of the basilar artery (Alford et al., 1965; Anderson and Kubicek, 1971; Bernstein and Silverstein, 1966; Shambaugh, 1923). Stroke is a likely candidate in producing VBI and deafness may result from the lack of blood flow within the vertebrobasilar system because the inner ear has a complete absence of collateral circulation and a very high energy metabolism (Lee and Baloh, 2005). It is not known if ischemia of the vessels that supply the middle ear could potentially result in sudden hearing loss. Of particular interest to us is the correlation between sudden-onset deafness and dental procedures performed in dogs and cats while under anesthesia (Stevens-Sparks and Strain, 2010).

During dental procedures, jaw manipulation is inevitable. It is therefore logical that a study of the anatomy of the blood vessels close to the temporomandibular joint be performed before live-animal physiological studies are performed to determine if vascular occlusion could result in subsequent loss of hearing. The goals of this study were to confirm the labyrinthine artery as the main blood supply to the canine inner ear and to identify juxta-articular branches of the maxillary artery as sources of blood supply to the middle ear.

There are two components of the inner ear, the cochlea, which houses the organ of hearing and the vestibular component that aids in the maintenance of balance. The organ of hearing is well guarded within a bony enclosure called the promontory, and entrance to the promontory is gained through the internal acoustic meatus. The labyrinthine artery enters the internal acoustic meatus to supply the membranous labyrinth (both cochlear and vestibular components) of the inner ear. However, there is much debate concerning the parent vessel from which this artery originates. The depiction by Evans (1993) of the labyrinthine artery as a direct branch of the basilar artery is in agreement with *Nomina Anatomica Veterinaria* (2005). Evans shows the artery entering the meatus along with cranial nerves VII and VIII. From this, it must be concluded that the bilateral branches originating from the basilar artery in the current study were the left and right labyrinthine arteries (Fig. 4.2 and 4.3). The torturous labyrinthine arteries extended rostrolaterally toward the roots of cranial nerves VII and VIII (Fig. 4.2) and were observed entering the internal acoustic meatus along with these cranial nerves (Fig. 4.3). The right corrosion cast specimen revealed that the labyrinthine artery enters the meatus and forms a loop, where the distal limb of this loop extends away from the meatus (Fig. 4.13). A similar observation of an arterial loop occurring within the internal acoustic meatus has been made by several authors: Sunderland (1945) referred to this vessel as the anterior inferior cerebellar artery in the human; Bernstein and Silverstein (1966) referred to this branch as the anterior cerebellar artery in the cat; and Anderson and Kubicek (1971) referred to this artery as the

anterior inferior cerebellar artery in the dog. All stated that the labyrinthine (auditory) artery arose from either the proximal limb, or in most cases, from the apex of this arterial loop and therefore do not acknowledge the parent vessel that branches directly from the basilar artery and provides this loop within the meatus as the labyrinthine artery. In the corrosion casted specimen, no branches were observed that actually extended into the depths of the meatus to supply the structures within the promontory. If the observations by the previous authors were correct, a branch should have been seen originating from either the proximal limb or from the apex of the vascular loop. The lack of visualization in this regard is likely the result of insufficient vascular filling due to the minute size (0.4 mm) of the vascular branch, or perhaps from the delicacy of the cast, where the vessel may have been inadvertently broken while pruning the cast. Another possibility is that there is variation in the branching pattern of the labyrinthine artery and it does not originate from this arterial loop within the meatus. Sunderland (1945) described variable origins of the internal auditory artery (labyrinthine artery) from the basilar and anterior inferior cerebellar arteries in humans. One observation in this study by Sunderland was the origin of the labyrinthine artery from the basilar artery, independent of the anterior inferior cerebellar artery in 17 percent of the dissected specimens. If this were the case in the present study, two separate arteries should have been seen entering the mouth of the meatus; there were no additional arterial branches observed entering the meatus in either the dissected or the corrosion casted specimens.

There were two branches of the maxillary artery that supplied structures of the middle ear and adnexa. One branch arose from the caudal deep temporal artery and extended dorsally between the maxillary artery and the lingual nerve. This vessel supplied the proximal region of the auditory tube, surrounding nasopharynx, and chorda tympani nerve (Figs. 4.4 – 4.7). According to Evans (1993) and Ghoshal (1975), the rostral tympanic artery may branch from the caudal deep temporal artery or directly from the maxillary artery. In the current study, the rostral auditory tube branch was

the only vessel noted branching from the caudal deep temporal artery. A second vessel of interest, supplying the middle ear, branched from the maxillary artery, just caudal to the retroarticular process. This vessel served as the parent branch of three small branches: one of these branches extended dorsally toward the ventral aspect of the tympanic bulla and bony portion of the external ear canal; a second branch extended rostrally, ventral to the retroarticular process, to supply the joint capsule of the TMJ; a third branch extended rostr dorsally along the caudal aspect of the retroarticular process (Fig. 4.9). This branch coursed medially between the retroarticular process and the tympanic bulla to enter a small foramen that was located medial to the mandibular fossa (Figs. 4.10 – 4.16). As this vessel coursed toward the foramen, it provided several branches to the caudal joint capsule of the TMJ (Fig. 4.15). Fine branches of this vessel extended caudoventrally toward the tympanic bulla as the vessel passed through its bony canal (Fig. 4.12). Complete injection did not occur in any of the dissected specimens, as neither the vasculature of the tympanic membrane nor the vasculature of the epithelial lining of the tympanic bulla was visible. It therefore cannot be stated with certainty that the fine branches of this vessel that were seen extending toward the tympanic bulla provided circulation to the internal lining of, or to other structures within, the tympanic bulla. However, it is the opinion of the author that this vasculature does supply structures within the tympanic bulla even though the complete course of the vessel could not be visualized. In Evans' (1993) description of the rostral tympanic artery, he stated that the vessel enters one of the small foramina located in a depression medial to the joint and courses through the temporal bone into the middle ear. Although the origin of the vessel observed in this study varied from the origin of the rostral tympanic artery as described by Evans (1993), the distribution of these vessels were similar in that they both entered a foramen within a depression located medial to the TMJ and extended through the temporal bone within a bony canal. It is therefore concluded that the vessel described in this study as arterial branch 3 is the rostral tympanic artery.

Evans (1993) identified the mandibular branch (*r. mandibularis*) as being the first branch of the canine maxillary artery. This branch leaves the dorsal surface of the maxillary artery 5 to 15 mm distal to the origin of the superficial temporal artery and provides the main supply to the caudal part of the temporomandibular joint capsule. This vessel may give off two or three small, threadlike branches. Davis and Story (1943) identified the *A. auricularis profundus* as the first branch of the feline maxillary artery. This vessel bifurcates into lateral and medial branches, with the medial branch providing articular and auricular branches. The articular branch supplies the posterior part of the capsule of the TMJ; the auricular branch extends dorsomedially and supplies the bony external auditory meatus, tympanic membrane and malleus. The course of the lateral branch is described as running across the postglenoid (retroarticular) process and supplying twigs to the posterior part of the mandibular capsule. The vessel identified in the present study as the parent branch that gave off the three small branches as discussed above and demonstrated in Fig. 4.9, appears to be synonymous with the canine *r. mandibularis* and the feline *A. auricularis profundus*. Each of the three branches of the parent branch identified in this study can be related to each of the three branches of the *A. auricularis profundus* described by Davis and Story (1943). Branch one, as described in this study as extending dorsally toward and appearing to supply the ventral aspect of the tympanic bulla and bony external ear canal is similar to the auricular branch; branch two, as described in this study as extending rostrally, ventral to the retroarticular process, to supply the joint capsule, is similar to the described articular branch; and branch three, as described in this study as extending rostrodorsally along the caudal aspect of the retroarticular process and providing small branches to the joint capsule is similar to the lateral branch. One clear difference is that the third branch in this study has been identified as the rostral tympanic artery because its entry into a small foramen located medial to the mandibular fossa was noted in several specimens (Figs. 9, 10 and 15), matching the description of Evans (1993) of the distal distribution of the rostral tympanic artery.

Anomalies of arterial branching patterns in the dog are not uncommon. When these variations are noted to occur frequently, especially in the large to medium sized vessels, it is important to document these differences because surgeons often use vessels as landmarks during surgical procedures. A variation of the branching pattern of the caudal auricular artery was identified in the current study. In *Miller's Anatomy of the Dog* (Evans, 1993) (Fig. 4.8), the caudal auricular artery is shown to branch from the maxillary artery distal to where the lingual and facial arteries originate and just proximal to the origin of the superficial temporal artery. It was seen in both left and right sides of the CT three-dimensional reconstruction that the caudal auricular artery branched opposite the lingual artery, just distal to the origin of the occipital artery (Fig. 4.21). This variation was also seen in both the left and right halves of the corrosion casted specimens. In 14 of 56 latex-injected heads (25%), the origin of the caudal auricular artery occurred immediately distal to the origin of the ascending pharyngeal and occipital arteries and opposite the origin of the lingual artery. In the remaining 42 specimens, the well-accepted branching pattern of the caudal auricular artery was seen.

This study was limited by the lack of filling of very small vessels by the different injection media. An explanation for the lack of small vessel filling by radio-opaque materials, including Microfil, was provided by Mondy et al. (2009), who reported that a toxic response of the vascular endothelial cells to the heavy metals used in contrast media results in cytoplasmic swelling of these cells. This results in a narrowing of the lumen of the microvasculature, subsequently blocking the contrast agent's perfusion and resulting in incomplete filling of the microvasculature. It is possible that there is an endothelial cell reaction to the formaldehyde used during embalming, which may prevent filling of the microvasculature by any injection media.

Subjecting specimens to micro-CT would be ideal for determining the microvasculature supply to a given region. This would eliminate the need for decalcification, micro-dissection, and histological serial sectioning in mapping out the blood supply to a specific region of interest. If the

region of interest is larger scale, a micro-CT scanner that accommodates specimen samples larger than 1cm x 1cm x 2cm is necessary. Three dimensional reconstructions of these vascular models can be challenging. Radio-opaque agents do not produce sufficient contrast between the agent-filled capillary lumen and the surrounding tissues, so the background levels must be modified to visualize the vasculature more clearly. This often compromises the visualization of the finer vessels. Mondy et al. (2009) proposed a method for microvasculature visualization that would eliminate the need for radio-opaque, metal-containing reagents. They suggested that micro-CT scanning of corrosion casts prepared with modified Batson's injection media would eliminate the need for radio-opaque materials to visualize the vasculature and at the same time would decrease, and perhaps eliminate, background noise upon digital reconstruction of these models. This method would also allow for specific areas of the fragile vascular model to be viewed in three dimensions without having to physically preen and subsequently destroy the surrounding vasculature. The success of this procedure would be limited to the dimensions of the sample size limitation of the scanner.

Evidence from this study supports the following conclusions.

1. The labyrinthine artery originates from the basilar artery (Fig. 4.2) and was seen in the right corrosion casted specimen to consist of a proximal limb entering the internal acoustic meatus, a loop within the meatus, and a distal limb exiting the meatus (Fig. 4.13). This loop configuration was lost in the dissected specimen upon removal of the brain; however, it was confirmed that the labyrinthine artery does enter the depths of the meatus, accompanied by the seventh and eighth cranial nerves (Fig. 4.3).
2. The rostral tympanic artery, as described by Evans (1993) and Ghoshal (1975) as being either a direct branch of the maxillary artery occurring opposite the caudal deep temporal artery or as a branch of the caudal deep temporal artery was not seen in any of the dissections.

3. The rostral tympanic artery is a derivative of the mandibular ramus (*r. mandibularis*), which is the first branch of the maxillary artery. The mandibular ramus supplied two additional branches, one to the joint capsule and one to the bony external ear canal.
4. The rostral tympanic artery enters a foramen that is located rostralateral to the foramen of the chorda tympani nerve (Figs. 4.10 and 4.11) and supplies portions of the joint capsule and tympanic bulla.
5. Only one vessel originated from the caudal deep temporal artery at its origin. This vessel, referred to as the rostral auditory tube branch, extended dorsally between the maxillary artery and lingual nerve to supply the proximal portion of the auditory tube, surrounding nasopharynx, and chorda tympani nerve (Figs. 4.4 – 4.7).

More suitable methods are necessary for microvascular studies. Although it is the author's opinion that the rostral tympanic artery, a branch of the mandibular ramus of the maxillary artery, supplies structures within the tympanic bulla, it cannot be confirmed due to the lack of vascular filling and the difficulty in tracking this vessel through bone. The extent of the regions supplied by the rostral auditory tube branch and the prevalence of the labyrinthine arterial loop and its branches within the internal acoustic meatus is also not known due to lack of vascular filling. These issues can potentially be resolved through micro-CT imaging of corrosion casts that have been injected with modified Batson's media, as long as the dimensions of the sample size limitation of the scanner are not too restricted.

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CHAPTER 5

THE CANINE JAW-EAR CONNECTION: THE MALLEOMANDIBULAR AND TYMPANOMANDIBULAR LIGAMENTS

Introduction

Acute onset deafness in dogs and cats can occur following dental and ear procedures done while the animal is under anesthesia (Stevens-Sparks and Strain, 2010). The majority (66%) of reported acute onset deafness cases following a procedure done under anesthesia were dental related (Stevens-Sparks and Strain, 2010). This raises the question of a potential jaw-ear connection in the dog. If such a connection exists, it would lay the basis for future mechanical studies where jaw manipulation could be potentially linked to the disruption of the proper articulation of the ossicles of the middle ear which could, in turn, lead to immediate onset conductive hearing loss. Such a jaw-ear connection has been documented ontogenetically and phylogenetically through the development of Meckel's cartilage, which induces the development of the mandible distally. The proximal end of the cartilage is replaced by bone, giving rise to two of the auditory ossicles (malleus and incus) (Ji et al., 2009; Martin and Ruf, 2009; Meng et al., 2011; Weil, 2011). In man, two ligaments derived from the first embryonic branchial arch that unite the mandible and TMJ with the middle ear have been identified as the sphenomandibular ligament (SML), also known as the malleomandibular ligament (MML), and the discomalleolar ligament (DML) (Burch, 1966; Cesarani et al., 1991; Cheynet, 2003; Meng et al., 2011; Pinto, 1962; Rodriguez Vazquez et al., 1992, 1993, 1998; Sencimen et al., 2008). The sphenomandibular ligament is described as a fibrous remnant of Meckel's cartilage. There is controversy concerning the derivation and even the factual existence of the discomalleolar ligament in the human adult (Burch, 1966; Cheynet, 2003; Harpman and Woollard, 1938; Loughner et al., 1989; Pinto, 1962; Sencimen et al., 2008; Smeele, 1988). A third ligament often discussed in the

literature, the anterior ligament of the malleus (AML), specifically refers to the ligamentous attachments of the DML and/or SML to the malleus within the tympanic cavity.

The course of the sphenomandibular ligament, as described in humans, is fairly consistent. The ligament attaches distally at the mandibular foramen to the lingula of the mandible and courses caudodorsally, medial to the temporomandibular joint (TMJ). Medial to the glenoid (mandibular) fossa of the temporal bone, some of the fibers of the SML enter the petrotympanic fissure and course through Huguier's canal (or chordal canal) along with the chorda tympani nerve and rostral tympanic artery. Those fibers coursing through the chordal canal attach to the malleus. Many authors, including Henry Gray (*Gray's Anatomy*, 1995), state that the majority of the proximal fibers of the SML attach to the spine of the sphenoid bone, hence the name sphenomandibular ligament. Varying degrees of attachment of the ligament to the spine of the sphenoid have been observed (Burch, 1966; Cameron, 1915; Loughner et al., 1989). Some authors maintain that the ligament has no sphenoid attachments and is therefore a true malleomandibular ligament (Burch, 1966; Cheynet et al., 2003; Merida Velasco et al., 1990; Rodriguez Vazquez et al., 1992; Toledo Filho et al., 1985).

There have been different proposals for the embryological origin and morphology of the discomalleolar ligament. Several authors believe that the DML is a remnant of the tendon of the primitive lateral pterygoid muscle (Cheynet et al., 2003; Eckerdal, 1991; Harpman and Woollard, 1938). In reptiles, the bones of the viscerocranium that are analogous to the auditory ossicles of the definitive mammalian middle ear are used for structural support of the jaw. For example, the articular, quadrate and columella bones in reptiles are homologous to the malleus, incus and stapes, respectively, in mammals. The primitive lateral pterygoid muscle (represented in mammals by the medial portion of the adductor mandibulae) was inserted on the articular bone and for this reason some have suggested that it is logical that the DML is a remnant of the tendon of this muscle. Others state that there is no evidence that the tendon of the lateral pterygoid muscle inserts onto the malleus

at any stage of development (Furtsman, 1963; Yuodelis, 1966). A mesenchymal origin of the DML as a fibrous tract with no muscle intermediate has been suggested by Rodriguez Vazquez *et al.* (1993). The origin of a fibrous tract from the mesenchyme surrounding Meckel's cartilage was described as extending "from the posterior area of the TMJ disc to the most lateral aspect of the tympanosquamosal fissure, finally inserting into the area of continuity of Meckel's cartilage with the malleus."

The discomalleolar ligament is described by most authors as an intrinsic ligament of the temporomandibular joint capsule that extends from the posterosuperior and medial end of the articular disc and enters the middle ear through the petrotympanic fissure to attach to the neck and anterior process of the malleus. There is disagreement regarding the strata of the temporomandibular joint capsule and the layer from which the ligament is derived. Pinto (1962) and Reese (1954) believed the ligament to be the continuation of the upper stratum of a bilaminar zone located dorsal to the disc of the TMJ. Smeele (1988) described the ligament specifically as the continuation of the intermediate lamina of a trilaminar zone that exists at the dorsal aspect of the temporomandibular disc. Variations have also been reported for the fibrous distribution of the DML. For example, reports of the degree of fibrous attachment to the walls of the petrotympanic fissure and to the malleus differed (Eckerdal, 1991; Rowicki and Zakrzewska, 2006; Smeele, 1988), and in some cases the ligament was reported to terminate within the walls of the petrotympanic fissure and therefore never reach the malleus (Loughner *et al.*, 1989; Smeele, 1988). There is debate over the factual existence of the DML due to the variation of its presence among specimens and/or the difficulty of dissection and visualization of the structure using modern imaging techniques. For example, Loughner *et al.* (1989) found the ligament in only 15 out of 52 dissections; Rowicki and Zakrzewska (2006) observed the ligament by endoscope as a band of thickened, flaccid tissue in the posteromedial aspect of the upper joint compartment in only 4 out of 14 specimens.

The anterior ligament of the malleus (AML) is the ligamentous component that originates from the anterior wall of the epitympanum and traverses the tympanic cavity to attach to the head and/or neck of the malleus. Some authors propose that the AML is formed by the convergence of the DML and SML (Burch, 1966, 1970; Cesarini *et al.*, 1991; Toledo Filho *et al.*, 1985), yet there is lack of agreement concerning the contribution of fibers from the DML to the AML (Burch, 1970; Cesarini *et al.*, 1991; Coleman, 1970; Eckerdal, 1991; Loughner *et al.*, 1989; Rodriguez Vazquez *et al.*, 1992; Sencimen *et al.*, 2008; Toledo Filho *et al.*, 1985). Others propose that the DML and AML are two unique structures in their attachments to the malleus (Coleman, 1970; Komori *et al.*, 1986; Rodriguez Vazquez *et al.*, 1998; Sencimen *et al.*, 2008). Rodriguez Vazquez *et al.* (1998) propose that the DML and AML are two clearly differentiated fibrous structures that connect the TMJ (DML) and mandible (SML via the AML) to the middle ear and that the AML represents the tympanic portion of the SML.

Anomalies of the temporomandibular joint, jaw malocclusion and dentition have been linked to various hearing problems. Temporomandibular joint disorders and jaw malocclusion may result in symptoms such as otalgia, tinnitus, vertigo, a stuffy sensation or auricular fullness, and hearing loss (Anagnostopoulou *et al.*, 2008; Candido dos Reis *et al.*, 2000; Lawrence *et al.*, 2001; Pinto, 1962). Loughner *et al.* (1989) reported two cases of disruption of articulation of the auditory ossicles during surgical procedures on the TMJ where partial or total deafness resulted on the ipsilateral side. In a case report by Candido dos Reis *et al.* (2000), otologic symptoms of a patient were directly related to jaw occlusive factors, and symptoms improved following correction with dentures. Lawrence *et al.* (2001) discussed the possible role of dentition as an exogenous factor contributing to presbycusis, indicating that the progressive decline of audition that occurs during aging is more pronounced with tooth loss. This study revealed that the loss of jaw structural support through the loss of molar teeth had an impact on hearing decline in both the ipsilateral and contralateral ears.

Due to the structural affiliation of both the discomalleolar and sphenomandibular ligaments to the malleus, a physiological relationship between these structures is likely, but has not been established. Several authors support a physiological role of the SML and its ability to transfer movement to the malleus (Burch, 1970; Dai *et al.*, 2007; Loughner *et al.*, 1989). Movement of the malleus has been observed when tension was placed on the ligament (Burch, 1970; Loughner *et al.*, 1989); however, the degree of tension on the malleus, by way of the SML, is dependent upon numerous features including the level of fibrous attachment to the walls of the petrotympanic fissure, the level of fibrous attachment to the walls of the spine of the sphenoid bone, the length and diameter of Huguier's (chordal) canal, and the volume of the ligament (Cheynet *et al.*, 2003; Rodriguez Vazquez *et al.*, 1998; Sencimen *et al.*, 2008). The ability of the DML to move the malleus is disputed (Coleman, 1970; Eckerdal, 1991; Komori *et al.*, 1986; Loughner *et al.*, 1989; Sencimen *et al.*, 2008; Smeele, 1988). Only a few reports have confirmed movement of the malleus when tension was placed on the DML by pulling on either the ligament itself or the caudal aspect of the joint capsule (Pinto, 1962; Rowicki and Zakrzewska, 2006). According to Rodriguez Vazquez *et al.* (1998), the movement of the malleus by the DML is dependent upon the degree of closure of the petrotympanic fissure during development and the degree of adhesion between the ligament and the edges of the petrotympanic fissure. Anagnostopoulou *et al.* (2008) stated that there is a correlation between the length of the petrotympanic fissure and symptoms of TMJ disorders, i.e., a long petrotympanic fissure favors hearing loss, otic fullness, and vertigo, and a short petrotympanic fissure favors tinnitus, otalgia, and vertigo. Dai *et al.* (2007) demonstrated that both the SML and AML provide mechanical support to the malleolar head and that structural change of these ligaments may affect middle ear sound transmission, resulting in conductive hearing loss.

Since hearing loss can result following dental procedures in dogs and since the ligaments connecting the TMJ and the malleus in man have clinical significance, it is hypothesized that the

equivalent of the sphenomandibular ligament as described in man also exists in the dog. The verification and description of this anatomical structure in the dog would support the existence of a jaw-ear connection and a potential physiological role in conductive hearing loss following jaw manipulation. The SML, rather than the DML, was examined in this study since the SML is a more likely candidate for effecting malleus movement, based on human studies. Further, because of the direct derivation of the SML from Meckel's cartilage and continuing questions of the existence of the DML in humans, there was a greater chance of identifying this structure in the dog.

Materials and Methods

Human Dissection Studies

An embalmed, adult human cadaver head obtained from the LSU Department of Kinesiology was sagittally sectioned and then reduced to two manageable blocks containing the temporal region. The two blocks were then decalcified in RDO rapid decalcifying agent (Apex Engineering Products Corporation, Aurora, IL) for eight days. The acid was neutralized by placing the blocks into a saturated solution of sodium bicarbonate and agitated. The blocks were maintained in a fresh solution of saturated sodium bicarbonate overnight. The blocks were then flushed under running water for several hours. The epitympanum was approached dorsally by dissection through the floor of the cranium using dissecting forceps to expose the head, neck, and ligaments of the malleus.

The remaining studies were performed using canine specimens. Approval for these studies was granted by the Louisiana State University Institutional Animal Care and Use Committee.

Developmental Studies

The cranial cartilages of two canine fetuses (Crown-Rump Length (CRL) = 11 cm and 6.5cm) were stained with Alcian blue to demonstrate the course and location of Meckel's cartilage. One fetus (CRL = 11 cm) had already been removed from the uterus and had been maintained in fixative for several years. The second fetus was obtained from the isolated and fixed uterus of a

mongrel dog that had been maintained for some time in a dilute formaldehyde solution. After removal from the zonary placenta and embryonic membranes, the fetus was placed in a formaldehyde solution for storage and later use. Before transferring into a 50% ethyl alcohol solution, the fetus was rinsed thoroughly under running water overnight. The fetus was stored in 50% ethyl alcohol for 5 months, with the solution replaced once during this period. The fetus was dehydrated in absolute ethyl alcohol for four days before staining. An Alcian blue staining solution was prepared (60% absolute ethyl alcohol, 40% acetic acid, 0.3 mg/ml Alcian blue) and the specimen was maintained in solution for 6 days with slow stirring. It was then transferred into a saturated sodium bicarbonate solution and left to slowly stir for twelve days, with the sodium bicarbonate solution replaced every three days. The specimen was rinsed thoroughly under running water overnight. The first specimen (CRL = 11 cm) was bleached using a solution of hydrogen peroxide (15 ml of 3% H₂O₂) and potassium hydroxide (85 ml of 1% KOH) for several days. Unfortunately, the head of this specimen collapsed due to over-bleaching and maceration and so was discarded and bleaching time was reduced for the second specimen. The second specimen was transferred to a 70% ethyl alcohol solution for storage following neutralization. The head was removed from the body and sagittally sectioned using a carbon steel razor blade. The specimen was then examined and photographed using a Stereomaster Digital Zoom dissecting microscope (Fisher Scientific, Waltham, MA).

A litter of lab-mix puppies that had aborted at full term, still enclosed within the embryonic membranes and zonary placenta, was donated for this study. These specimens were frozen until used. Two of the specimens were removed from the embryonic sac and placenta after thawing and the heads removed and sectioned sagittally. Four un-embalmed ears were dissected under a Stereomaster Digital Zoom dissecting microscope using dissecting forceps to carefully remove tissues obscuring the landmark branches of the mandibular nerve and the ligamentous remnant of Meckel's cartilage. A partially dissected left ear from one of the specimens was placed in water and macerated for four

days to clear away the soft tissues that concealed the view of the ligament. Structures were photographed using a Stereomaster Digital Zoom dissecting microscope.

Postnatal Dissection Studies

The following specimen were used for dissection: embalmed adult canine ears (n=5), embalmed and decalcified adult canine ears (n=8), un-embalmed adult canine ears (n=4), one embalmed two-month-old puppy ear and one embalmed one-month-old deaf puppy ear. Specimens were prepared for dissection by cutting into manageable blocks containing the tympanic region with affiliated ear structures, the TMJ and proximal mandible, including the mandibular foramen. Of these specimens, five embalmed and two un-embalmed specimens were used for preliminary studies.

All adult specimens were mongrel dog heads that were obtained from one of three sources. Eight of the specimens were purchased from Sargeant's Wholesale Biological (Bakersfield, CA). One specimen was obtained from the hound colony at the LSU School of Veterinary Medicine. The remaining specimens were procured following terminal surgery teaching labs. The specimen from the hound colony had previously been used for vascular studies. This specimen was heparinized (200U/kg, IV) thirty minutes prior to anesthesia administration (1mg/kg acepromazine (IM), 1 mg/kg small animal xylazine (IV), 12 mg/kg ketamine (IV)). The left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated caudally. The head was perfused at constant pressure (4 psi) with a 0.9% saline solution until fluids from the opened cranial vena cava ran clear. The head was then removed with cannulas in place and submerged in an ice bath for 2.5 hours before freezing. The specimen was thawed prior to injection of Microfil (Flow Tech, Inc., Carver, MA), a radio-opaque material, into the non-fixed vasculature. Two 35 cc syringes were used to simultaneously inject the prepared media into both common carotid arteries. A total of 210 cc was injected. Following x-ray and CT scanning, the head was sectioned into blocks and placed in 10% buffered neutral formalin (BNF) for fixation. The block containing the right ear structures and

proximal jaw was decalcified with Cal-Ex (Fisher Scientific, Pittsburgh, PA) for three months prior to dissection. The dogs obtained from the terminal surgery labs were decapitated prior to perfusion through the carotid with 10% BNF. The common carotid arteries were cannulated (#10 cannula) and both jugular veins were clamped prior to perfusion. Following perfusion, heads were submerged in a formaldehyde solution overnight. The heads were then sawed down into smaller blocks and stored in formaldehyde. Eight of the embalmed adult specimen blocks were decalcified using either Cal-Ex (Microfil specimen block (n=1), Sergeant's Wholesale specimen block (n=1)) or RDO (surgery lab specimen blocks (n=4), Sergeant's Wholesale specimen blocks (n=2)). Blocks were maintained in Cal-Ex for a minimum of 2 weeks and RDO for 9 days.

The two-month-old puppy (Labrador mix) was procured from the East Baton Rouge Animal Control facility following euthanasia. The carotid arteries were cannulated (#12 cannula) cranially and the caudal portion of the artery was ligated. The head was perfused with approximately 3 liters of 4% formaldehyde at a constant pressure of 4 psi. The head was removed from the body and sectioned along the mid-sagittal plane into right and left halves which were then submerged in a formaldehyde solution.

The one-month-old puppy (a Catahoula) was donated and euthanized after testing deaf in both ears. The left and right common carotid arteries were cannulated (#12 cannula) cranially and then ligated caudally. The specimen was then perfused with 10% BNF (4 L). Following perfusion, the head was removed and sectioned along the mid-sagittal plane into right and left halves which were submerged in a formaldehyde solution. The left ear was dissected for this study.

After preliminary dissections (n = 7) of adult specimens, it was determined that ligamentous attachments to the head and neck of the malleus could not be visualized through a dorsal approach into the epitympanic region using a rotary drill. A ventromedial approach was instead pursued, using

decalcified specimens, locating the region of distal attachment of the ligament at the mandibular foramen and then following proximally. The pharynx was removed and the lateral pterygoid muscle and distal attachment of the medial pterygoid muscle to the caudal margin of the ramus of the mandible were located and removed to expose the medial aspect of the TMJ and tympanic bulla. The mandibular foramen was observed upon reflection of the mylohyoid and pterygoid muscles. The three branches of the mandibular division of the trigeminal nerve (mylohyoid, inferior alveolar and lingual) and the chorda tympani nerve were used as landmarks in locating the mandibular foramen and the ligament. Once the distal portion of the ligament was located, it was traced proximally using a rotary drill and bone rongeurs of various sizes to carve through the tympanic bone in the calcified specimens. In the decalcified specimens, dissecting forceps were used to scrape through and pick away portions of the tympanic bone to follow the course of the ligament, visualized with a dissecting microscope (Stereomaster Digital Zoom). Specimens were photographed with a Powershot S3IS digital camera (Canon USA, Lake Success, NY) and Stereomaster Digital Zoom microscope.

Histological Studies

Four adult canine ears were submitted for histological serial sectioning. Two tissue blocks were from an East Baton Rouge Animal Control facility specimen. Following anesthesia, the left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated distally. The specimen was then perfused with 10% BNF (5 L). Following perfusion, the head was removed and cut down to two blocks containing the ear structures. The blocks were maintained in 10% BNF for one week. The blocks were rinsed thoroughly, cut down further and placed in a 5% formic acid solution for decalcification. Following four weeks in formic acid, the blocks were cut down further and placed into histology cassettes and transferred into RDO rapid decalcifier for an additional 5 days because of inadequate decalcification. A third specimen was acquired from an embalmed dog half head from Sergeant's Wholesale Biological. After cutting this specimen to fit into a histology

cassette, it was submerged in Cal-Ex for two weeks. A fourth specimen was submitted for serial sectioning following gross dissection of the distal ligament (refer to preparation method for Sergeant's Wholesale block using RDO in the Canine Post-partum Dissection Studies section above). All tissues were processed using a Leica TP1020 tissue processor (Leica Microsystems, Wetzlar, Germany) and then embedded in paraffin for histological sectioning. Sections were cut at 12 μ m using a rotary microtome (Lipshaw Mfg. Co., now ThermoFisher, Waltham, MA). Slides from two specimens were then double stained using Verhoff elastin and Masson trichrome stains. The stained slides were viewed under the dissecting microscope and photographed.

Jaw Manipulation Studies

The tympanic bulla in ten un-embalmed adult canine ears was opened so that the manubrium of the malleus could be viewed and used to gauge ossicle movement while the jaw and tongue were manipulated. The mandibular nerve branches, chorda tympani nerve and ligament were dissected and tension placed on each of the structures, in combination and then individually, to observe any movement of the malleus.

Results

Human Dissection Studies

Human dissections were used to identify the attachments and courses of the malleolar ligaments for application in locating the canine SML equivalent. Dissection of the human ear revealed two distinct components of the anterior malleolar ligament (Fig. 5.1). A band of fibrous tissue was observed attaching to the head of the malleus and in its anterior extent, the lateral component of this fibrous structure fanned out and appeared to become continuous with the joint capsule of the TMJ. This observation supports previous descriptions of the human discomalleolar ligament (Burch, 1966; Cesarani *et al.*, 1991; Cheynet, 2003; Meng *et al.*, 2011; Pinto, 1962; Rodriguez Vazquez *et al.*, 1992, 1993, 1998; Sencimen *et al.*, 2008). The medial component of the

anterior malleolar ligament (SML portion) was more cord-like and paralleled the chorda tympani nerve in its course. This portion of the ligament appeared to occupy a bony passageway separate from that of the chorda tympani nerve. The distal attachments of this ligament were not observed in either of the two specimens.

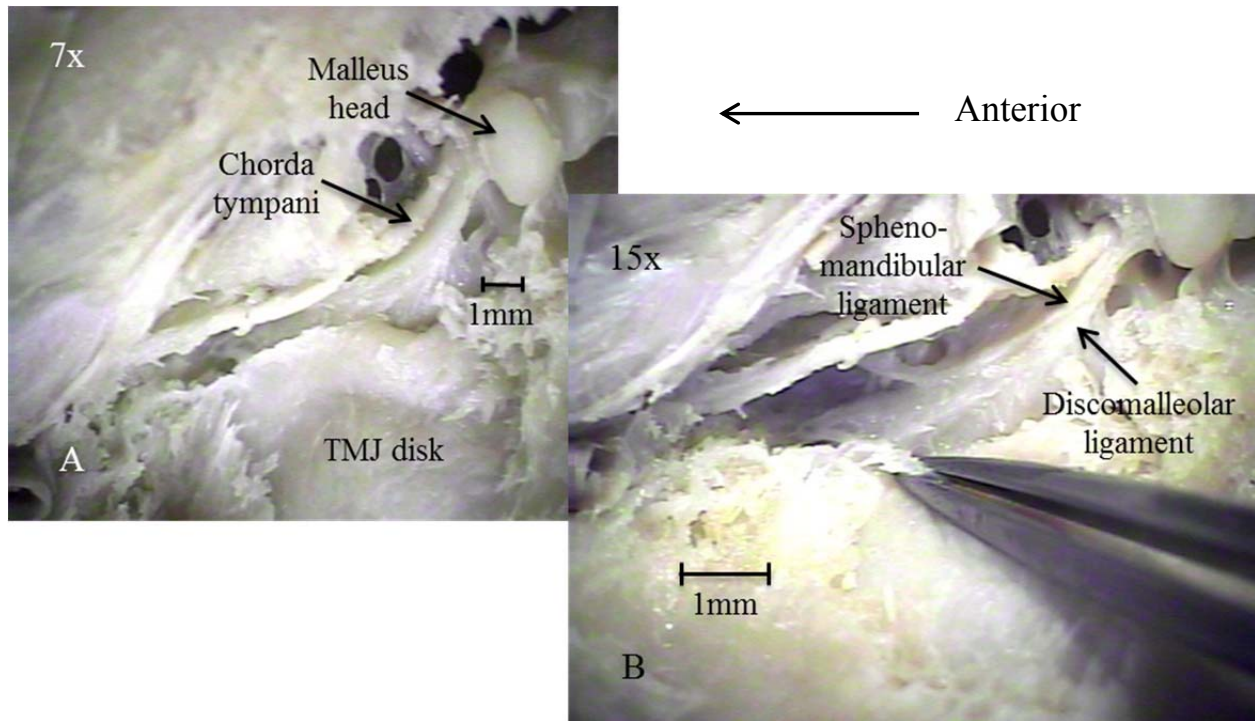


Figure 5.1. Dissection of the human left ear revealed two distinct components of the anterior malleolar ligament, the discomalleolar ligament laterally and the sphenomandibular ligament medially. (A) 7x magnification. (B) 15x magnification. Left dorsal view.

Developmental Studies

Alcian blue staining of a canine fetus of approximately 6 weeks gestational age showed the continuity of Meckel's cartilage from the mandible to the malleus (Fig. 5.2). The distal component of this cartilaginous structure was contained within a canal that was located within the ventral region of the body of the mandible, with its distal-most extent at the mandibular symphysis. At the level of the origin of the mandibular ramus, the cartilaginous structure extended caudodorsally at about a 120° angle and terminated as the malleus and incus.

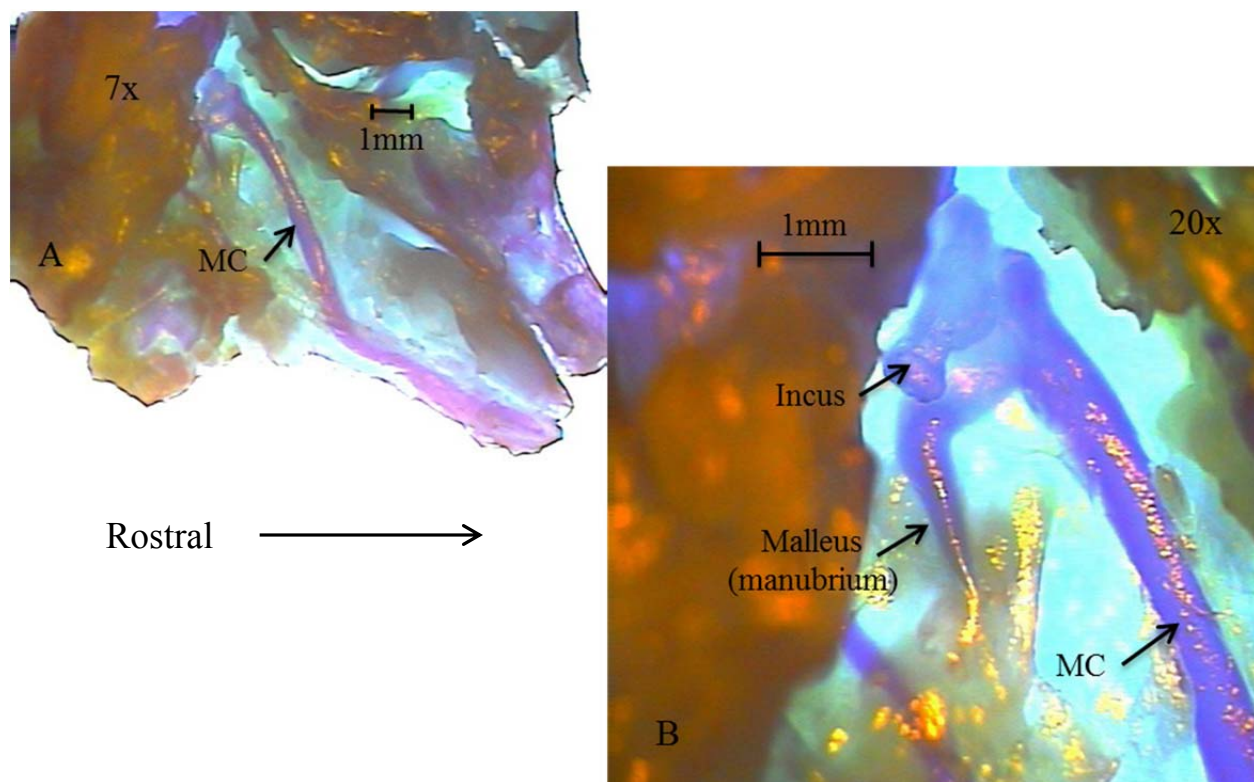


Figure 5.2. Alcian blue staining of a canine fetus at approximately 6 weeks gestational age showed the continuity of Meckel's cartilage (MC) from the mandible to the malleus. (A) Left side of the sagittally sectioned head at 7x magnification, demonstrating the entire course of Meckel's cartilage. (B) Proximal portion of Meckel's cartilage and its continuity with the malleus at 20x magnification. Left medial view.

A ligamentous connection between the mandible and malleus was observed in all dissected newborn puppy ears ($n = 4$). The ligament was semitransparent and located medial to the branches of the mandibular nerve, a branch of cranial nerve V (Fig. 5.3). The ligament extended from the medial aspect of the mandible to the rostral process of the malleus; therefore, the ligament in the newborn puppy is a true malleomandibular ligament (MML). Following maceration of the soft tissues in one of the specimens, it was clearly noted that the rostral process of the malleus passed lateral to the rostradorsal limb of the tympanic annulus (Fig. 5.3). The MML was located medial to the branches of the mandibular nerve and appeared to converge with the chorda tympani nerve proximally (Fig.

5.4). In one of the specimens, the malleus was excised and the distal part of the ligament was cut from the medial jaw to demonstrate the connection between the malleus and the ligament (Fig. 5.5).

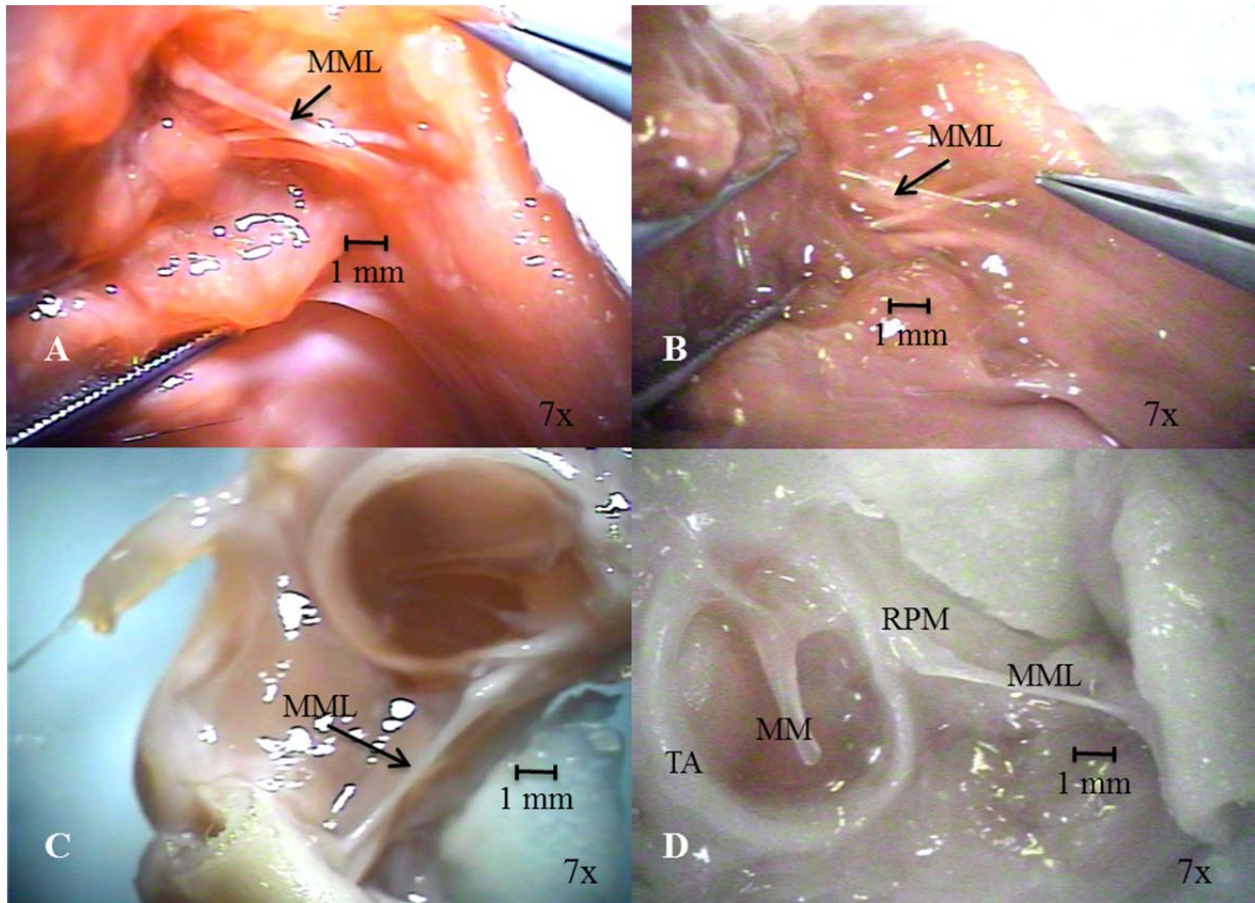


Figure 5.3. Dissection of the right ear of a newborn puppy revealed a ligamentous connection between the mandible and malleus (MML). (A) Fresh tissue. (B) Macerated for two days. (C) and (D) Macerated for four days. The rostral process of the malleus was observed passing lateral to the rostro-dorsal limb of the tympanic annulus (C) and (D). MM = manubrium of malleus, MML = malleomandibular ligament, RPM = rostral process of malleus, TA = tympanic annulus. Right ventromedial view.

Postnatal Dissection Studies

A ligament was observed extending from the medial side of the mandible to the rostroventral aspect of the tympanic bulla in a 2 month old puppy (Fig. 5.6). Distally, the ligament was located medial to the branches of the mandibular nerve and ventral to the chorda tympani nerve. As the

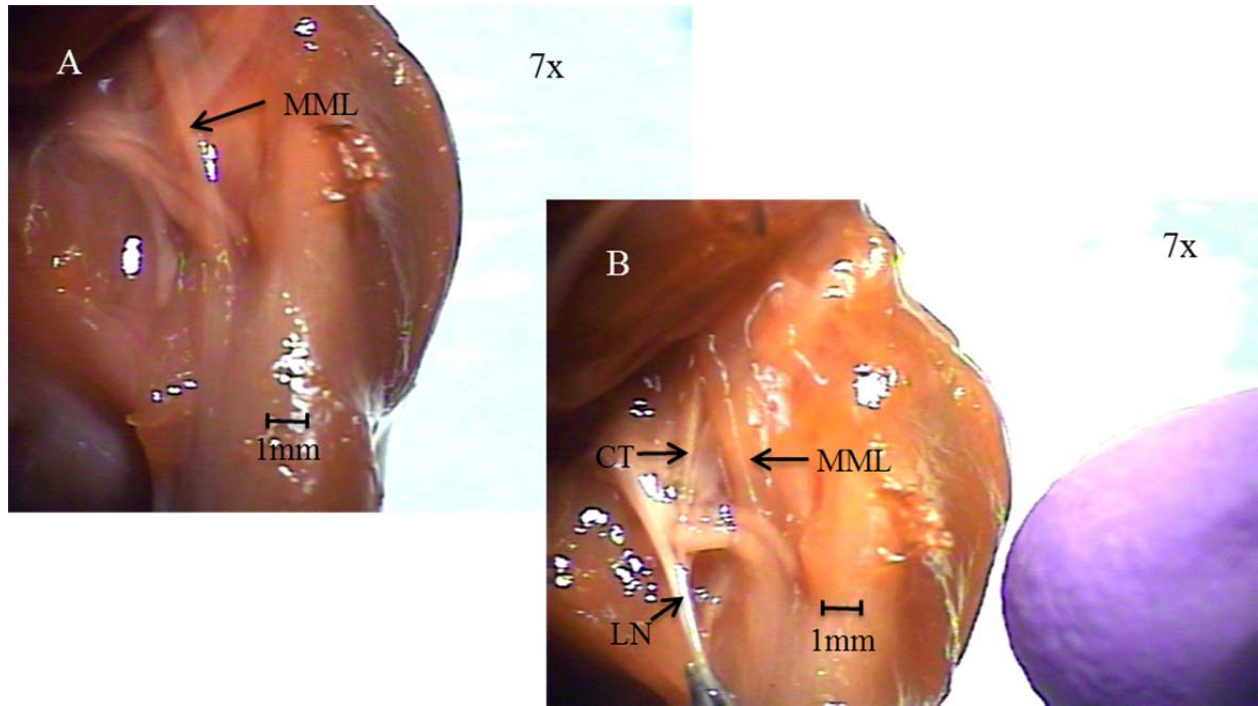


Figure 5.4. (A) The MML was located medial to the branches of the mandibular nerve and (B) appeared to converge with the chorda tympani nerve proximally. Tension has been placed on the lingual nerve in (B) to show the chorda tympani. CT = chorda tympani nerve, LN = lingual nerve, MML = malleomandibular ligament. Right ventral view.

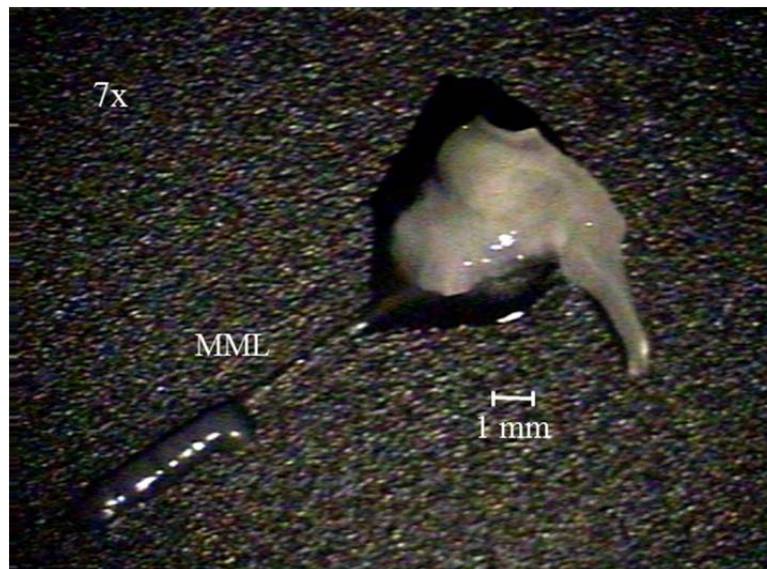


Figure 5.5. Excised malleus and the distal portion of the ligament cut from the medial jaw to demonstrate the connection between the malleus and the malleomandibular ligament (MML).

ligament extended proximally, it coursed lateral and caudal to the chorda tympani nerve. The majority of fibers of the proximal ligament had a strong attachment to the rostroventral region of the tympanic bulla, lateral to the tympanic annulus; however, a limited number of fibers did not attach at the bulla but instead coursed along the rostradorsal limb of the tympanic annulus, immediately caudal and lateral to the chorda tympani nerve. The ligament could not be followed to an attachment on the malleus due to an apparent loss of fibers to the walls of the bony canal in which it coursed.

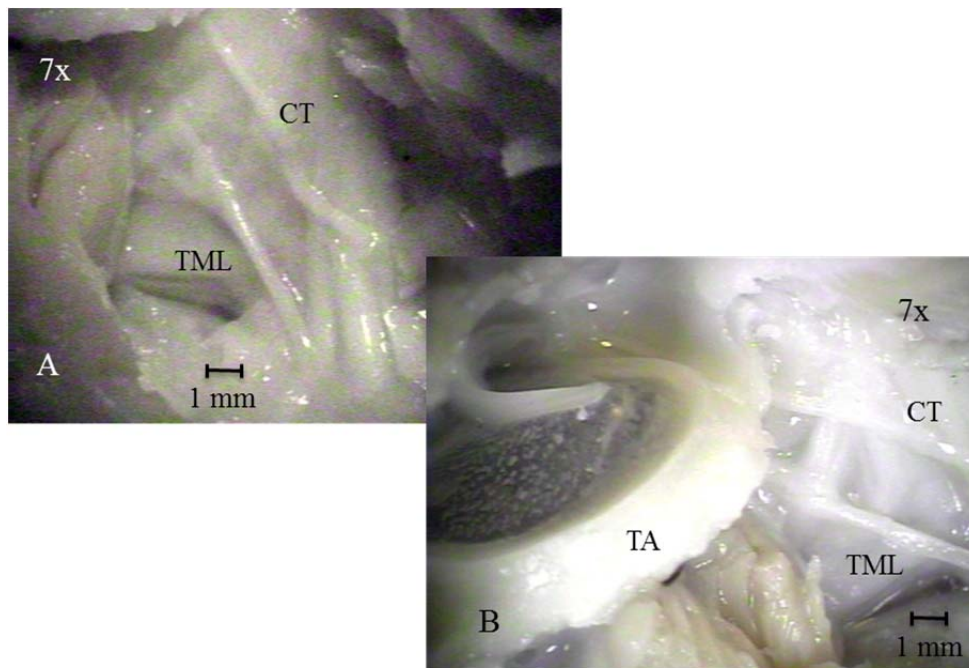


Figure 5.6. A ligament was observed extending from the medial side of the mandible to the rostroventral aspect of the tympanic bulla in this 2 month old puppy. (A) Medial view of the distal portion of the ligament (TML), where it is located medial to the branches of the mandibular nerve and ventral to the chorda tympani. (B) Attachment of the proximal portion of the ligament to the rostro-ventral region of the tympanic bulla, lateral to the tympanic annulus (TA). The chorda tympani (CT) was observed coursing dorsal and then rostral to the ligament. The medial and ventral portions of the tympanic bulla have been removed in this specimen. Left medial view.

In all adult canine and the one and two-month-old puppy specimens, the distal portion of the ligament appeared as a thickening of the rostral border of a semi-transparent fascia that covered the medial side of the TMJ (Fig. 5.7). Bifurcation of the ligament was observed in several adult specimens ($n = 9$) (Fig. 5.8). The majority of the proximal fibers had a strong attachment to the

rostroventral aspect of the tympanic bulla, lateral to the tympanic annulus (tympanomandibular ligament, TML), while a smaller component of the ligament traversed a foramen with the chorda tympani and then coursed caudal and lateral to the nerve. In most specimens, the thin ligamentous structure broke while attempting to follow its course. In several specimens ($n = 4$), it appeared that both the nerve and ligament coursed within the same canal; in other specimens ($n = 2$) there appeared to be a thin bony separation between the ligament and the nerve. In some specimens ($n = 4$), the ligament was followed through the entire canal but was broken where it could not be followed past a fibrous loop that perpendicularly crossed the rostral process of the malleus (Fig. 5.9). In one adult canine specimen, a portion of the ligament was observed attaching to the medial side of the joint capsule, while a small branch entered a foramen along with the chorda tympani (Fig. 5.10). The continuity of the ligament to the malleus could not be documented in any of the adult specimens. The ligament could be followed proximally from its attachment to the medial aspect of the mandible to the tympanic bulla, but the smaller component of the ligament could not be tracked completely through its bony canal to terminate as an attachment to the malleus. What appeared to be ligamentous attachments to the head of the malleus were observed in one specimen; however, continuity between these attachments and the distal ligament could not be demonstrated (Fig. 5.11).

Histological Studies

Four adult specimens were serially sectioned for histological examination of the middle ear to determine any ligamentous attachments to the head, neck, and/or rostral process of the malleus and the relationship of this ligament to the chorda tympani nerve. A tissue block from the dissected specimen depicted in Fig. 5.9 was sectioned and stained (Verhoef-Masson). A connective tissue, distinct from the neighboring bone, was observed attaching to the head and rostral process of the malleus (Fig. 5.12). This unique tissue also appeared to line the bony canal containing the chorda tympani nerve.

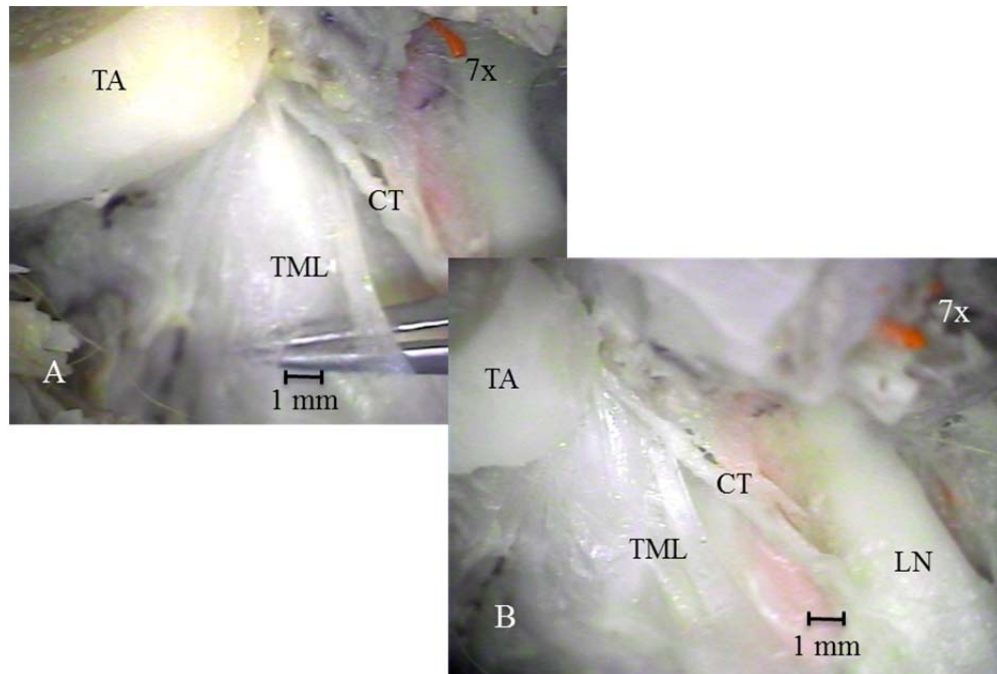


Figure 5.7. In all adult dog head dissections, the ligament appeared as a thickening of the rostral border of a semi-transparent fascia that covered the medial side of the TMJ. The forceps have been placed lateral to the fascia in (A). CT = chorda tympani nerve, LN = lingual nerve, TA = tympanic annulus, TML = tympanomandibular ligament. Left medial view.

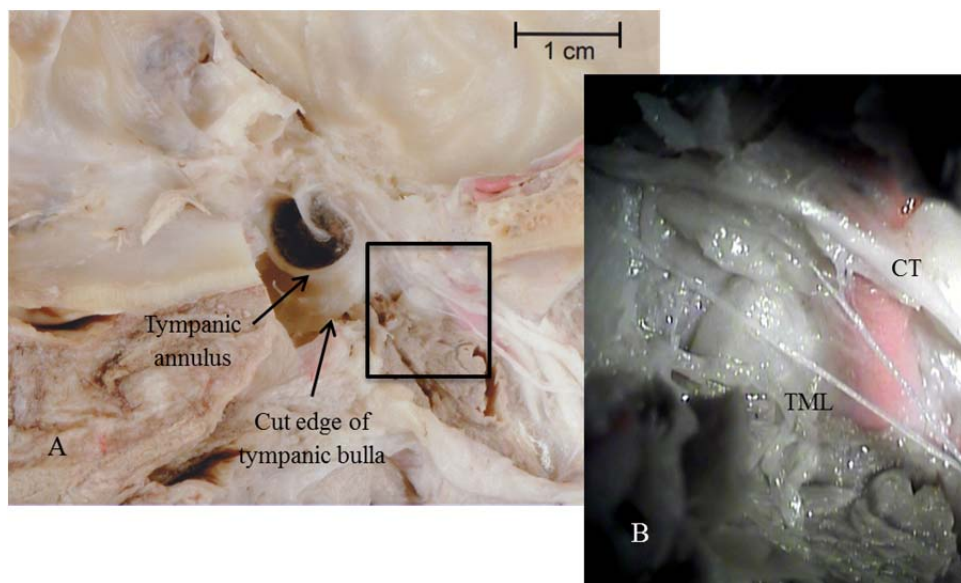


Figure 5.8. Bifurcation of the TML was observed in several adult specimens. The majority of fibers attached to the rostro-ventral aspect of the tympanic bulla (tympanomandibular ligament, TML). A smaller quantity of fibers coursed caudal and lateral to the chorda tympani nerve (CT). The medial and ventral portions of the tympanic bulla have been removed in this specimen. (B) Enlargement of the boxed region shown in (A). Left medial view.

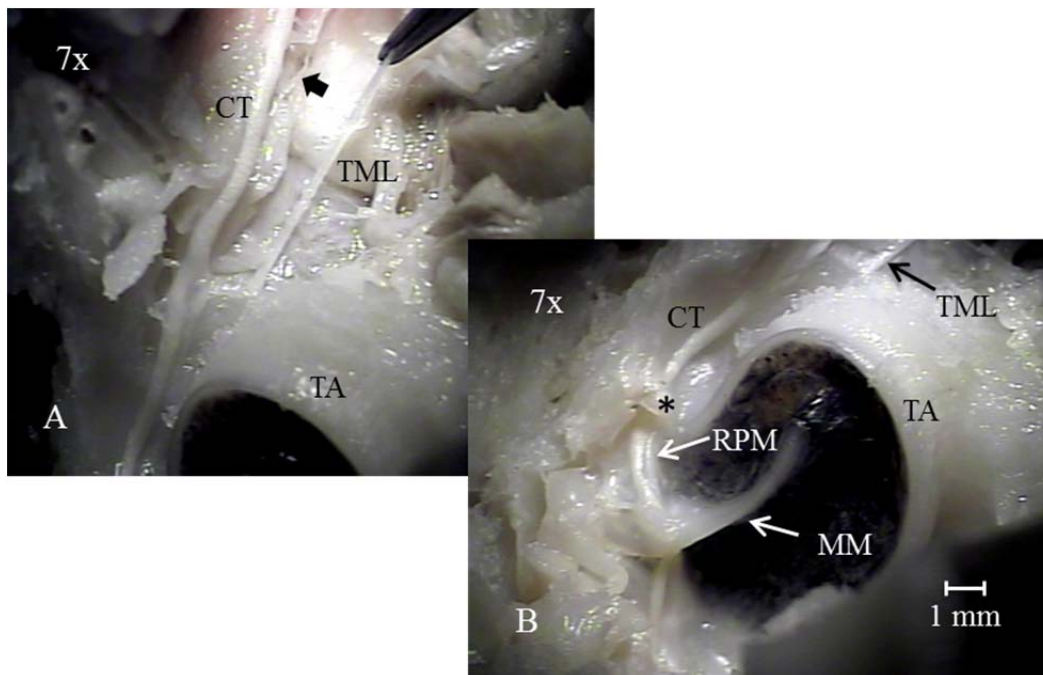


Figure 5.9. In several specimens, the ligament (TML) was broken where it could not be followed past a fibrous loop (asterisk in (B)) that perpendicularly crossed the rostral process of the malleus (RPM). The finer component of the ligament is demonstrated in (A) by the thick arrow. The medial and ventral portions of the tympanic bulla have been removed in this specimen. CT = chorda tympani nerve, MM = manubrium of malleus, TA = tympanic annulus. Left medial view.

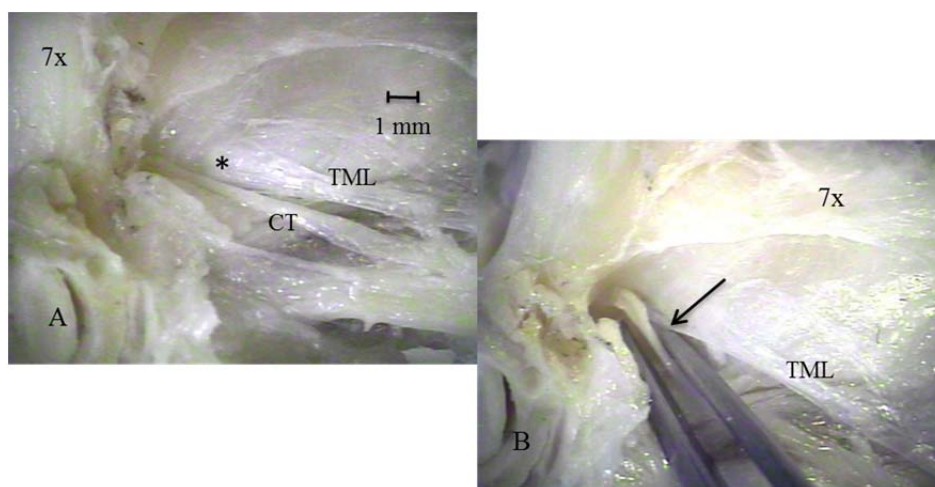


Figure 5.10. In one adult specimen, a portion of the ligament (TML) was observed attaching to the medial side of the joint capsule (asterisk in (A)). A small component of the ligament entered a foramen along with the chorda tympani nerve (arrow in (B)). The chorda tympani nerve (CT) in (B) has been displaced medially by forceps. Right ventral view.

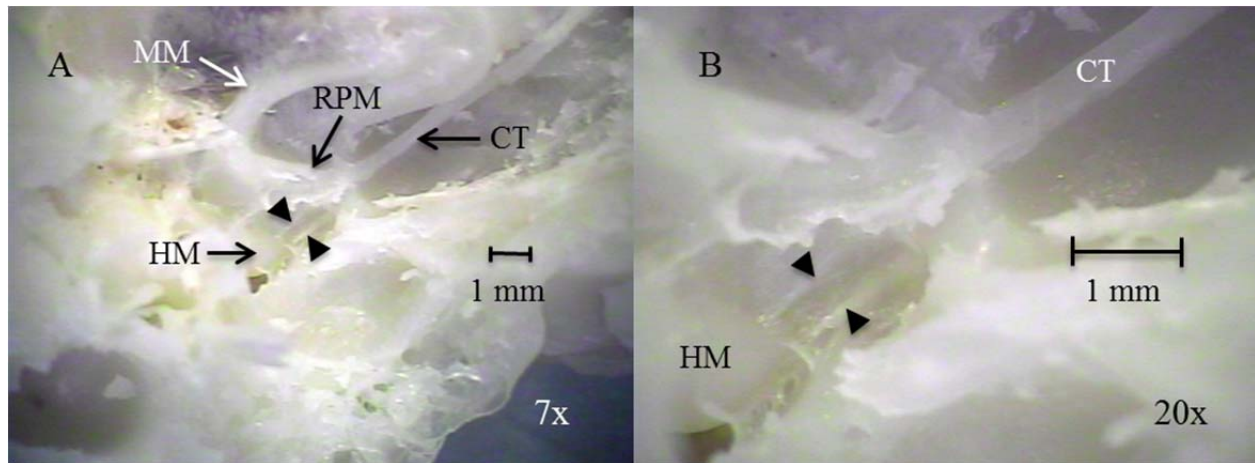


Figure 5.11. Continuity of the ligament to the malleus could not be documented in any of the adult specimens. What appeared to be ligamentous attachments (arrowheads) to the head of the malleus (HM) were observed in this specimen; however, continuity between these attachments and the distal ligament could not be demonstrated. (A) 7x magnification. (B) 20x magnification. CT = chorda tympani nerve, MM = manubrium of malleus, RPM = rostral process of malleus. Right dorsal view.

Three tissue blocks containing the tympanic bulla, structures of the middle ear, and tympanic cavity (epitympanum, tympanic cavity proper, and hypotympanum) were submitted for histological sectioning. Results from two of these blocks were inconclusive. The serial sections from one of the blocks revealed a connective tissue structure coursing through a bony canal and then becoming continuous with a sheet-like connective tissue that had attachments to the malleus, incus and chorda tympani nerve (Figs. 5.13 – 5.16). The canal containing the ligamentous structure was located between the tympanic annulus and the adjacent tympanic bone. In several sections, fibers from the ligament were observed attaching to the wall of the bony canal within which it was contained (Fig. 5.14). It was noted that the ligament occupied a canal separate from that of the chorda tympani; however, connective tissue was noted within the canal of the chorda tympani nerve that appeared to be continuous with the sheet-like connective tissue observed attaching to the malleus and incus (Figs. 5.15 and 5.16).

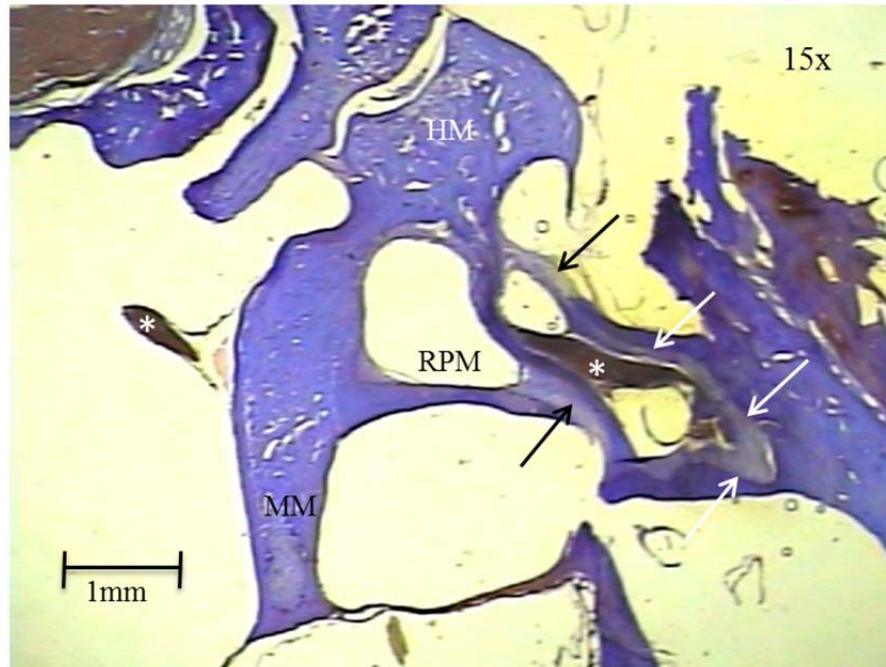


Figure 5.12. Oblique sagittal histological section through the canine middle ear that has been stained using Verhoeff-Masson. A connective tissue distinct from the neighboring bone was observed attaching to the head (HM) and rostral process (RPM) of the malleus (black arrows). This unique tissue also appeared to line the bony canal (white arrows) containing the chorda tympani nerve (asterisks). MM = manubrium of malleus.

Jaw Manipulation Studies

The tympanic bulla in ten un-embalmed adult ears was opened so that the manubrium of the malleus could be viewed and used to gauge movement of the malleus while the jaw and tongue were manipulated. Movement of the malleus did not occur when the jaw was hyperextended or laterally displaced, nor was movement observed when the tongue was pulled laterally. Pulling on the mandibular nerve branches, chorda tympani nerve and the distal ligament, individually and in then in combination, did not affect movement of the malleus.

Discussion

The equivalent of the sphenomandibular ligament (SML), a remnant of Meckel's cartilage that makes a connection between the jaw and middle ear in humans, also exists in the dog. In humans, the SML courses from the medial aspect of the mandible, with the majority of fibers

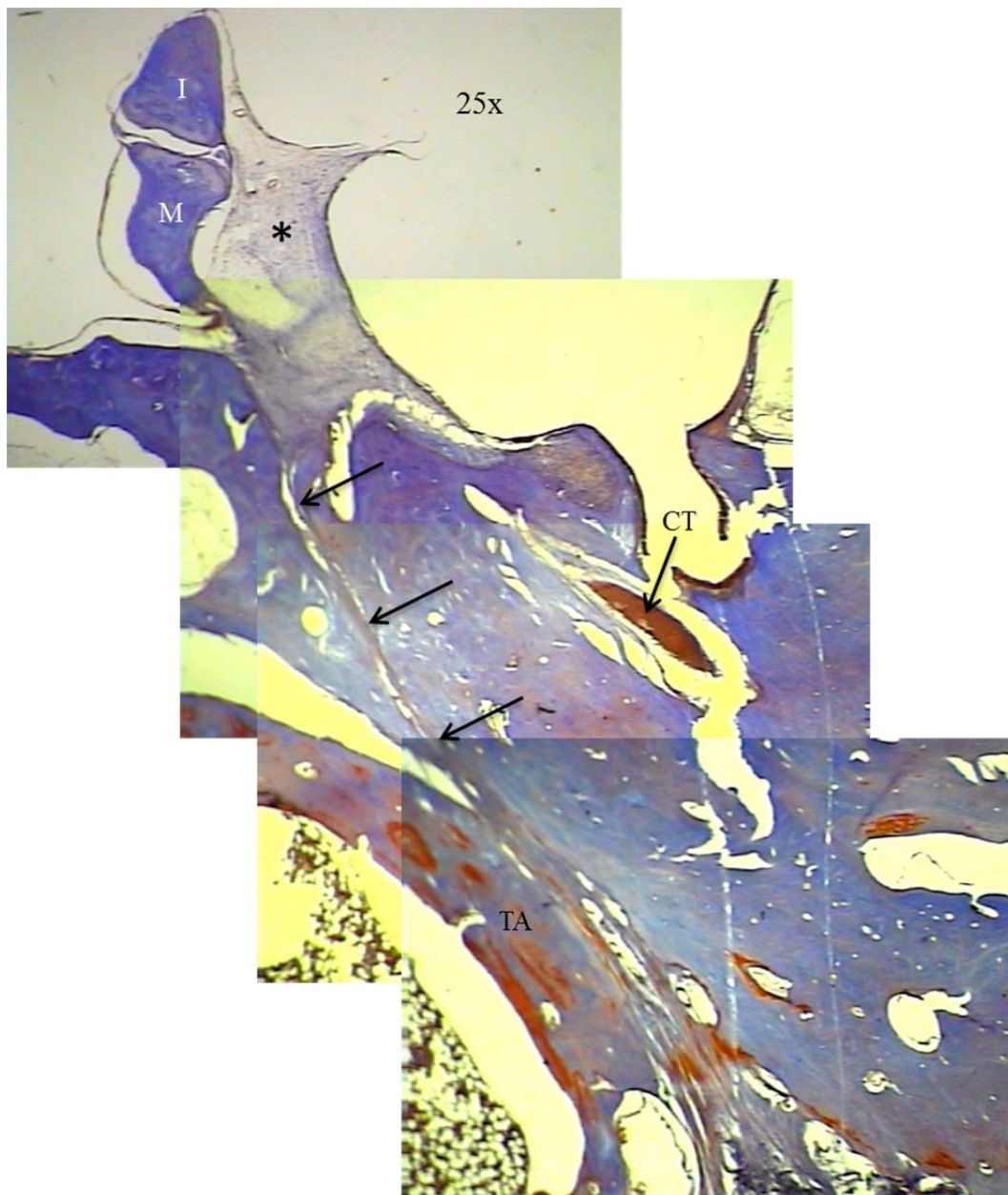


Figure 5.13. Sagittal histological section through the canine middle ear that has been reconstructed from several regions of the slide that were photographed at 25x magnification. A connective tissue structure was observed coursing through a bony canal (arrows) located between the tympanic annulus and the surrounding tympanic bone. The ligament became continuous with sheet-like connective tissue (asterisk) that had attachments to the malleus (M) and incus (I).

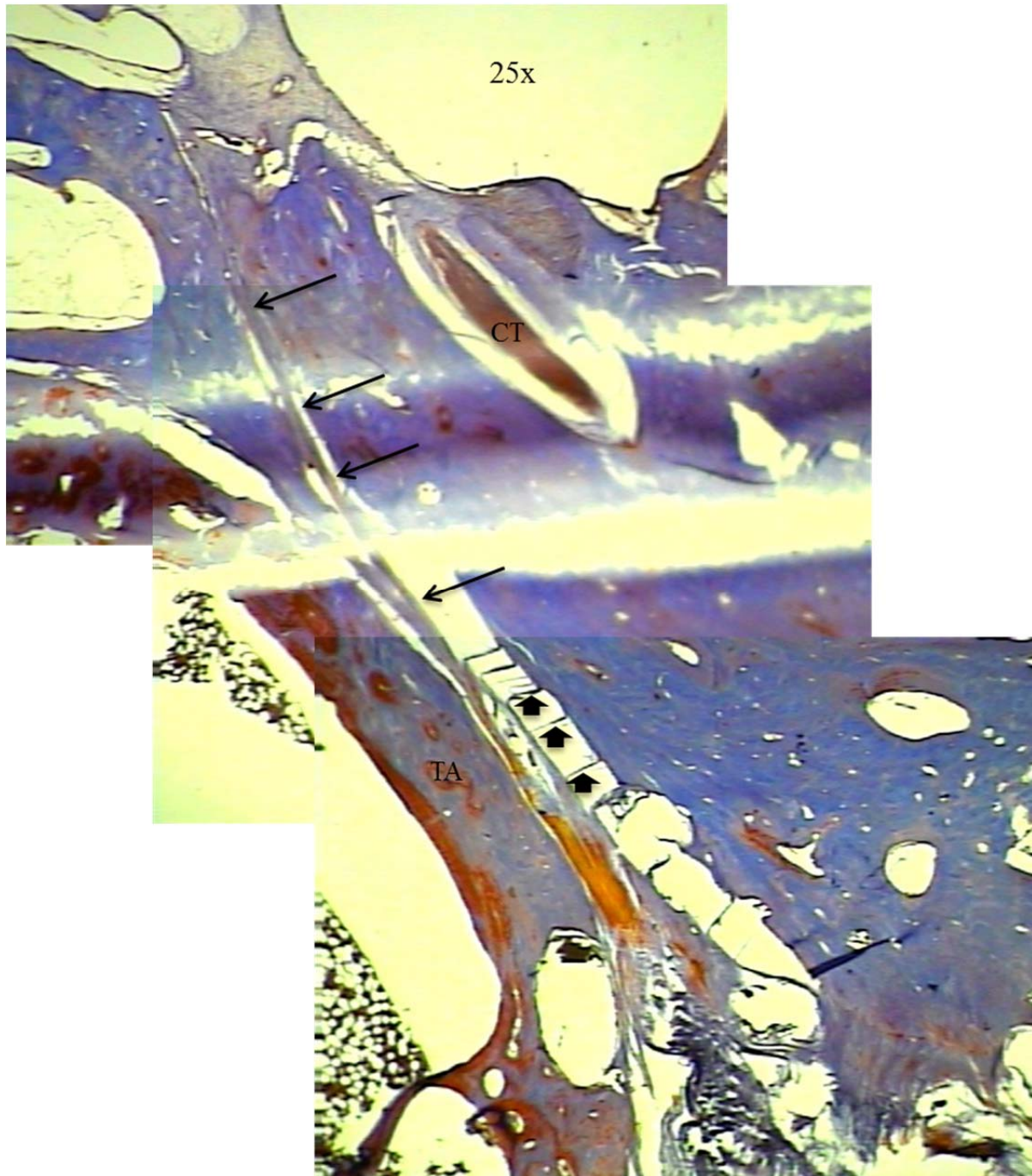


Figure 5.14. Sagittal histological section through the canine middle ear that has been reconstructed from several regions of the slide that were photographed at 25x magnification. The canal containing the ligament (thin arrows) was located between the tympanic annulus (TA) and the surrounding tympanic bone. The fibrous attachments of the ligament to the canal wall are demonstrated in this section (thick arrows).

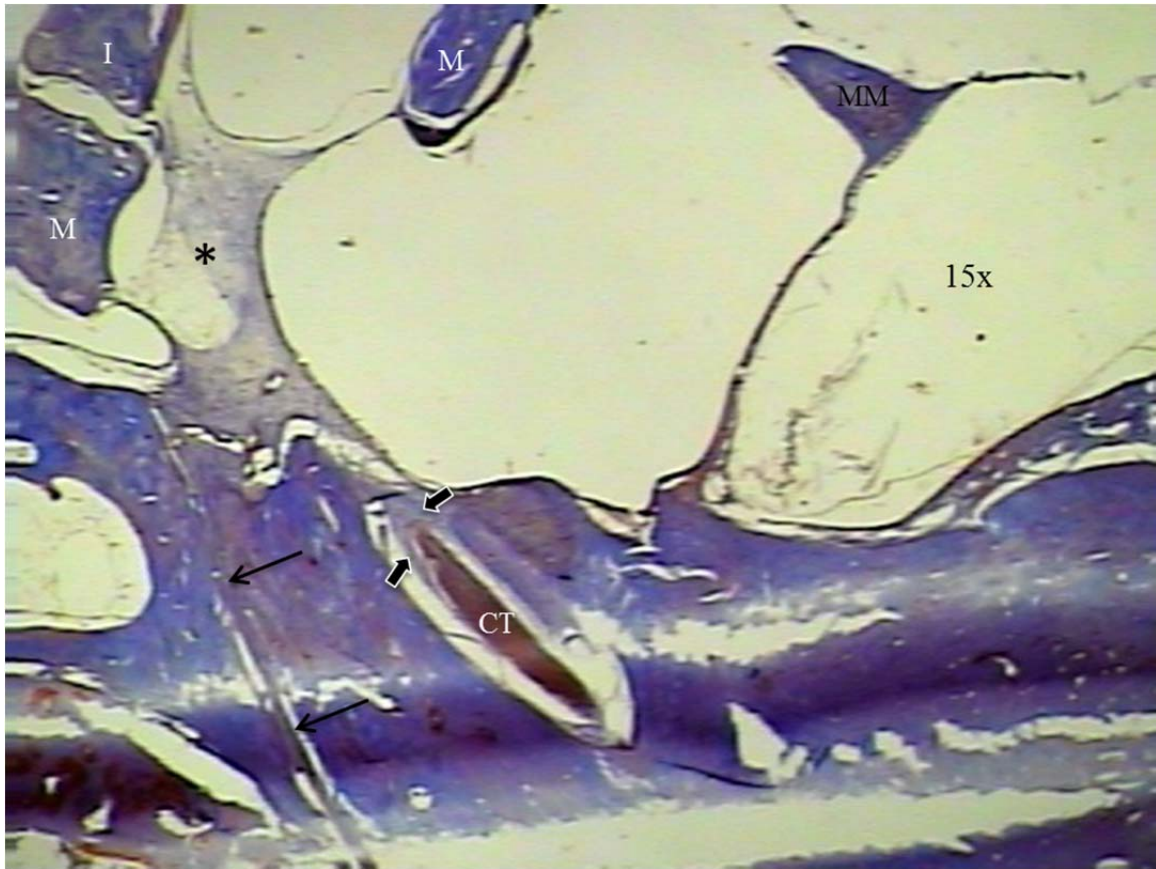


Figure 5.15. The attachments of the sheet-like connective tissue (asterisk) to the malleus (M) and incus (I) and the connective tissue (thick arrows) extending into the canal of the chorda tympani nerve (CT) are demonstrated in this section.

attaching to the spine of the sphenoid bone. The spine of the sphenoid bone does not exist in the dog, so an exact equivalent of this structure between man and dog cannot exist. Comparison of this structure between species must therefore be from a developmental standpoint. In a 6 week-old canine fetus, Meckel's cartilage is demonstrated as a continuous structure from the lower jaw to the malleus and incus, two of the bones of the middle ear (Fig. 5.2). Dissection of the newborn puppy ear demonstrated a ligamentous connection between the medial mandible and rostral process of the malleus (RPM) (Fig. 5.3). The RPM in these newborn specimens extended rostrally and beyond the enclosure of the tympanic annulus. This pronounced extension of the RPM was not observed in any of the older specimens. To verify that the observed ligamentous structure was indeed attached to the

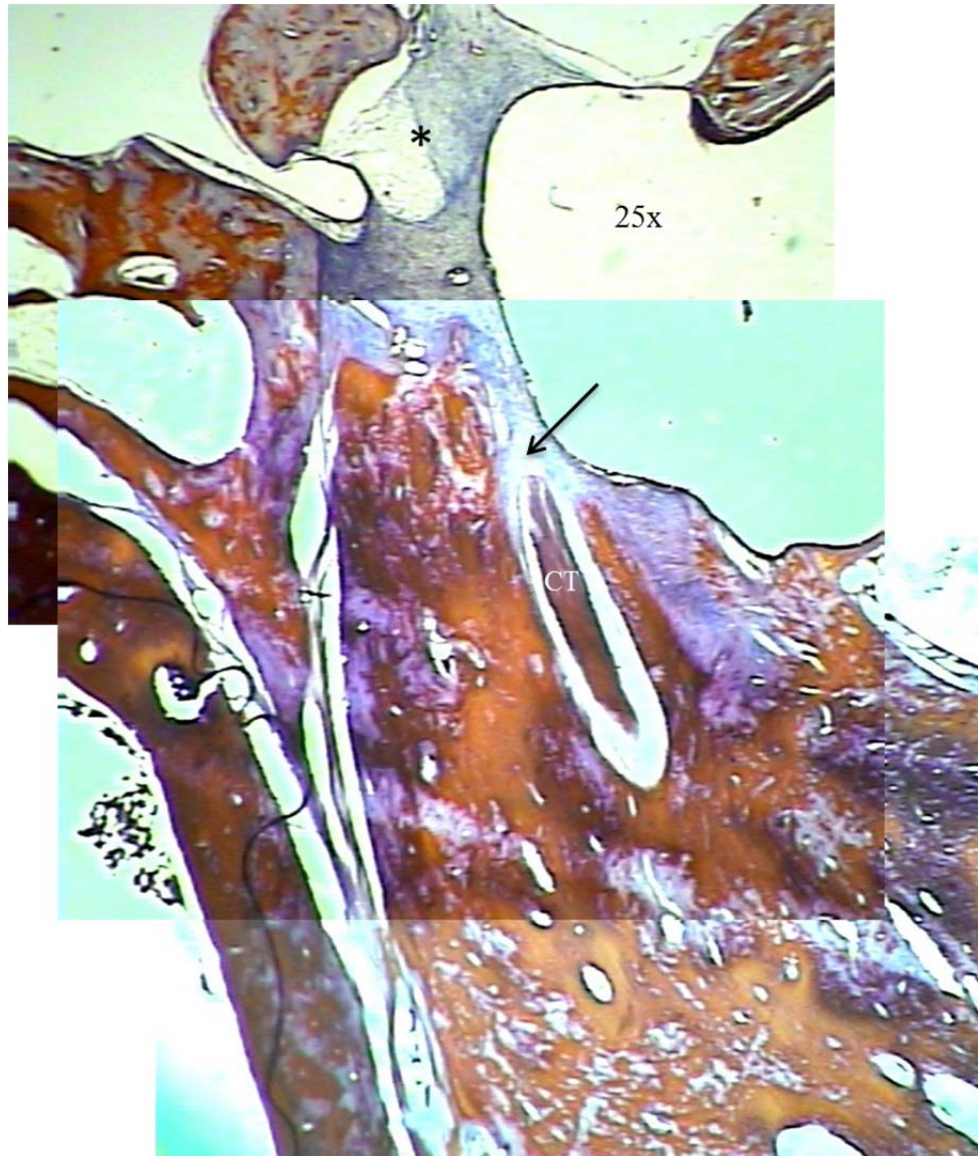


Figure 5.16. Sagittal histological section through the canine middle ear that has been reconstructed from several regions of the slide that were photographed at 25x magnification. In this section, the connective tissue (arrow) extending into and partially lining the canal of the chorda tympani nerve (CT) appears to be continuous with the sheet-like connective tissue (asterisk) that attaches to the malleus and incus.

RPM rather than a component of the annulus, the malleus was excised and the distal portion of the ligament was cut from the medial mandible (Fig. 5.5). From these findings, the ligamentous structure observed in the newborn puppy is presumed to be the remnant of Meckel's cartilage and is therefore comparable with the human SML. A ligamentous structure extending from the medial aspect of the canine jaw to the rostral tympanic bulla was observed in all adult specimens (Figs. 5.7 – 5.9). This

structure was also observed in a two-month-old puppy (Fig. 5.6) and a one-month-old deaf puppy. There was a noted bifurcation of this connective tissue structure in several specimens, with the majority of fibers attaching to the rostroventral aspect of the tympanic bulla, lateral to the tympanic annulus (Figs 5.6 – 5.9). The larger component of the ligament, that part attaching to the tympanic bulla, has been dubbed the tympanomandibular ligament (TML). Burch (1966) also described a tympanomandibular ligament in humans, which corresponds to the distal part of the sphenomandibular ligament extending from the lingula of the mandible and attaching to the spine of the sphenoid bone. Burch observed that in the majority of specimens (45 out of 51) the fibers of the SML did not attach directly to the sphenoidal spine, but rather attached to the tympanic bone at the petrotympanic fissure. For this reason, Burch stated that the SML might be more correctly termed the TML.

The smaller component of the ligament identified in this study did not attach at the tympanic bulla, but was observed coursing in a canal parallel and caudolateral to the chorda tympani nerve. A continuation of the ligament to the malleus was not observed grossly in any of the dissected adult specimens. Only in the newborn puppy was this ligament grossly identified as a true malleomandibular ligament (MML), extending continuously from the medial aspect of the mandible to the rostral process of the malleus. It should be noted that in the newborn puppy, the tympanic bulla has not developed. The tympanic bulla in the dog, a structure not present in man, expands the hypotympanum, and by extension the cavity of the middle ear. It seems likely that the development and ossification of the bulla encloses the ligament between the tympanic annulus and the adjacent tympanic bone. Indeed, in several histological serial sections of the tympanic region, a fibrous structure was noted coursing between the tympanic annulus and surrounding tympanic bone to a point of attachment to the malleus and incus via a connective tissue expansion within the tympanic cavity (Figs. 5.13 and 5.14). Additional developmental studies of this region are necessary to

understand the conformational changes that take place with the development and ossification of the tympanic bulla.

The attachment of the SML to the head of the malleus is grossly visible in the human middle ear (Fig. 5.1). There are two ligamentous attachments to the malleus in man, the SML and DML, which converge as the anterior ligament of the malleus. An equivalent structure was not seen grossly in the canine middle ear; however, histological serial sectioning revealed a sheet-like connective tissue structure that attached to the canine malleus and incus (Figs. 5.12 – 5.13, 5.15 – 5.16). In some sections, an extension of this sheet-like tissue appeared to occupy and partially line the bony canal of the chorda tympani nerve (Fig. 5.12, 5.15 – 5.16).

It was not conclusive from the current study if a DML equivalent exists in the dog. The jaw articulation in relation to the head of the malleus is located farther rostrally (anteriorly) in the dog than in humans. This orientation, along with the fact that the canine anterior (rostral) ligament of the malleus was not visible grossly, would make it very difficult to visualize the DML in the canine if it does exist. Histological sectioning targeted to the region between the joint capsule and the middle ear could potentially reveal this structure in the dog, but a large bony expansion between these two structures would make sectioning difficult without complete decalcification.

It was hypothesized that tension on the canine SML equivalent at its distal (mandibular) end would produce movement of the malleus. Ten tympanic bullae from 5 adult un-embalmed dogs were opened to determine malleus displacement while the jaw, tongue, branches of the mandibular nerve and TML were manipulated. Overextension and lateral displacement of the jaw did not produce movement of the malleus in any of these specimens. Malleolar movement was not observed while the tongue was pulled laterally, nor when tension was applied to the mandibular nerve branches, and/or the distal component of the TML ligament. From these studies, overextension of the jaw, tongue

manipulation, and/or tension on the tympanomandibular ligament (TML) in the adult dog does not produce movement of the malleus.

An estimate of the rate of occurrence of deafness in dogs following dental procedures under anesthesia cannot be made as the annual average number of dental procedures in dogs performed under anesthesia is not known. These outcomes are rare, as there were only 62 reported cases of hearing loss following anesthesia between the years 2002 and 2009 (Stevens-Sparks and Strain, 2010). If there is sufficient anatomic variability among dogs so that in some the ligament between the jaw and malleus can move, it is unknown how many specimens would need to be dissected and manipulated to discover one where jaw extension or overextension would produce movement of the malleus. In humans, it is speculated that the degree of tension on the malleus by means of the SML is dependent upon numerous features, including the level of fibrous attachment to the walls of the petrotympanic fissure, the level of fibrous attachment to the walls of the spine of the sphenoid bone, the length and diameter of Huguier's (chordal) canal, and the volume of the ligament (Cheynet *et al.*, 2003; Rodriguez Vazquez *et al.*, 1998; Sencimen *et al.*, 2008). Based on the current study, the canine TML appears to occupy a bony passageway adjacent and parallel to the chorda tympani nerve. The canal of the TML is likely formed due to the ossification of the tympanic bulla around the ligament. It is probable that ability of the TML to produce movement of the canine malleus is determined by the same conditions applicable in humans, namely the degree of fibrous attachments to the canal walls, the length and diameter of the canal itself and the volume of the ligament. Further studies are needed to follow and identify the conformational changes that take place in this region as the tympanic bulla develops and ossifies.

Another factor for consideration is that of age. Stevens-Sparks and Strain (2010) noted that an association between age and hearing loss following anesthesia in dogs and cats may exist, with older animals more likely to be affected. This cannot be confirmed because of the absence of data on

numbers of dogs undergoing the procedures at different ages and the percentage developing deafness. An association between age and osteoporosis is well known. In a study by Wong, *et al.* (1985), bone composition and viability decreased with age and microfracture occurrence increased with age. It might be that changes in bone density could affect the conformation and diameter of cranial bony canals and fissures. It is also noted that aging leads to an increase in the stiffness of ligaments (Osakabe *et al.*, 2001). According to Anagnostopoulou *et al.* (2008), in humans “an aged and rigid ligament with sufficiently small initial length (equal to that of the petrotympanic fissure) may exert a high enough force to the malleus to cause a critical displacement of the auditory ossicles and elicit tinnitus.” Apparently, the human petrotympanic fissure, which contains both the DML and SML, can vary in length. It is not known if the canal containing the canine tympanomandibular ligament varies in length among species. Given that the jaw articulation is located much further rostrally to the middle ear in the dog in comparison to its immediate ventral location to the middle ear in the human, a small initial length of the canine TML is never likely. However, increased rigidity of the canine TML with age is possible. A combination of ligament stiffening and changes in bone conformation, as may occur with age, may create suitable conditions for middle ear effects with TML displacement. Further studies on age-related changes of the canine tympanic bone are required to assess this potential effect.

In summary, the equivalent of the human SML does exist in the canine and is represented as a fibrous remnant of Meckel’s cartilage. In the newborn puppy, the ligament is a true malleomandibular ligament because it extends from the medial mandible to the rostral process of the malleus with no intermittent attachments. The development and ossification of the tympanic bulla is what likely entraps the ligament within a bony passageway, making it difficult to grossly view the complete course of the ligament. In the adult, the majority of the ligamentous fibers attach to the tympanic bulla, thus this portion of the ligament has been dubbed the tympanomandibular ligament.

Based on histological findings, it appears that a smaller component of the ligament continues through a canal, located between the tympanic annulus and the surrounding tympanic bone, to become continuous with a connective tissue sheet within the cavity of the middle ear that has attachments to the malleus and incus. Tension on the canine TML does not result in movement of the malleus. Future studies on the development and ossification of the tympanic bulla in relation to Meckel's cartilage, the MML and the TML are necessary for a complete understanding of the ligaments of the canine middle ear. Further studies are also needed to define age related changes of the canine TML and tympanic bone to determine if older dogs may be more susceptible to a dislocation of the middle ear ossicles as a result of jaw manipulation.

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CHAPTER 6
A STUDY OF THE CANINE TYMPANIC ANNULUS, CHORDAL CANAL, AND
FORAMINA OF THE CHORDA TYMPANI NERVE AND
ROSTRAL TYMPANIC ARTERY

Introduction

The tympanic annulus is an important landmark structure in reference to the location and course of the chorda tympani nerve and ligaments of the malleus. Although the annulus is mentioned as the attachment site of the tympanic membrane and the rostral ligament of the malleus (Evans, 1993a), a thorough anatomical description of the orientation of the canine tympanic annulus has not been reported.

There are two small foramina that are located medial to the jaw articulation that are not identified in canine anatomy textbooks. One of these foramina is located caudally and lies medial to the retroarticular process; the other has a more rostral location and is located in a depression that is medial to the mandibular fossa. Foramina adjacent to the medial jaw articulation have been mentioned in earlier sources in reference to the rostral tympanic artery and the chorda tympani nerve but have not been specifically identified (Evans, 1993b; Ghoshal, 1975). According to Evans and Kitchell (1993), the chorda tympani nerve usually passes through a small canal in the rostradorsal wall of the tympanic bulla and emerges from the petrotympanic fissure at a small opening medial to the retroarticular process. He further states that when this canal fails to develop, the opening is through the rostrolateral wall of the tympanic bulla. In Evan's (1993b) description of the rostral tympanic artery, he states "It usually leaves the maxillary artery medial to the temporomandibular joint and enters one of the small foramina located in a depression medial to the joint." Similarly, Ghoshal (1975) describes the rostral tympanic artery as entering the temporal bone through a small opening located in a depression medial to the temporomandibular joint. Because no diagrams

accompany these descriptions, it is difficult to specifically identify which of the two foramina located medial to the joint articulation accommodates the chorda tympani nerve and which accommodates the rostral tympanic artery.

The purpose of this study was to provide a complete description of the location and orientation of the tympanic annulus and to specifically identify which structures occupy the two foramina that are located medial to the temporomandibular joint.

Materials and Methods

Skeletal Studies

The tympanic bulla was removed in two adult macerated skulls and a two-month-old puppy macerated skull, leaving the tympanic annulus intact for observation. A portion of the tympanic bone that contained the tympanic annulus, malleus and chordal canal was isolated and observed under higher magnification using a Stereomaster Digital Zoom dissecting microscope (Fisher Scientific, Pittsburgh, PA). The ventral aspects of twenty macerated adult canine skulls of unknown age were examined to determine the prevalence of the foramina at the medial aspect of the TMJ. This region was also observed in 2 two-month-old macerated puppy skulls. After removing as much tissue as possible from the head, the adult canine skulls were put into a warm water bath and allowed to macerate for two weeks. The skulls were rinsed with water and then put into a 3.5% hydrogen peroxide solution for three to five days. The skulls were rinsed and scrubbed under running water and then allowed to dry at room temperature for several days. The two-month-old puppy heads were defleshed and then placed in a warming chamber for desiccation. The heads were then macerated for two weeks using dermestid beetles at the LSU Natural Science Museum. This method of maceration was preferred over water maceration in the underdeveloped skulls to maintain the fibrous connective tissues holding the bony elements of the skull together. The skull was rinsed under running water and left to dry at room temperature for several days.

The head of a puppy (CRL = 18 cm) from a litter of lab-mix puppies that had been aborted at full term, still enclosed within the embryonic membranes and zonary placenta, was donated for this study. After removal from the placenta and embryonic membranes, the puppy's head was imaged in the LSU SVM Veterinary Teaching Hospital by computed tomography (CT) (GE Lightspeed 16-Slice CT, Waukesha, WI) at 0.625 mm resolution.

Dissection Studies: Chorda Tympani Nerve

The following specimens were used for dissection: embalmed adult canine ears (n=5), embalmed and decalcified adult canine ears (n=7), un-embalmed adult canine ears (n=4), one embalmed two-month-old puppy ear and one embalmed one-month-old deaf puppy ear. Specimens were prepared for dissection by cutting into manageable blocks containing the tympanic region with affiliated ear structures, the TMJ and proximal mandible, including the mandibular foramen. Of these specimens, five embalmed and two un-embalmed specimens were used for preliminary studies.

All adult specimens were mongrel dog heads that were obtained from one of two sources. Eight of the specimens were purchased from Sargeant's Wholesale Biological (Bakersfield, CA), pre-embalmed and latex-injected. The remaining specimens were procured following terminal surgery teaching labs. The dogs obtained from the terminal surgery labs were decapitated prior to perfusion through the carotid arteries with 10% buffered neutral formalin (BNF). The common carotid arteries were cannulated (#10 cannula) and both jugular veins were clamped prior to perfusion. Following perfusion, heads were submerged in a formaldehyde solution overnight. The heads were then sawed into smaller blocks and stored in formaldehyde. Seven of the embalmed adult specimen blocks were decalcified using either Cal-Ex (Sergeant's Wholesale specimen block (n=1)) or RDO (surgery lab specimen blocks (n=4), Sergeant's Wholesale specimen blocks (n=2)). Blocks were maintained in Cal-Ex for a minimum of 2 weeks and RDO for 9 days.

The two-month-old puppy (Labrador mix) was procured from the East Baton Rouge Animal Control facility following euthanasia. The carotid arteries were cannulated (#12 cannula) cranially and the caudal portion of the artery was ligated. The head was perfused with approximately 3 liters of 4% formaldehyde at a constant pressure of 4 psi. The head was removed from the body and sectioned along the mid-sagittal plane into right and left halves which were then submerged in a formaldehyde solution.

The one-month-old puppy (a Catahoula) was donated and euthanized after testing deaf in both ears. The left and right common carotid arteries were cannulated (#12 cannula) cranially and then ligated caudally. The specimen was then perfused with 10% BNF (4 L). Following perfusion, the head was removed and sectioned along the mid-sagittal plane into right and left halves which were submerged in a formaldehyde solution. The left ear was dissected for this study.

A ventromedial approach was used to locate the chorda tympani nerve where it separated from the lingual nerve. The pharynx was removed and the lateral pterygoid muscle and distal attachment of the medial pterygoid muscle to the caudal margin of the ramus of the mandible were located and removed to expose the medial aspect of the TMJ and tympanic bulla. The lingual nerve was located and followed caudodorsally to where the chorda tympani nerve was observed leaving its caudal surface and crossing the inferior alveolar and mylohyoid nerves. The chorda tympani was traced through its bony canal using a rotary drill and bone rongeurs of various sizes to carve through the tympanic bone in the calcified specimens. In the decalcified specimens, dissecting forceps were used to scrape through and pick away portions of the tympanic bone to follow the course of the nerve, visualized with a dissecting microscope (Stereomaster Digital Zoom). Specimens were photographed with a Powershot S3IS digital camera (Canon USA, Lake Success, NY) and Stereomaster Digital Zoom microscope.

A litter of Labrador-mix puppies that had aborted at full term, still enclosed within the embryonic membranes and zonary placenta, was donated for this study. These specimens were frozen until used. Two of the specimens were removed from the embryonic sac and placenta after thawing and the heads removed and sectioned sagittally. Four un-embalmed ears were dissected under a Stereomaster Digital Zoom dissecting microscope using watchmaker and Graefe forceps. Structures were photographed using a Stereomaster Digital Zoom dissecting microscope.

Dissection Studies: Rostral Tympanic Artery

Embalmed specimens dissected for this study (n=8) included: latex-injected adult canine ears (n=3) and latex-injected and decalcified adult canine ears (n=5). Most specimens were prepared for dissection by reducing the heads to manageable blocks of the tympanic region that contained the ear structures, the temporomandibular joint (TMJ) and the proximal mandible. Specimens were decalcified by submerging in either Cal-Ex (Fisher Scientific, Pittsburgh, PA) (n=1) for a minimum of 14 days, or RDO rapid decalcifying agent (Apex Engineering Products, Aurora, IL) (n=4) for 9 days. Following decalcification, acid in the decalcifying agent was neutralized by placing the blocks into a saturated solution of sodium bicarbonate and agitated. The blocks were maintained in a fresh solution of saturated sodium bicarbonate overnight. The blocks were then flushed under running water for several hours and then transferred to a 4% formaldehyde solution for storage.

Most specimens were mongrel dog heads that were obtained from one of three sources.

- Six specimens were obtained from dogs purchased from Sargeant's Wholesale Biological (Bakersfield, CA).
- One dog was obtained from the hound colony at the LSU SVM. One ear from this specimen was dissected.
- One dog was obtained following a terminal surgery lab. One ear from this specimen was dissected.

The study was approved by the LSU Institutional Animal Care and Use Committee. The specimens purchased from Sergeant's Wholesale Biological were pre-embalmed and latex-injected dogs purchased for dissection by first year veterinary students. The dog (approximately 16 kg) obtained from the LSU SVM hound colony was heparinized (200U/kg, IV) thirty minutes prior to anesthesia administration (1mg/kg acepromazine (IM), 1 mg/kg small animal xylazine (IV), 12 mg/kg ketamine (IV)). The left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated caudally. The head was perfused at constant pressure (4 psi) with a 0.9% saline solution (9 L) until fluids from the opened cranial vena cava ran clear. The head was removed with cannulae still in place and submerged in an ice bath for 2.5 hours before freezing. The specimen was later thawed and prepared for perfusion and injection. The head was perfused at constant pressure (4 psi) with 10% BNF through the common carotid arteries. Following perfusion, the head was placed neck down into a solution of 10% BNF and stored under refrigeration for 10 days. The vasculature was injected via the left and right common carotid arteries with 20 ml of 1% ammonium hydroxide to neutralize the acidity of the formaldehyde in preparation for latex injection. Two 35 cc syringes were filled with filtered red latex (Carolina Biological, Burlington, NC) and the latex was slowly injected into the common carotid arteries. Cheesecloth soaked in acetic acid was placed into the vertebral venous sinus and spinal central canal to minimize latex seepage. The head was then stored in a formaldehyde solution for later dissection.

The dog obtained following the terminal surgery lab was heparinized (200U/kg, IV) before euthanasia. The left and right common carotid arteries were cannulated (#10) cranially and tied off caudally before perfusion with a 1% saline solution (10 L). The head was then perfused with 4% formaldehyde. The head was removed from the body, sagittally sectioned, and placed in a formaldehyde solution. The left half was used to cannulate and latex-inject the external carotid artery at its origin. In this specimen, the maxillary artery was ligated immediately proximal to the caudal

alar foramen to maintain the pressure and enhance vascular filling of the maxillary artery and its branches in the region adjacent to the jaw articulation.

Specimens were dissected under a Stereomaster Digital Zoom dissecting microscope so that minute branches of the maxillary artery could be identified and traced. The maxillary artery was approached medially following removal of the tongue, pharynx, and pterygoid muscles. Of special interest were the branches of the maxillary artery that were in close proximity to the temporomandibular joint. The inferior alveolar and caudal deep temporal arteries were used as landmarks to describe the origin and orientation of other small arterial branches in this region. A rotary drill tool with ball-end miniature carbide bur attachment and bone rongeurs were used to track the vessels through the tympanic bone in the calcified specimens. In the decalcified specimens, dissecting forceps were used to scrape through and pick away portions of the tympanic bone to follow the course of the arteries. Photographs of these dissections were made using the Stereomaster Digital Zoom dissecting microscope.

Corrosion Casting Studies

A dog (approximately 16 kg) from the LSU SVM hound colony was anesthetized (1 mg/kg acepromazine (IM), 1 mg/kg small animal xylazine (IV), 12 mg/kg ketamine (IV)) and the left and right common carotid arteries were cannulated (#10 cannula) cranially and ligated caudally. The head was perfused with a 0.9% saline solution until fluids from the opened cranial vena cava ran clear. The head was then removed with cannulae still in place and submerged in an ice bath for 2.5 hours before freezing. The specimen was thawed for vascular injection with Batson's #17 solution (Polysciences, Inc., Warrington, PA). A freshly prepared Batson's #17 working solution consisted of 200 ml base, 24 ml catalyst, and 24 drops of promoter. Red color pigment (5 mg) was added for clear observation of the vessel casts. All of the prepared media was injected simultaneously into both common carotid arteries under a constant pressure (5 psi) while the specimen was submerged in an

ice bath. Both jugular veins were ligated and paper towels were used to block seepage from the vertebral venous sinus, the transverse vertebral canals and the spinal central canal. The specimen was left in an ice water bath for three hours until complete polymerization of the Batson's solution had occurred. The specimen was then transferred into a container and frozen in water. A band saw was used to cut the specimen while still embedded in ice to prevent tearing of the corrosion-casted vasculature. The head was sagittally sectioned and then two axial cuts were made in each half, one immediately caudal to the orbit and the other immediately caudal to the nuchal crest, to generate a block that contained the entire temporal bone. The two specimens were macerated in water for one month. The specimens were next flushed in running water for two days. The left block was further macerated in a 1% potassium hydroxide solution for 2 days. The right block was further macerated in water for two weeks, followed by maceration with 5% potassium hydroxide for 2 days. Photographs were taken to document these dissections using a Powershot S3IS digital camera (Canon USA, Lake Success, NY). Higher magnification images of the finer vasculature were taken using the Stereomaster Digital Zoom dissecting microscope.

Results

Skeletal Studies

The tympanic annulus forms a loop, with its rim located just inside the external auditory meatus. The external meatus forms a complete bony ring; however, the rim of the tympanic annulus is not complete dorsally. The rostradorsal region of the annular rim makes a 90° bend, just ventral to the rostral process of the malleus, and extends toward the petrous bone where it terminates at the petrotympanic fissure, while the caudodorsal rim of the annulus terminates opposite the rostral process of the malleus (Figs. 6.1 and 6.2). The annulus is more oblong than circular in shape, with its apex directed rostromedially. There are dorsal and ventral limbs of the annular rim, where the dorsal limb is located rostrally and the ventral limb is located caudally (Fig. 6.2). The tympanic annulus was

fully developed and ossified within the newborn puppy, while the tympanic bulla was not (Figs. 6.3 and 6.4). The chorda tympani nerve in the newborn puppy passed lateral to the rostradorsal limb of the tympanic annulus and dorsal to the rostral process of the malleus (Fig. 6.4). This same orientation was noted in the adult as well (Fig. 6.5). The rostral extension of the rostradorsal limb of the tympanic annulus formed a small loop-like structure immediately rostral to the rostral process of the malleus (Figs. 6.5 and 6.6). This loop appeared to have a very high fibrous content as it always had to be cut and could not be broken away or pulled away during dissection.

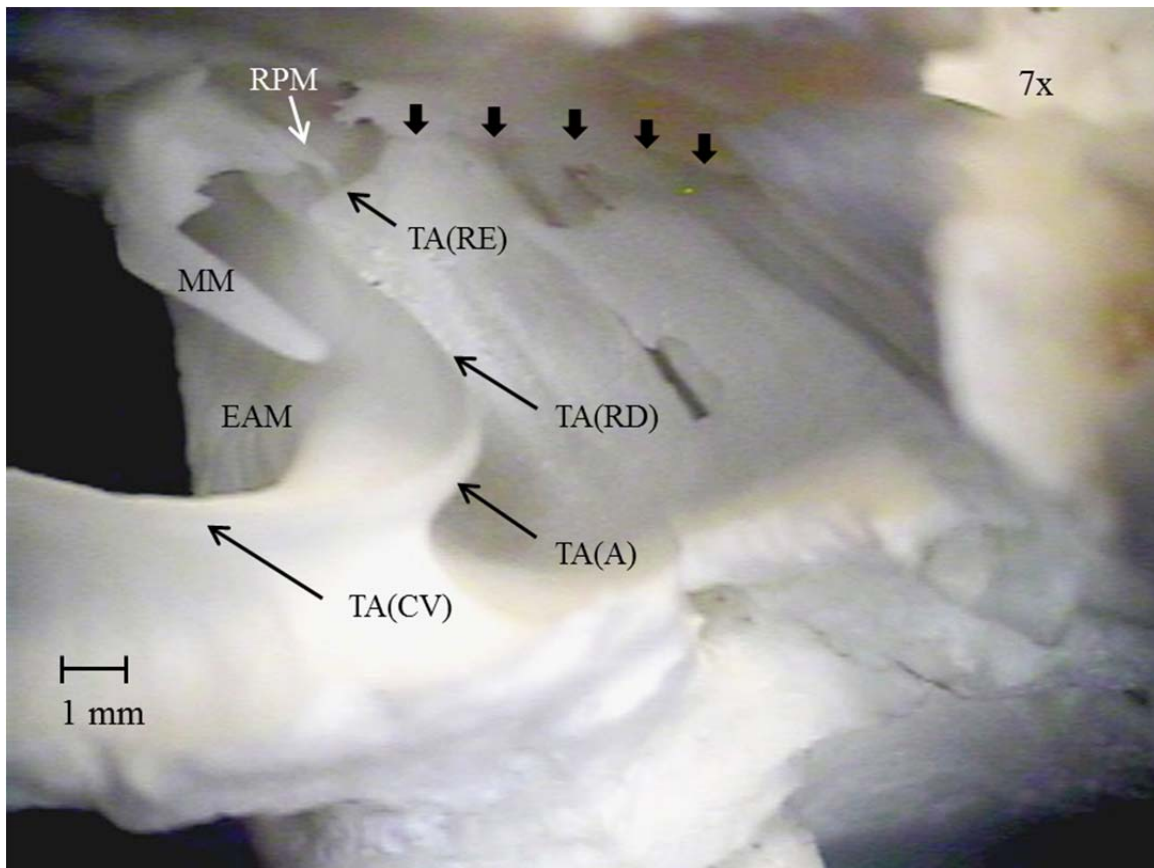


Figure 6.1. Medial view of the tympanic annulus and surrounding structures in an adult canine skull. The tympanic annulus is an incomplete loop, with its rim located just inside the external auditory meatus (EAM). The rostradorsal region ((TA(RD))) of the annular rim makes a 90° bend, just ventral to the rostral process of the malleus (RPM), and extends (TA(RE)) toward the petrous bone where it terminates at the petrotympanic fissure. The extent of the petrotympanic fissure is outlined by the thick arrows. MM = manubrium of malleus, TA(A) = apex of tympanic annulus, TA(CV) = caudoventral limb of tympanic annulus.

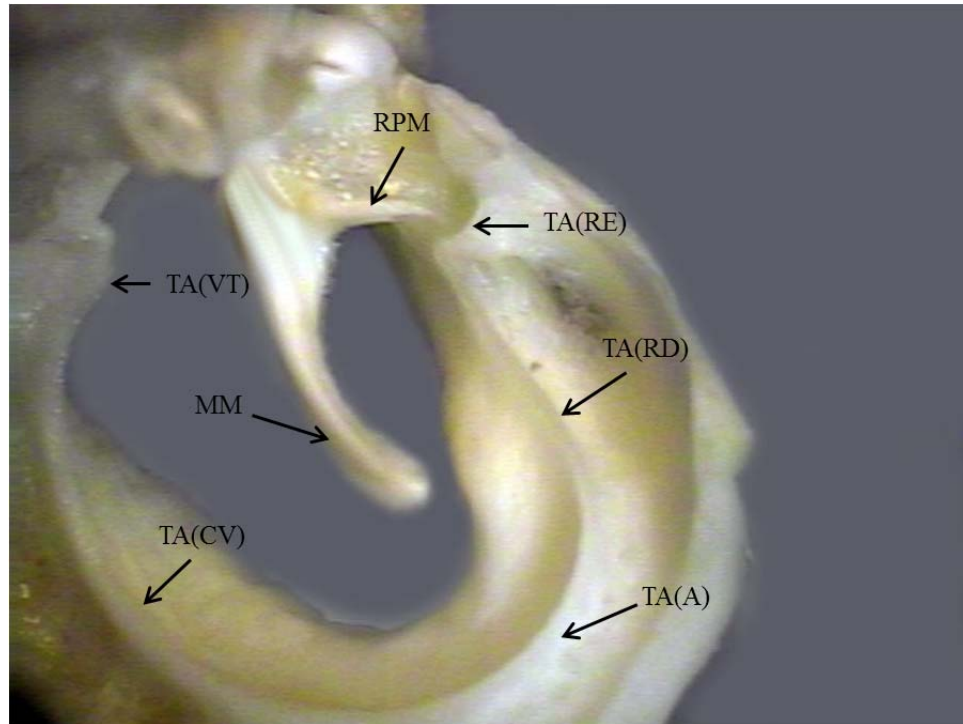


Figure 6.2. Medial view of an isolated region of the left tympanic bone of a two-month-old puppy skull. The tympanic annulus has a rostradorsal limb (TA(RD)), a caudoventral limb (TA(CV)), and an apex (TA(A)). The rostral process of the malleus (RPM) does not extend beyond the annulus, but is located caudal to the rostral extent (TA(RE)) of the rostradorsal limb of the annulus. MM = manubrium of the malleus.

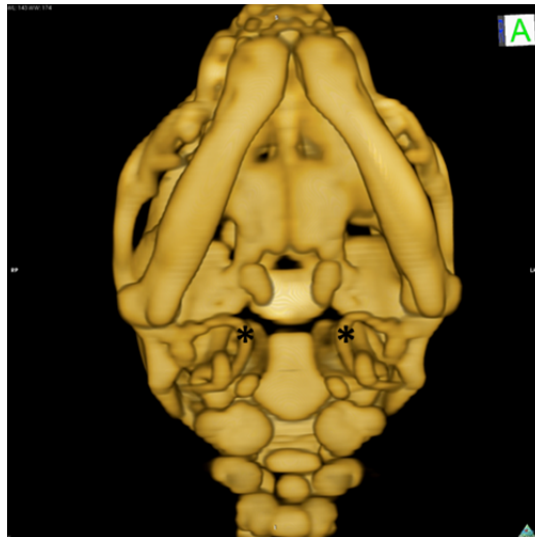


Figure 6.3. Ventral view of a CT three-dimensional reconstruction of a newborn puppy head. The left and right tympanic annuli are visible (asterisks); the marked absence of the tympanic bullae demonstrates that they have not yet developed and ossified.

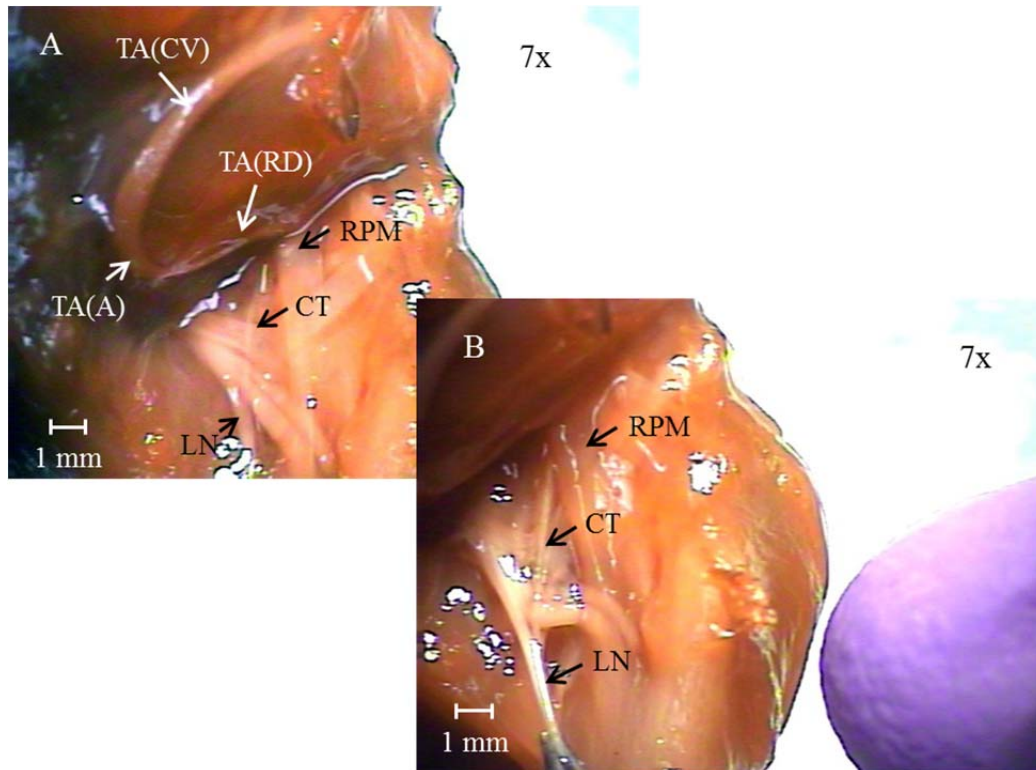


Figure 6.4. Ventral views of a newborn puppy head. The tympanic annulus is clearly visible in (A). (B) Tension is being placed on the lingual nerve (LN) to better demonstrate the chorda tympani nerve (CT). The chorda tympani nerve passed lateral to the rostradorsal limb of the tympanic annulus (TA(RD)) and dorsal to the rostral process of the malleus (RPM). TA(A) = apex of tympanic annulus, TA(CV) = caudoventral limb of tympanic annulus.

In all skulls (n=22), both foramina were observed in consistent locations. The cranial foramen was always located within a depression, medial to the mandibular fossa. This foramen appeared to develop within the squamotympanic fissure (Fig. 6.7). The more caudal foramen was observed medial to the retroarticular process and extended as a canal within the tympanic bone and not as a fissure between bony elements (Fig. 6.8).

Dissection Studies: Chorda Tympani Nerve

In all 25 specimens (inclusive of specimens dissected for arterial studies), the chorda tympani nerve was observed entering a foramen medial to the retroarticular process and extending through a

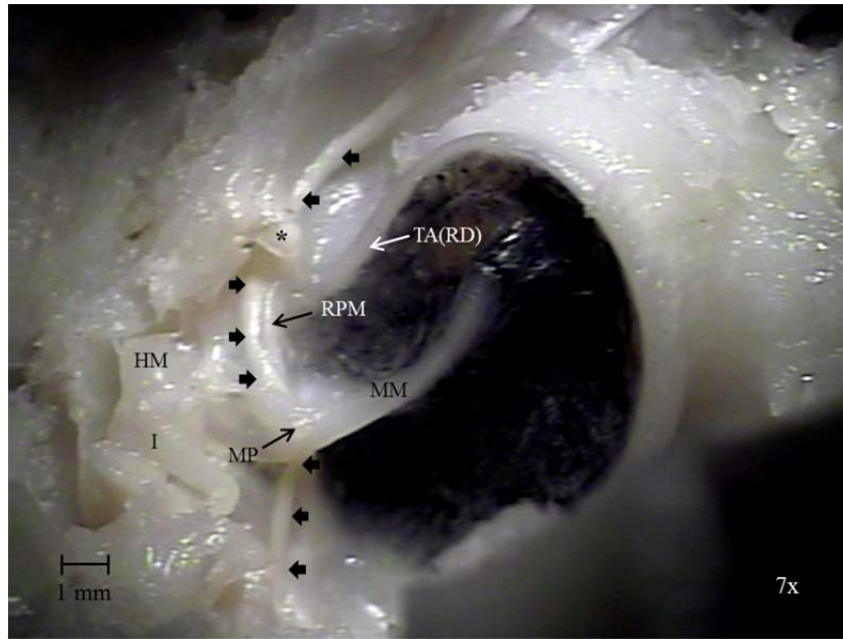


Figure 6.5. In the adult, the chorda tympani nerve (thick arrows) passed lateral to the rostral extension (asterisk) of the rostradorsal limb of the tympanic annulus (TA(RD)) and dorsal to the rostral process of the malleus (RPM). HM = head of malleus, I = incus, MM = manubrium of malleus, MP = muscular process of malleus.

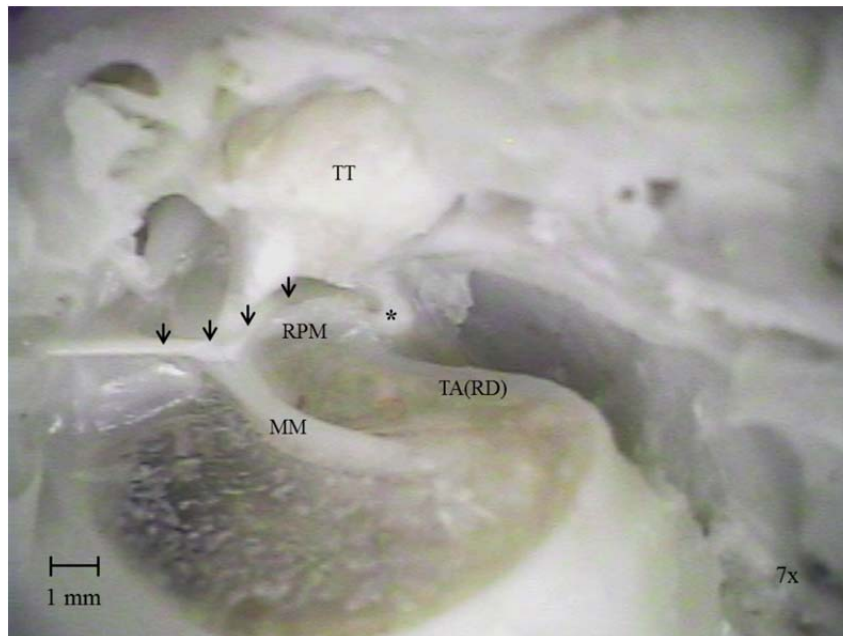


Figure 6.6. The rostral extension (asterisk) of the rostradorsal limb of the tympanic annulus (TA(RD)) formed a small loop-like structure immediately rostral to the rostral process of the malleus (RPM). The chorda tympani nerve (arrows) is located dorsal to the rostral process of the malleus and extends lateral to the rostral extension of the rostradorsal limb of the tympanic annulus. MM = manubrium of malleus, TT = tensor tympani muscle.

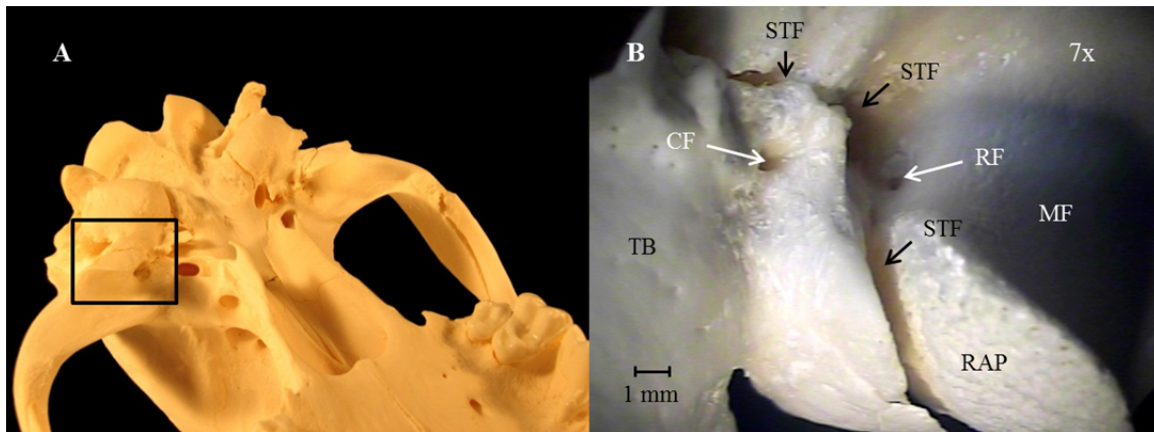


Figure 6.7. (A) Ventral oblique view of the canine skull. (B) Magnified (7x) view of boxed region in (A). The caudal foramen (CF) was located within the tympanic bone, medial to the retroarticular process (RAP). The rostral foramen (RF) was located medial to the mandibular fossa (MF) and appeared to have developed within the squamotympanic fissure (STF). TB = tympanic bulla.

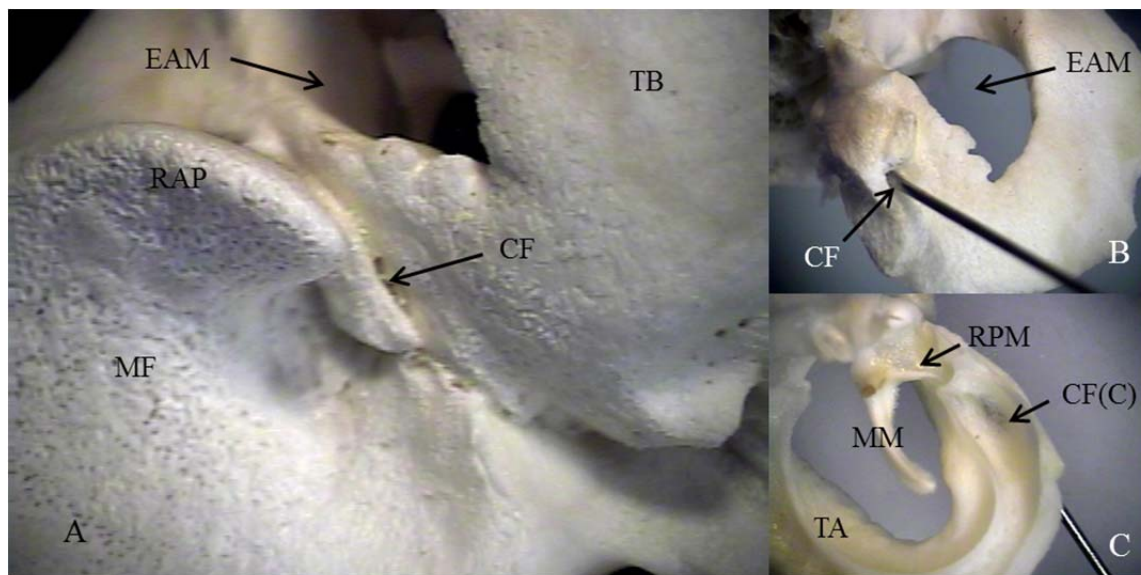


Figure 6.8. (A) Left ventral view of the temporal bone of a two-month-old puppy. (B) Ventrolateral and (C) medial views of a region of the tympanic bone that was isolated from (A). A pin has been inserted into the caudal foramen (CF) to demonstrate the location of the canal (CF(C)) with which it is continuous. EAM = external acoustic meatus, MF = mandibular fossa, MM = manubrium of malleus, RAP = retroarticular process, RPM = rostral process of malleus, TA = tympanic annulus, TB = tympanic bulla

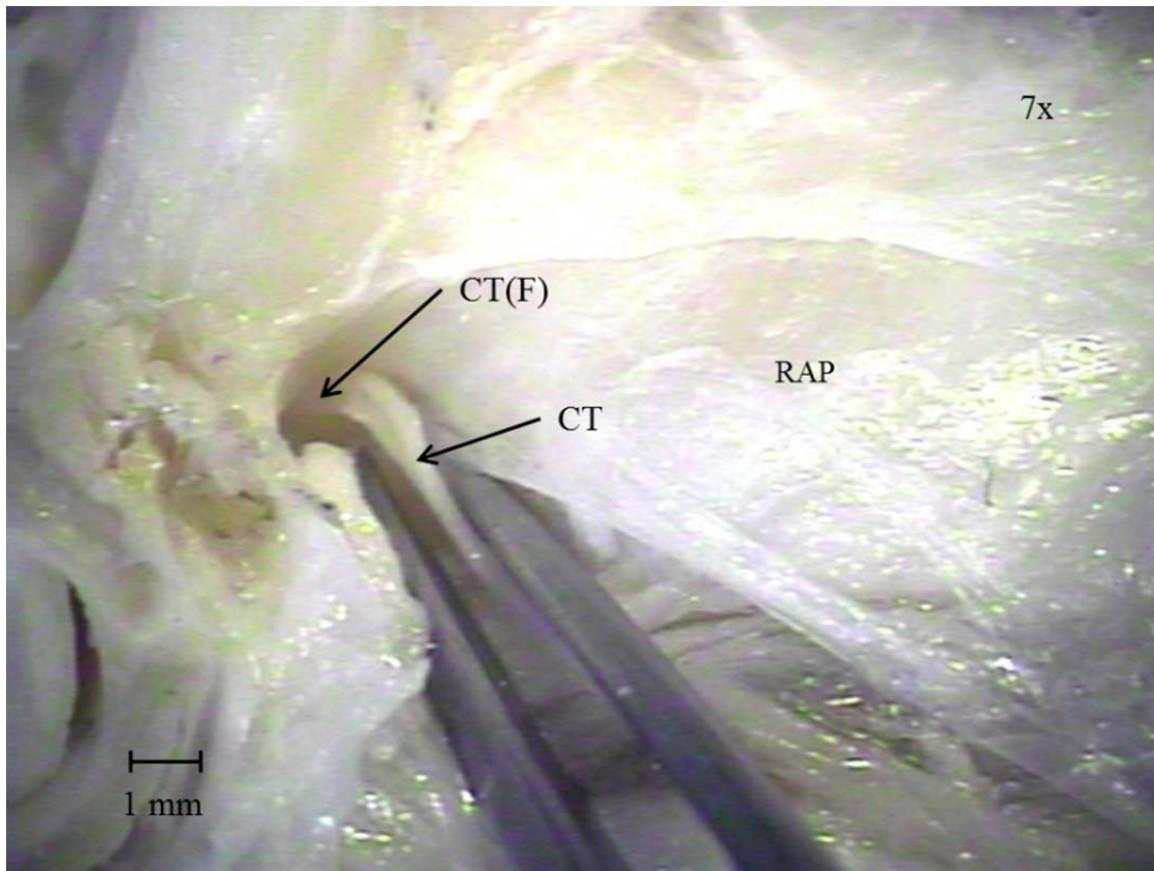


Figure 6.9. The chorda tympani nerve (CT) passes into a foramen (CT(F)) that is located medial to the retroarticular process (RAP). Right ventral view.

canal that was located within the tympanic bone rostral and dorsal to the rostro-dorsal limb of the tympanic annulus (Figs. 6.9 and 6.10).

Dissection Studies: Rostral Tympanic Artery

In the five dissected specimens, the rostral tympanic artery was observed curving around the caudal aspect of the retroarticular process and extending medially toward a foramen located immediately medial to the mandibular fossa of the temporal bone. The foramen for the chorda tympani nerve was located medial and slightly caudal to the foramen that accommodated rostral tympanic artery (Fig. 6.11).

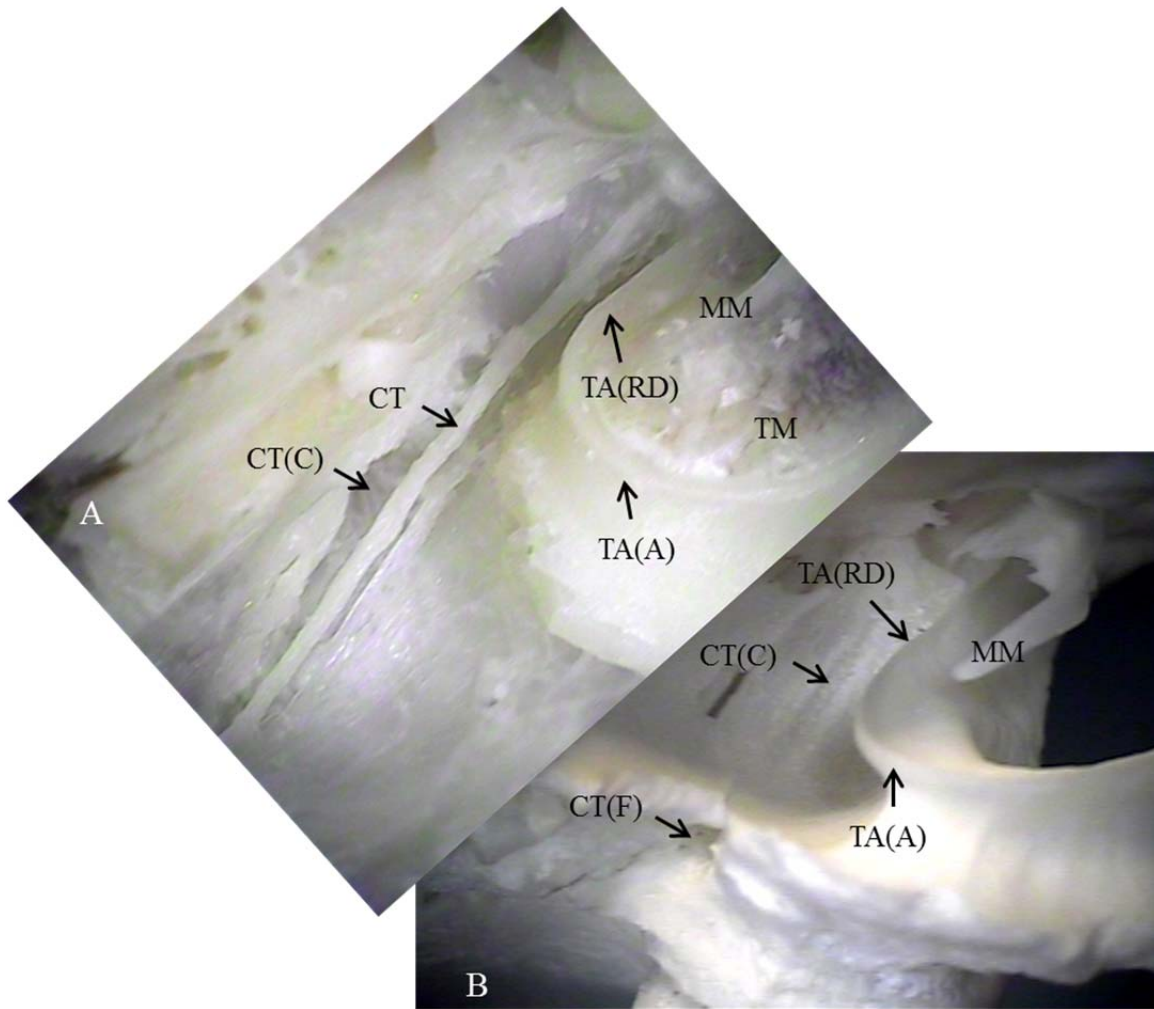


Figure 6.10. (A) The chorda tympani nerve (CT) coursed within a canal of the tympanic bone that was located rostral and dorsal to the rostradorsal limb of the tympanic annulus (TA(RD)). (B) The foramen of the chorda tympani nerve (CT(F)) is seen to be continuous with the chordal canal (CT(C)) in this dissected skull. Right medial views. MM = manubrium of malleus, TA(A) = apex of tympanic annulus, TM = tympanic membrane.

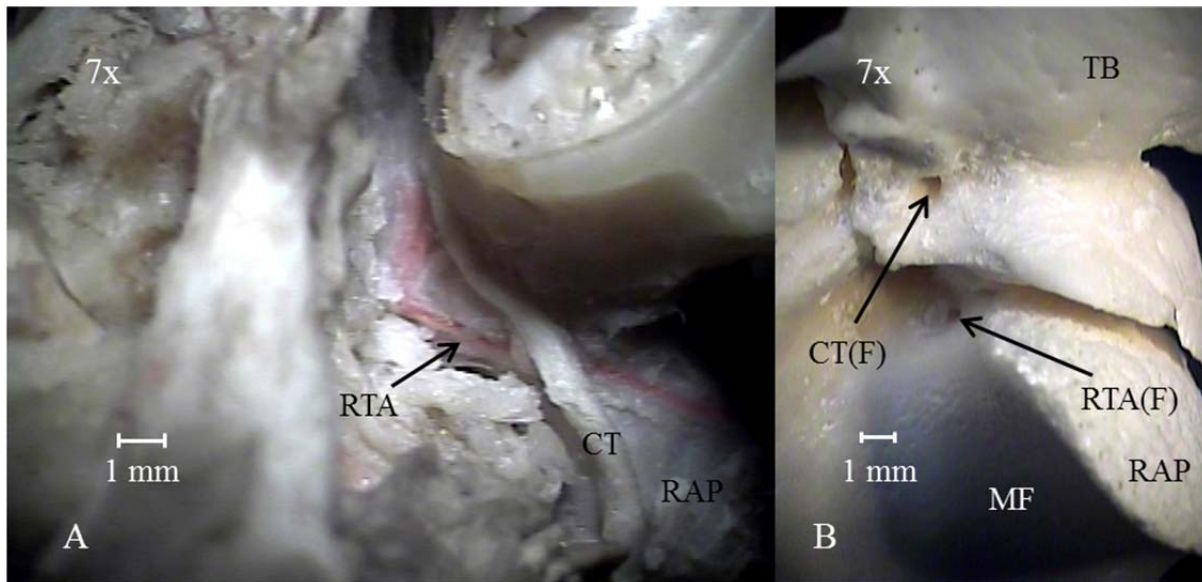


Figure 6.11. (A) Right ventromedial view showing the location of the chorda tympani nerve (CT) relative to that of the rostral tympanic artery (RTA). (B) Right ventromedial view of the skull showing the location of the foramina for the chorda tympani nerve (CT(F)) and the rostral tympanic artery (RTA(F)). The artery curved along the ventral aspect of the retroarticular process (RAP) and entered a foramen located immediately medial to the mandibular fossa (MF). The chorda tympani nerve crossed the artery ventrally and extended toward a foramen that was located caudal and ventral to the foramen of the rostral tympanic artery. TB = tympanic bulla.

Corrosion Casting Studies

Results from the single corrosion cast were inconsistent, with greater resolution obtained from the right half than the left. However, the bony structures of the right half were not well maintained, making it difficult to visualize the relationships between the vessels and bony landmarks. Vascular filling of the left Batson's-injected specimen was not as complete as the right; however, the rostral tympanic artery was identified. The bone remained intact in this specimen, so the relationship of the rostral tympanic artery to the surrounding bony elements could be determined. The artery coursed medially along the caudal aspect of the retroarticular process and gave off several branches to the region of the joint capsule (Fig. 6.12). The vessel then entered a small foramen that was located within a depression immediately medial to the mandibular fossa of the temporal bone (Fig. 6.13).

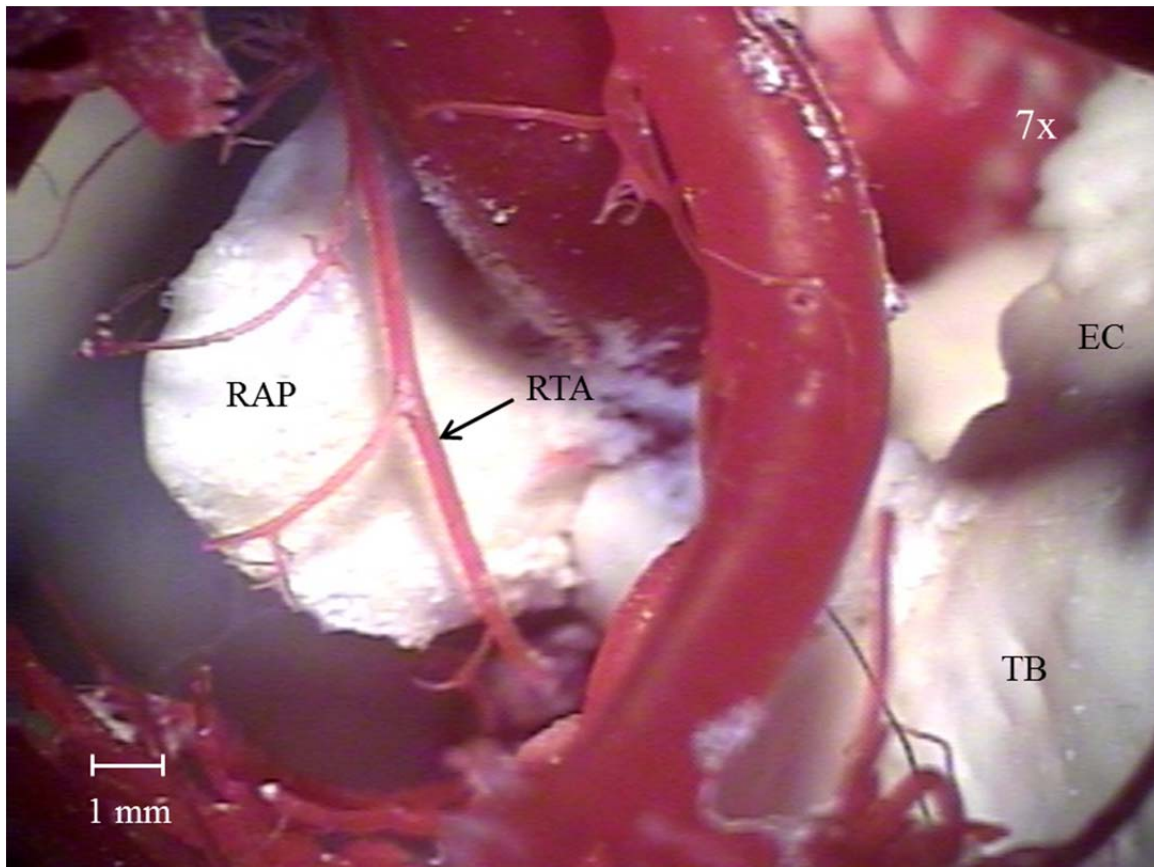


Figure 6.12. The rostral tympanic artery (RTA) coursed medially along the caudal aspect of the retroarticular process (RAP) and gave off several branches to the region of the joint capsule. EC = external ear canal, TB = tympanic bulla. Left ventromedial view.

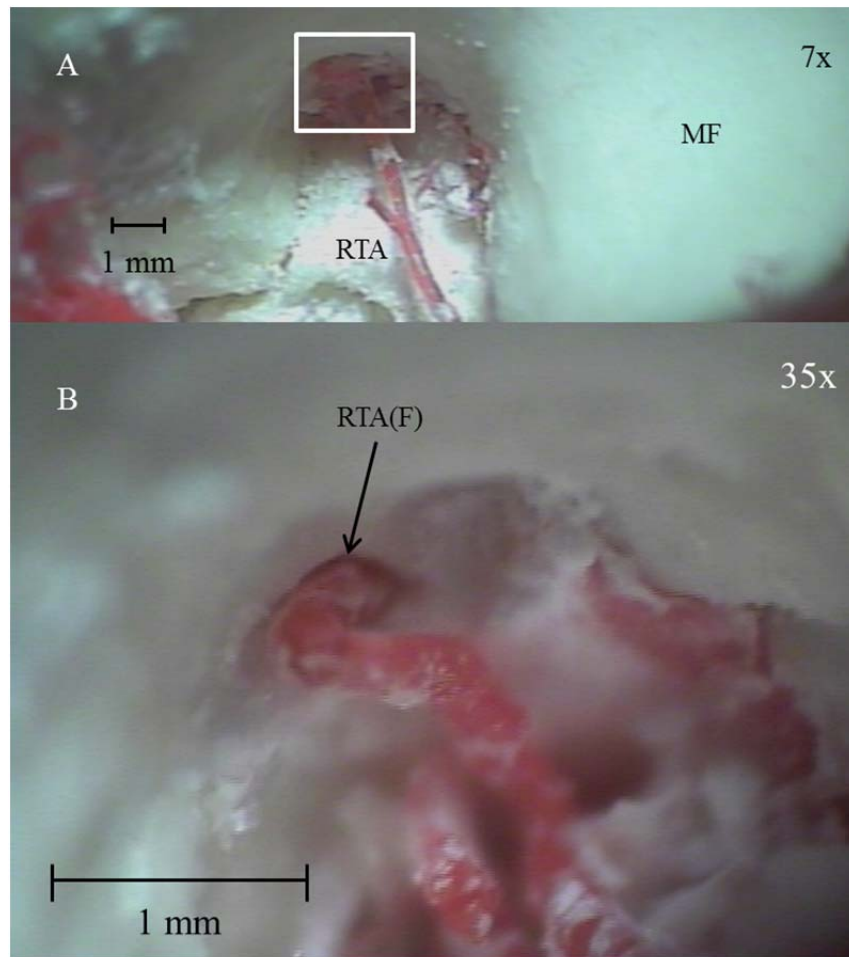


Figure 6.13. The rostral tympanic artery (RTA) entered a small foramen (RTA(F)) that was located within a depression immediately medial to the mandibular fossa (MF) of the temporal bone.

Discussion

The tympanic annulus is a bony rim that is located immediately inside the external acoustic meatus of the temporal bone and suspends the tympanic membrane within the cavity of the middle ear. The orientation of the tympanic annulus is difficult to visualize because it is obscured by the tympanic bulla and other portions of the tympanic bone. Because the tympanic annulus is an important point of reference in relation to the tympanic membrane, malleus, and chorda tympani nerve, it is important to understand its configuration. The term “annulus” is Latin for “little ring”, thus giving the impression that the tympanic annulus is a complete circular structure. This term is

therefore misleading because the tympanic annulus is more of an oblong loop rather than a circular structure and it is not complete dorsally (Fig. 6.2). There are dorsal and ventral limbs of the annulus, where the dorsal limb is located rostrally and the ventral limb is located caudally (Fig. 6.2). Both limbs meet at the ventrally located apex, which is directed rostromedially.

In the human, the chorda tympani nerve has been described as entering the petrotympanic fissure in its course from the middle ear (Morgan *et al.*, 1995; Swartz, 2009). This is clearly not the case in dog. The chorda tympani nerve passes lateral to the loop-like rostral extension of the rostradorsal limb of the tympani annulus, ventral to the petrotympanic fissure (Figs. 6.1, 6.5 and 6.6). The chorda tympani nerve occupies a canal (chordal canal) within the tympanic bone and does not lie within a bony fissure, a groove formed between two separate bones (Figs. 6.8 and 6.10). The nerve exits the chordal canal through a foramen that is located more caudal and medial to the foramen of the rostral tympanic artery. It is an important finding that the chorda tympani nerve exits through a foramen separate from that of the rostral tympanic artery, as it is generally believed in humans that these two structures occupy the same fissure (Anagnostopoulou *et al.*, 2008; Gulya, 2010).

Both Evans (1993b) and Ghoshal (1975) have mentioned foramina adjacent to the medial jaw articulation in relation to the chorda tympani nerve and the rostral tympanic artery, but did not discuss the specific orientation of either of these foramina in relation to the structures that penetrate them. The results of this study clearly demonstrate that the foramen of the chorda tympani nerve is located caudomedially relative to the foramen of the rostral tympanic artery and lies between the retroarticular process and the tympanic bulla (Fig. 6.11). The foramen of the chorda tympani nerve is continuous with the chordal canal, which is located rostralateral to the rostradorsal limb of the tympanic annulus. The foramen of the rostral tympanic artery is located within a depression that is located immediately medial to the mandibular fossa and this foramen appears to develop within a

fissure (squamotympanic) between two parts of the temporal bone, the squamous and tympanic temporal components (Figs. 6.7, 6.11, and 6.13).

Because of their locations, the chorda tympani nerve and the rostral tympanic artery may be susceptible to disruption during manipulation of the jaw; this is especially the case with the rostral tympanic artery. If the artery is occluded as a result of jaw manipulation, the blood supply to structures of the middle ear would be compromised and this could lead to a disruption in hearing (Anagnostopoulou *et al.*, 2008). According to Mikheev and Tsybul'kin (1988), when the human mandibular head (condyle) is shifted backward or medially the chorda tympani nerve is not affected; however, pathological processes occurring around the temporomandibular joint may impact the anterior tympanic artery. Further studies are needed to determine the likelihood of stressing the chorda tympani nerve and the rostral tympanic artery during jaw manipulation. Additional studies are necessary to determine if occlusion of the rostral tympanic artery could lead to audiologic abnormalities, with the most severe being acute-onset deafness.

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CHAPTER 7

CONCLUSIONS

Overall Summary of Findings

Reported cases of hearing loss in dogs and cats following dental and ear procedures that were performed under anesthesia prompted the current study. The most likely causes for the deafness were considered to be mechanical or vascular. The possibility of jaw manipulation was considered as a cause of acute-onset deafness in the dog. Structures adjacent to the temporomandibular joint were of particular interest because changes in jaw orientation could disrupt vessels and nerves in this area. Another considered cause was a jaw-ear connection via a ligamentous remnant of Meckel's cartilage. Following review of the published literature, it became clear that descriptions of the anatomy of the vasculature supplying the canine ear were either incomplete or inconsistent, and further that a ligament extending from the jaw to the ear, while recognized in humans, had never been described in the dog. The goal of this research was to lay the foundation for future physiological studies by providing more accurate anatomical descriptions of the relevant canine juxta-articular structures.

In Chapter 3, the occurrence of hearing loss in dogs and cats following procedures performed under anesthesia was documented. Information obtained from pet owners, postings on the Veterinary Information Network web site, and surveys sent to LSU SVM alumni and to members of the American Veterinary Dental College were used to compile data on the number of these deafness cases and potentially relevant details. The number of identified cases was low, with only 62 identified between 2002 and 2009 from the sources listed above and five additional cases reported by survey respondents, but the actual prevalence may be much greater. Most subjects did not recover auditory function, but vestibular dysfunction resolved. Older animals may be more vulnerable, but animals of all ages can be affected. No relationship was observed between hearing impairment and species (dog, cat), breed, gender, size of dogs, or anesthetic drug use. Older animals appeared more

susceptible; however, there may be a bias since these procedures are more often performed on older animals.

In Chapter 4, the blood supply to the canine ear was studied using micro-dissection, corrosion casting and computed tomography. Hearing loss due to occlusion of the labyrinthine artery subsequent to jaw manipulation could be ruled out following confirmation of the location and course of the vessel. The maxillary artery was the main focus of this study due to its proximity to the temporomandibular joint (TMJ). Arteries potentially affected by jaw manipulation would most likely be derivatives of the maxillary artery due to its proximity to the TMJ; so branches that originated near the jaw articulation were dissected and followed to determine if they distributed to ear structures.

The labyrinthine artery originated from the basilar artery (Fig. 4.2) and was seen in the corrosion casted specimen to consist of a proximal limb entering the internal acoustic meatus, a loop within the meatus, and a distal limb exiting the meatus (Fig. 4.13). This loop configuration was lost in the dissected specimen following removal of the brain; however, it was confirmed that the labyrinthine artery does enter the depths of the meatus, accompanied by the seventh and eighth cranial nerves (Fig. 4.3). This vessel is well protected within the cranium and it is unlikely to be susceptible to occlusion from jaw manipulation.

The rostral tympanic artery, described by Evans (1993) and Ghoshal (1975) as being either a direct branch of the maxillary artery occurring opposite the caudal deep temporal artery, or as a branch of the caudal deep temporal artery, was not seen as originating from either of these arteries in any of the dissections. It was concluded from the current vascular studies that the rostral tympanic artery is a derivative of the mandibular ramus (*r. mandibularis*), which is the first branch of the maxillary artery. It was demonstrated that the rostral tympanic artery enters a foramen that is located rostralateral to the foramen of the chorda tympani nerve (Figs. 4.10 and 4.11) and supplies portions

of the joint capsule and tympanic bulla. The mandibular ramus supplied two additional branches, one to the joint capsule of the temporomandibular articulation and one to the bony external ear canal.

Only one vessel originated from the caudal deep temporal artery at its origin and had not been previously described. This vessel, identified in this study as the rostral auditory tube branch, extended dorsally between the maxillary artery and lingual nerve to supply the proximal portion of the auditory tube, surrounding nasopharynx, and chorda tympani nerve (Figs. 4.4 – 4.7).

The locations of the rostral tympanic artery and the rostral auditory tube branch make these vessels susceptible to disruption by jaw manipulation. Pressure on the anterior (rostral) tympanic artery has been reported to cause disturbance to the blood supply of the middle ear (Anagnostopoulou, 2008) and could have a negative impact on hearing. A link between the occlusion of the rostral auditory tube branch and hearing impairment is a new possibility, as this vessel has not been previously described. The blood supply to the proximal auditory tube has been previously reported as the auditory tube branch of the ascending pharyngeal artery (Evans, 1993 and Ghoshal, 1975), and not the rostral auditory tube branch of the caudal deep temporal artery, as was shown in the current study.

An additional anatomical variation of the vasculature was observed in this study. In *Miller's Anatomy of the Dog* (Evans, 1993, Fig. 4.8), the caudal auricular artery is shown to branch from the maxillary artery distal to the origins of the lingual and facial arteries, and just proximal to the origin of the superficial temporal artery. However, it was shown in this study that the caudal auricular artery branched opposite the lingual artery, just distal to the origin of the occipital artery from both left and right sides of the CT three-dimensional reconstruction (Fig. 4.21). This variation was also seen in both the left and right halves of the corrosion casted specimens. In 14 of 56 latex-injected heads (25%), the origin of the caudal auricular artery occurred immediately distal to the origin of the ascending pharyngeal and occipital arteries and opposite the origin of the lingual artery. In the

remaining 42 specimens, the branching pattern of the caudal auricular artery as described in Evans (1993) was seen. Anomalies of arterial branching patterns in the dog are not uncommon. When these variations are noted to occur frequently, especially in the large to medium sized vessels, it is important to document these differences because surgeons often use vascular landmarks during surgical procedures.

In Chapter 5, a jaw-ear connection is established through studies of ligamentous structures connecting the jaw and ear. The equivalent of the sphenomandibular ligament (SML), a remnant of Meckel's cartilage that makes a connection between the jaw and middle ear in humans, was shown to also exist in the dog. In humans, the SML courses from the medial aspect of the mandible, with the majority of fibers attaching to the spine of the sphenoid bone. The spine of the sphenoid bone does not exist in the dog, so an exact equivalent of this structure cannot exist in the dog. The ligament is a true malleomandibular ligament in the newborn puppy because it extends from the medial mandible to the rostral process of the malleus, with no intermittent attachments. The development and ossification of the tympanic bulla likely entraps the ligament within a bony passageway, making it difficult to grossly view the complete course of the ligament. In the adult, the majority of the ligamentous fibers attach to the tympanic bulla, thus the appropriate name for this portion of the ligament is the tympanomandibular ligament. Based on histological findings, a smaller component of the ligament appears to continue through a canal, located between the tympanic annulus and the surrounding tympanic bone, to become continuous with a connective tissue sheet within the cavity of the middle ear that has attachments to the malleus and incus. Tension on the canine TML does not result in movement of the malleus, making it unlikely that ligament movement with jaw manipulation could result in hearing loss.

In Chapter 6, an anatomical description of the canine tympanic annulus was provided and two foramina located medial to the jaw articulation were identified as foramina for the chorda

tympani nerve and the rostral tympanic artery. The tympanic annulus is an oblong loop that it is not complete dorsally (Fig. 6.2). There are dorsal and ventral limbs of the annulus, with the dorsal limb located rostrally and the ventral limb located caudally (Fig. 6.2). Both limbs meet at the ventrally located apex, which is directed rostromedially. The foramen identified for the chorda tympani nerve is located caudomedially relative to the foramen of the rostral tympanic artery, and lies between the retroarticular process and the tympanic bulla (Fig. 6.11). The foramen for the chorda tympani nerve is continuous with the chordal canal, which is located rostralateral to the rostradorsal limb of the tympanic annulus. The foramen identified for the rostral tympanic artery is located within a depression that is located immediately medial to the mandibular fossa. This foramen appears to develop within a fissure (squamotympanic) between two parts of the temporal bone, the squamous and tympanic temporal components (Figs. 6.7, 6.11, and 6.13). Because of their locations, the chorda tympani nerve and the rostral tympanic artery may be susceptible to disruption during manipulation of the jaw; this is especially true for the rostral tympanic artery. If the artery is occluded as a result of jaw manipulation, the blood supply to structures of the middle ear would be compromised and this could lead to conductive hearing loss (Anagnostopoulou *et al.*, 2008). According to Mikheev and Tsybul'kin (1988), when the human mandibular head (condyle) is shifted backward or medially the chorda tympani nerve is not affected; however, pathological processes close to the temporomandibular joint could affect the anterior (rostral) tympanic artery.

Significance of Research

Acute-onset deafness in small animal species, documented in this study, has only been recently recognized. Although there are several documented reasons for acute-onset deafness in humans, the causes are often unknown (National Institute on Deafness and Other Communication Disorders, 2010). The acute-onset deafness in dogs and cats following dental and ear procedures performed under anesthesia has several possible etiologies. Jaw manipulation is a variable in these

procedures and may potentially impact relevant structures. This study identified two blood vessels, the rostral tympanic artery and the rostral auditory tube branch, whose locations make them potentially susceptible to compression, occlusion and/or possible severing. A new anatomical structure in the dog, the tympanmandibular ligament, was described. This ligament is a remnant of Meckel's cartilage and therefore represents a maintained connection between the jaw and the middle ear. Although tension on this ligament did not produce movement of the malleus in the current study, there may be other factors, such as age or bone disease, that could influence the stability of the ligament in relation to its bony attachments. The adhesions of the TML to the tympanic bulla and the walls of the bony canal within which it is contained may be inconsistent among individuals, where continuity from the TMJ to the ossicles could permit middle ear effects in a small number of individuals. It is only by examination of large numbers of specimens to identify possible variability, or by dissection of multiple donated animals that had experienced this form of deafness could these possibilities be evaluated. There is no certainty that only one etiology exists for this form of deafness.

Future Directions

More data are needed to accurately define the extent of occurrences of hearing loss in dogs and cats following dental procedures performed under anesthesia. Stevens-Sparks and Strain (2011) reported 67 deafness cases, but the prevalence of this type of deafness is not known unless the number of affected animals is known as a percentage of animals undergoing the procedures. It is important that veterinarians be made aware of this potential outcome so that they can closely monitor hearing changes in animals following procedures done under anesthesia. It is also important to better determine whether certain populations, such as geriatric subjects, are at greater risk. If jaw manipulation is a factor for acute-onset deafness, clinicians should exercise care when utilizing mouth specula and assure that the speculum size is appropriate. The length of time that the speculum is left in place should also be closely monitored. Overextension and/or manipulation of the jaw

during dental procedures, by specula or other means, should be avoided if this is indeed the basis for the deafness.

Improved methods are needed to visualize the finer vasculature. Micro-CT would be ideal for visualizing the microvascular supply to a given region. This would eliminate the need for decalcification, micro-dissection, and histological serial sectioning in mapping out the blood supply to a specific region. If the region of interest is larger scale, a micro-CT scanner that accommodates specimen samples larger than 1cm x 1cm x 2cm would be necessary. Micro-CT of corrosion casts may provide increased resolution; however, the success of this procedure would also be dependent on the dimensions of the size limitation of the scanner. Three-dimensional reconstructions of corrosion casts would allow specific areas of the fragile vascular model to be viewed in detail without having to physically preen and subsequently destroy the surrounding vasculature. Micro-surgical methods that permit experimental occlusion of the rostral tympanic artery and the rostral auditory tube branch would be useful in determining the significance of these vessels in hearing loss. If experimental occlusion produced deafness, subsequent studies of jaw mechanics would seek to determine the exact manipulations resulting in occlusion of these vessels. Due to the close proximity of the chorda tympani nerve to the rostral tympanic artery, future studies are also needed to determine if inflammation of the nerve could potentially compress or occlude the artery.

Interaction of Meckel's cartilage and the ossification of the tympanic bulla during development would be useful in determining the course of the canine tympanomandibular ligament. A developmental anomaly of incomplete closure of the tympanic bulla around the ligament could result in a free ligament potentially able to move the malleus. An understanding of the changes of the tympanic bulla and the TML with aging would also determine whether the TML could move the malleus in older animals. The feline SML equivalent has also not been previously described. Future studies should investigate the occurrence of this structure in the cat.

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APPENDIX A – SURVEY FIGURES AND TABLES

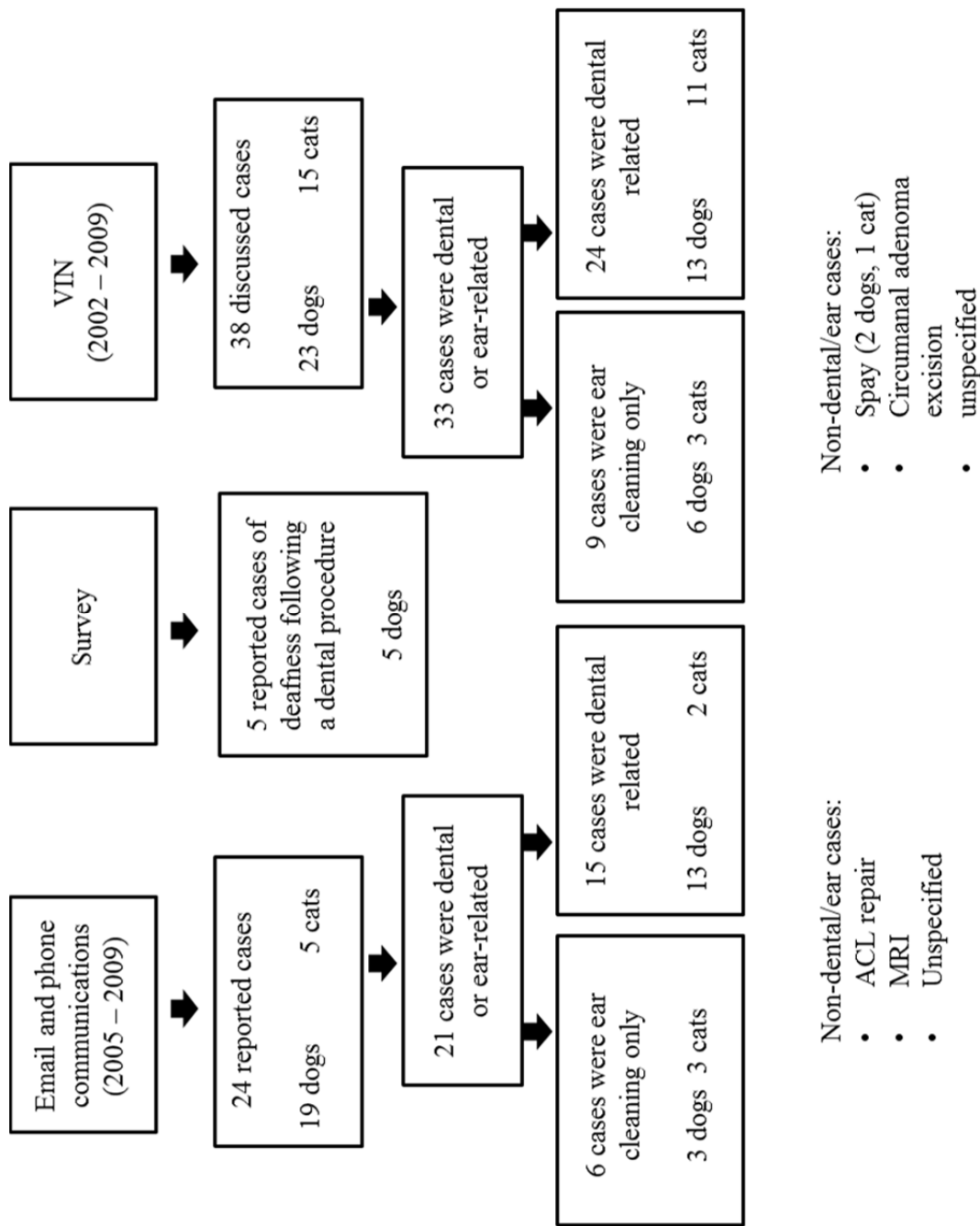


Figure A.1. Summary of reported deafness cases following anesthesia between the years 2002 and 2009.

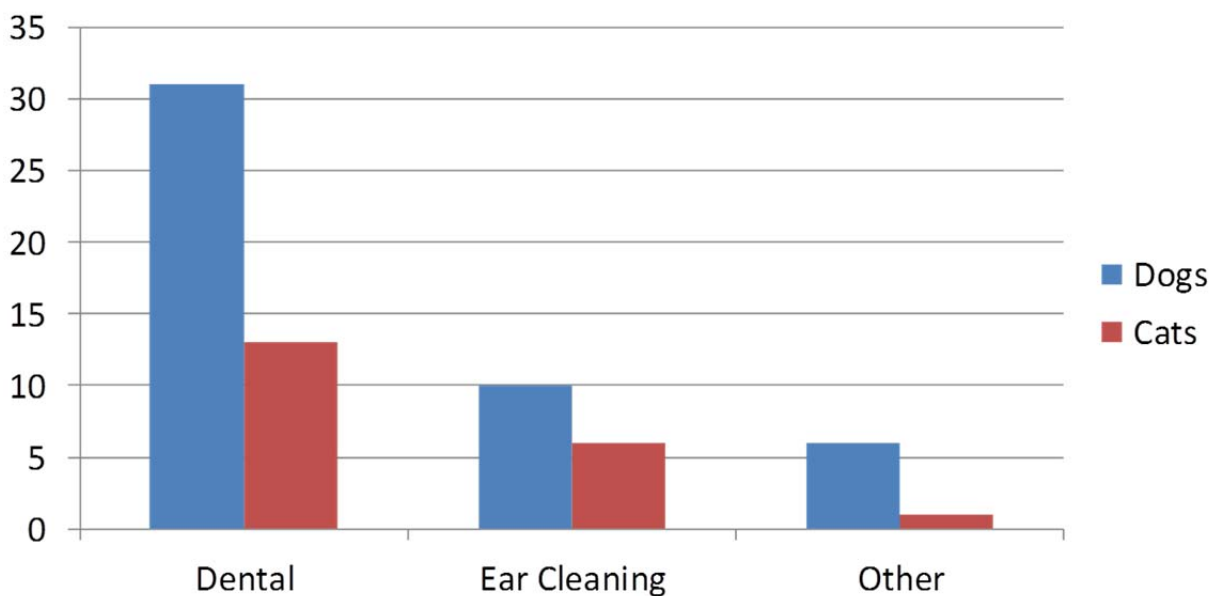


Figure A.2. The majority of reported anesthesia-associated hearing loss was subsequent to a dental procedure.

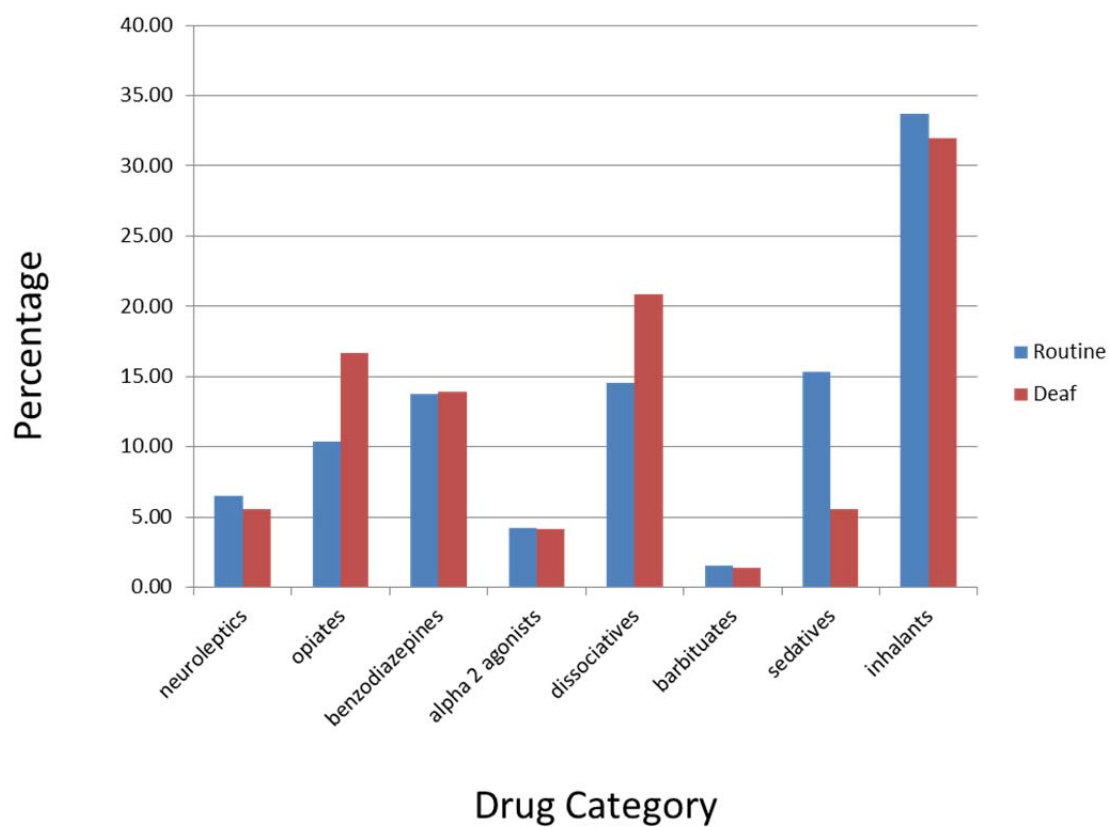


Figure A.3. Anesthetics used on animals that went deaf did not differ from those in routine use.

Table A.1. Summary of dog breeds with reported hearing loss, and gender and size of affected dogs. Data is inclusive of email, phone and VIN reports, where 37 out of 42 reported the dog's breed and 35 out of 42 reported gender.

Dog breed	Gender		Size		Number of reported cases
	female	male	small	med-large	
Bichon Frise	1	0	1		1
Boston Terrier	1	0	1		1
Brussels Griffon	0	1	1		1
Cairn Terrier	0	1	1		1
Chihuahua	-	-	1		1
Chow Chow	-	-		1	1
Cocker Spaniel	0	2		3	3
Collie	0	1		1	1
Dachshund	0	1	1		1
German Shepherd	0	3		3	3
German Shepherd mix	2	0		2	2
Golden Retriever	1	0		1	1
Jack Russell mix	0	1	1		1
Labrador Retriever	0	2		2	2
Lhasa Apso	0	1	1		1
Maltese	1	1	2		2
Mixed breed	1	0		1	1
Pit Bull Terrier	0	1		1	1
Poodle	1	1	1	1	2
Schnauzer	0	1		1	1
Scottish Terrier	-	-	1		1
Sheltie	3	0	4		4
Staffordshire Terrier	2	0		2	2
Terrier mix	0	2	2		2
Breed not identified	1	2			5
Total	14	21	18	19	42

Table A.2. Summary of procedures performed under anesthesia in dogs prior to deafness.

Procedure	VIN	Email	Total
	Number (%)	Number (%)	Number (%)
Dental prophylaxis only	2 (8.7%)	7 (36.8%)	9 (21.4%)
Dental prophylaxis with extractions or other oral procedure	6 (26.1%)	2 (10.5%)	8 (19.0%)
Extractions and/or other oral procedure	2 (8.7%)	0	2 (4.8%)
Dental prophylaxis with other procedure (non-dental, non-ear)	1 (4.3%)	0	1 (2.4%)
Dental prophylaxis/dental procedure with ear cleaning/ear procedure	2 (8.7%)	4 (21.1%)	6 (14.3%)
Ear cleaning/ear procedure only	5 (21.7%)	3 (15.8%)	8 (19.0%)
Ear cleaning with other non-dental procedure	1 (4.3%)	1 (5.3%)	2 (4.8%)
Non-dental, non-ear procedure	3 (13.0%)	2 (10.5%)	5 (11.9%)
Unreported	1 (4.3%)	0	1 (2.4%)
Total	23	19	42

Table A.3. Summary of procedures performed under anesthesia in cats prior to deafness.

Procedure	VIN	Email	Total
	Number (%)	Number (%)	Number (%)
Dental prophylaxis only	2 (13.3%)	2 (40.0%)	4 (20.0%)
Dental prophylaxis with extractions or other oral procedure	2 (13.3%)	0	2 (10.0%)
Dental prophylaxis with other procedure (non-dental, non-ear)	1 (6.7%)	0	1 (5.0%)
Dental prophylaxis/dental procedure with ear cleaning/ear procedure	6 (40.0%)	0	6 (30.0%)
Ear cleaning/ear procedure only	1 (6.7%)	3 (60.0%)	4 (20.0%)
Ear cleaning with other non-dental procedure	2 (13.3%)	0	2 (10.0%)
Non-dental, non-ear procedure	1 (6.7%)	0	1 (5.0%)
Total	15	5	20

Table A.4. Anesthetics reported on VIN postings for animals that went deaf. Twenty-eight out of 38 cases reported anesthetics used.

Drug Category	VIN n = 28 Number (%)
Neuroleptics	4 (14.3%)
Opiates	12 (42.9%)
Benzodiazepines	10 (35.7%)
α_2 -Agonists	3 (10.7%)
Dissociatives	15 (53.6%)
Barbiturates	1 (3.6%)
Sedatives	4 (14.3%)
Inhalants	23 (82.1%)

Table A.5. Geriatric classification guidelines. Dogs were considered to be geriatric based on a combination of age and typical breed body weight established from the age when dogs typically begin to have age-related diseases (from Goldston and Hoskins, 1995).

	Weight	Age
Small dogs	0 – 20 lbs	11.48 \pm 1.85 yrs
Medium dogs	21 – 50 lbs	10.90 \pm 1.56 yrs
Large dogs	51 – 90 lbs	8.85 \pm 1.38 yrs
Giant dogs	>90 lbs	7.46 \pm 1.94 yrs
Cats	–	11.88 \pm 1.94 yrs

Table A.6. Survey questions.

1. To your knowledge, have any animals been deaf upon recovery from a dental procedure in your clinic? *Yes* *No*
2. If you answered “Yes” to question 1, how many times has this occurred and what species and breed?
3. If you answered “Yes” to question 1, how would you describe the head type of the animal? *Brachycephalic* *Mesocephalic* *Dolicocephalic*
4. Have you ever heard of any cases of deafness in animals following routine dental procedures? *Yes* *No*
5. If you answered “Yes” to question 4, please explain.
6. Are teeth cleaning procedures performed by a veterinarian or by a technician in your clinic? *Veterinarian* *Technician* *Other*_____
7. What anesthesia do you use during dental procedures?
8. Do you use a mouth speculum? *Yes* *No*
9. If you answered “Yes” to question 8, what type of speculum do you use?
Commercial (spring-loaded) *Self-made – please describe*
10. If you answered “Yes” to question 8, typically how long is the speculum left in the animal’s mouth?
11. Are your procedures performed ultrasonically or mechanically?
Ultrasonically *Mechanically* *Other*_____

Table A.7. Summary of individuals performing routine dental prophylaxis. Tabulated response percentages are based on total number of answers rather than total number of survey participants.

Individual performing the procedure	LSU Alumni n = 57 Number (%)	AVDC n = 52 Number (%)	Total n = 109 Number (%)
Veterinarian	12 (21.1%)	7 (13.5%)	19 (17.4%)
Technician/clinical assistant	34 (59.7%)	19 (36.5%)	53 (48.6%)
Both (veterinarian and technician/assistant)	11 (19.3%)	17 (32.7%)	28 (25.7%)
Other:			
Students	0	6 (11.5%)	6 (5.5%)
Registered dental hygienist	0	2 (3.8%)	2 (1.8%)
Dentist	0	1 (1.9%)	1 (0.9%)

Table A.8. Anesthetics used during routine dental procedures. Responses recorded reflect the number of respondents who used drugs within each category. Tabulated response percentages are based on total number of survey participants rather than total number of drugs that were reported to be used during anesthetic procedures.

Drug Category	LSU Alumni n = 57 Number (%)	AVDC n = 39 Number (%)	Total n = 96 Number (%)
Neuroleptics	12 (21.1%)	5 (12.8%)	17 (17.7%)
Opiates	13 (22.8%)	14 (35.9%)	27 (28.1%)
Benzodiazepines	26 (45.6%)	10 (25.6%)	36 (37.5%)
α_2 -Agonists	7 (12.3%)	4 (10.3%)	11 (11.5%)
Dissociatives	29 (50.9%)	9 (23.1%)	38 (39.6%)
Barbiturates	4 (7.0%)	0	4 (4.2%)
Sedatives	21 (36.8%)	19 (48.7%)	40 (41.7%)
Inhalants	50 (87.7%)	38 (97.4%)	88 (91.7%)

Table A.9. Summary of mouth speculum use by veterinarians and technicians.

Speculum Type	LSU Alumni n = 39 Number (%)	AVDC n = 29 Number (%)	Total n = 68 Number (%)
Spring-loaded	36 (92.3%)	10 (34.5%)	46 (67.6%)
Self-made (syringe case)	0	14 (48.3%)	14 (20.6%)
Both	2 (5.1%)	4 (13.8%)	6 (8.8%)
Other	1 (2.6%)	1 (3.4%)	2 (2.9%)

Table A.10. Duration of mouth speculum use during dental procedures.

Time Category	LSU Alumni n = 30 Number (%)	AVDC n = 24 Number (%)	Total n = 54 Number (%)
1 – 10 minutes	7 (23.3%)	5 (20.8%)	12 (22.2%)
10 – 20 minutes	14 (46.7%)	4 (16.7%)	18 (33.3%)
20 – 30 minutes	7 (23.3%)	2 (8.3%)	9 (16.7%)
30 – 60 minutes	2 (6.7%)	6 (25.0%)	8 (14.8%)
>1 hour	0	7 (29.2%)	7 (13.0%)

Table A.11. Summary of dental cleaning techniques.

Cleaning Technique	LSU Alumni n = 57 Number (%)	AVDC n = 41 Number (%)	Total n = 98 Number (%)
Ultrasonic	44 (77.2%)	17 (41.5%)	61 (62.2%)
Mechanical	3 (5.3%)	0	3 (3.1%)
Both	10 (17.5%)	21 (51.2%)	31 (31.6%)
Other	0	3 (7.3%)	3 (3.1%)

APPENDIX B - SOLUTIONS, STAINS, AND PROTOCOLS

B.1. Buffered Neutral Formalin (10%) Solution

	<u>per Liter</u>
Formaldehyde, 37%	100 ml
Sodium phosphate, monobasic, monohydrate	4 g
Sodium phosphate, dibasic, anhydrate	6.5 g
ddH ₂ O (deionized water)	q.s. 1 L

B.2. Formaldehyde Embalming and Soaking Solution

For embalming, a 7% solution is used. Prepare a 1:5 solution using one part 37% formaldehyde and five parts warm water. A small amount of liquid detergent is added to aid in vascular filling. It is important that the solution be warm while embalming, as injecting cold solution will result in vascular constriction.

For soaking, a 2-4% solution is used. Prepare a 1:20 (2%) or 1:10 (4%) solution using 37% formaldehyde and warm water. A small amount of liquid fabric softener may be added to keep the tissues from drying out.

B.3. Embalming Protocol

Materials

Cannulae (x2) – use either #10 or #12, depending on the size of the dog
Scalpel (#4) with blade (#22)
String (cotton cable twine)
Hemostats
Scissors (artery scissors, curved)
12 cc syringe (x2)

Protocol

Place the animal in dorsal recumbency. Make a 2-3 inch incision on one side of the neck, beginning lateral to the larynx and extending caudally. Using fingers, separate the connective tissue between the neck muscles to gain access to the cervical visceral space. Locate and isolate the carotid sheath using hemostats. Separate the carotid artery from the vagosympathetic trunk and loosely loop two strings around the artery, leaving about a 1 inch space between the two strings. Tie the more caudal string in a tight knot around the artery. Use the artery scissors to cut a small opening in the artery between the two strings. Insert a cannula that is attached to a water-filled syringe into the artery, oriented toward the head. Tightly knot the string around the cannula to hold it in place. Fill embalming machine with warm formaldehyde embalming solution. Turn on machine and allow solution to run completely through the hose. Pinch off the hose and connect to the cannula and perfuse at 4 psi. Locate the external jugular vein on one side of the animal. Loosely loop two strings around the vein. When pressure starts to build in the vein, make a cut in the vein between the two strings. When fluids start to run clear, tightly tie off the two strings on either side of the vein incision. Perfuse about 1L embalming solution after the vein is tied off.

B.4. Paraffin Processing Protocol

Dehydration

	<u>Reagent</u>	<u>Time</u>
1.	Ethanol, 70%	5 hrs.
2.	Ethanol, 80%	1 hr.
3.	Ethanol, 95%	1 hr.
4.	Ethanol, 95%	1 hr.
5.	Ethanol, 100%	1 hr.
6.	Ethanol, 100%	1 hr.
7.	Ethanol, 100%	1 hr.
7.	Xylene	1 hr.
8.	Xylene	1 hr.
9.	Paraffin	1 hr.
10.	Paraffin	1 hr.
11.	Paraffin	1 hr.

Embedding

Tissues were embedded in Paraplast X-tra (McCormick) tissue embedding medium using a Leica EG 1160 embedding center.

B.5. Verhoeff Staining Protocol

Solutions and Reagents

Weigert's iodine solution

Potassium iodide	2 g
Iodine	1 g
Distilled water	100 ml

Use 5 ml distilled water to dissolve potassium iodide; add iodide. Once dissolved, dilute by adding 95 ml distilled water.

Verhoeff's working solution

5% alcoholic hematoxylin	20 ml
10% ferric chloride	8 ml
Weigert's iodine solution	8 ml

Staining Procedure

1. Deparaffinize and hydrate slides to distilled water.
2. Stain in Verhoeff's solution for 1 hour. Tissue should be completely black.
3. Rinse in tap water with 2-3 changes.
4. Differentiate in 2% ferric chloride for 1-2 minutes.
5. Stop differentiation with several changes of tap water.
6. Treat with 5% sodium thiosulfate for 1 minute.
7. Wash in running tap water for 5 minutes.
8. Counterstain with Masson's trichrome

B.6. Modified Masson Trichrome Counter-Staining Protocol

Solutions and Reagents

Masson Trichrome Modification

Ponceau 2R	2.7 g
Acid fuchsin	1.3 g
Orange G	2.0 g
1% Acetic acid water	400 ml

5% Phosphotungstic acid

Phosphotungstic acid	10 g
Distilled water	200 ml

Aniline Blue Solution

Aniline blue	2.5 g
Acetic acid, glacial	2 ml
Distilled water	100 ml

Counter Staining Procedure

1. Following the Verhoeff's staining procedure, rinse slides in running tap water for 5 min.
2. Stain in working Masson trichrome stain for 1 min.
3. Rinse in 0.2% acetic acid water – 2 changes.
4. Mordant in 5% phosphotungstic acid solution for 2 min. (discard solution).
5. Rinse in 0.2% acetic acid water 3 min.
6. Stain in aniline blue solution for 3 min.
7. Rinse and differentiate in 1% acetic acid water, 2 changes, 1 min. each.
8. Dehydrate in 2 changes each 95% alcohol and absolute alcohol.
9. Clear in xylene and mount in Permount.

B.7. Results for Modified Masson Trichrome Counter-Staining Protocol

Connective tissue (bone, collagen and elastic fibers)	Blue
Nuclei.....	Dark blue to black
Cytoplasm, keratin, muscle fibers, fibrin.....	Red

APPENDIX C – PERMISSIONS

From: Alexandra Lainsbury [mailto:A.Lainsbury@cabi.org]
Sent: Thursday, June 21, 2012 3:05 AM
To: George M Strain
Cc: Cathryn K Stevens-Sparks
Subject: Permission to use chapter in dissertation

Dear George,

CAB International hereby grants permission for Cathryn Stevens-Sparks to reproduce ‘Chapter 1 – Anatomy of the Ear’, from *Deafness in Dogs and Cats*, CABI, 2011, pp 1-22, in her doctoral dissertation at Louisiana State University, in both print format and electronic dissertation.

The original source of the material must be fully acknowledged thus: Author(s)/Editors(s); Year of publication; Title; Publisher (CAB International, Wallingford, UK).

Best wishes,

Alex

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Anatomy of the Auditory System

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Louisiana State University School of Veterinary Medicine
and Comparative Biomedical Sciences, Baton Rouge,
Louisiana 70803, USA

The ear is the sensory organ for hearing, balance and equilibrium. This organ can be divided into the outer, middle and inner ear; all of the inner ear structures are contained within the osseous labyrinth, a series of excavations within the temporal bone of the skull. The three components of the ear provide the ability for terrestrial mammals to transform sound pressure waves, i.e. longitudinal waves, into electrical energy. Pressure waves are transformed into mechanical energy in the middle ear, then into pressure waves in the fluid-filled inner ear and into electrical energy in the organ of Corti. This electrical energy is then processed and interpreted by the central nervous system. Sound waves are captured and transmitted to the middle ear by the structures of the external ear. The components of the external ear include the auricle, or pinna, and external auditory meatus, or ear canal, which receive sound waves and guide those to the eardrum, or tympanic membrane. The tympanic membrane separates the external ear from the middle ear. Components of the middle ear include the tympanic cavity and the three auditory ossicles (malleus, incus and stapes) and their associated ligaments and muscles. Components of the inner ear include the organ of hearing, the cochlea, and the organs of equilibrium, the semicircular canal ducts, the utricle and the saccule.

Anatomy of the External Ear

The external ear is comprised of the auricle (pinna) and external auditory meatus (ear canal). The auricle of the ear is shaped like a funnel in many

Re: Permission Request

FROM: [Howard Evans](#)
TO: [Cathryn Sparks](#)

Friday, May 18, 2012 9:38 AM

Dear Cathryn Sparks:

I am glad to hear that the illustrations in "Miller's Anatomy of the Dog" are of use to you. As the copyright executive for these Cornell University figures I hereby give you permission to use them, as you indicate, if reference as to their source (from "Miller's Anatomy of the Dog") is cited in the legend to each figure.

Next month a colored revision of "Miller's Anatomy of the Dog" by Evans and deLahunta will be published by Elsevier. To use the colored versions of these illustrations I think you will have to ask Elsevier Publishers.

Sincerely yours,
Howard Evans

On May 17, 2012, at 3:30 PM, Cathryn Sparks wrote:

Dear Dr. Evans,

I would like to thank you for granting your permission for use of images from *Miller's Anatomy of the Dog* (3rd ed.) in a recent book publication *Deafness in Dogs and Cats* (Stevens-Sparks CK. Anatomy of the Auditory System. In: Strain, GM (ed.) *Deafness in Dogs and Cats*. Wallingford, UK: CAB International; 2011. p. 1-22). I have been granted permission by the publishing company to use this book chapter, which would include your images (see below), in chapter 1 of my dissertation. I would like also to request your personal permission to use these images, as part of this book chapter, in my dissertation. The images used in the book chapter were from *Miller's Anatomy of the Dog*, 3rd edition, 1993, Saunders/Elsevier and included the following:

<u>Figure</u>	<u>Page</u>
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By this letter, I am also requesting permission to use one additional image, depicted in figure 11-15 (p. 607) from *Miller's Anatomy of the Dog*, 3rd edition, 1993, Saunders/Elsevier (arteries of the head in relation to the lateral aspect of the skull), in chapter 4 of my dissertation and perhaps in a future publication on a review of the blood supply of the canine ear. I greatly appreciate your consideration of these requests and look forward to hearing from you soon.

Sincerely,
Cathryn Stevens-Sparks
Instructor of Anatomy
Comparative Biomedical Sciences
Louisiana State University School of Veterinary Medicine
Baton Rouge, LA 70803

Professor Strain,

Yes, you and your student have Hill's permission to reprint the Hill's Atlas images contained within the book chapter written by your student.

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Jene' Haas

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From: George M Strain <strain@lsu.edu>
To: Jene Haas/USATP/NA/HILLS/COLPAL@COLPAL
Date: 06/13/2012 01:49 PM
Subject: Permission

Ms. Jene Haas:

As we discussed by phone, in August, 2010 Hill's Pet Nutrition gave me permission to reproduce two figures from *Hill's Atlas of Clinical Veterinary Anatomy* in a book titled *Deafness in Dogs and Cats* (which has subsequently been published), as outlined in the attached approval letter from Hill's.

One of those figures, "Normal Hearing Apparatus" was used as Figure 1.2 in the first chapter of the book, "Anatomy of the Auditory System." That chapter was written by doctoral graduate student Cathryn K. Stevens-Sparks. The publisher of the book, CAB International, located in the U.K., has given her permission to reproduce that chapter in her doctoral dissertation. By this note we are requesting permission to reproduce the Hill's figure within that chapter, exactly as originally published in the book, subject to the same conditions of the original permission.

Thank you in advance.

George M. Strain
Professor and Interim Head
Comparative Biomedical Sciences
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SHORT COMMUNICATION

Post-anesthesia deafness in dogs and cats following dental and ear cleaning procedures

Cathryn K Stevens-Sparks & George M Strain

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Abstract

Objective The present study was performed to document hearing loss in dogs and cats following procedures performed under anesthesia. Most cases of reported hearing loss were subsequent to dental and ear cleaning procedures.

Study design Prospective and retrospective case survey.

Animals Subjects were dogs and cats with deafness, personally communicated to one author, cases discussed on a veterinary information web site, and cases communicated through a survey of general practice and dental specialist veterinarians.

Methods Reported deafness cases were characterized by species (dog, cat), breed, gender, age, and dog breed size.

Results Sixty-two cases of hearing loss following anesthesia were reported between the years 2002 and 2009. Five additional cases were reported by survey respondents. Forty-three cases occurred following dental procedures. Sixteen cases occurred following ear cleaning. No relationship was observed between deafness and dog or cat breed, gender, anesthetic drug used, or dog size. Geriatric animals appeared more susceptible to post-anesthetic, post-procedural hearing loss.

Conclusions Deafness may occur in dogs and cats following anesthesia for dental and ear cleaning

procedures, but the prevalence is low. The hearing loss appears to be permanent.

Clinical relevance Deafness can be a consequence following anesthesia for dental or ear cleaning procedures. Older animals may have greater susceptibility.

Keywords anesthesia, cat, deafness, dental treatment, dog, geriatric.

Introduction

Deafness in dogs and cats can be hereditary or acquired; causes of acquired hearing loss include drug toxicity, noise trauma, infection, aging, middle ear effusion, excess cerumen production, and physical trauma (Strain 1996, 1999). The causes of acquired deafness are frequently unknown. A correlation between dental and/or ear cleaning procedures under anesthesia and the acute onset of deafness in dogs and cats may exist, based on personal communications with pet owners (Strain GM, unpublished observations), but clear prevalence data are unavailable and causes have not been established in these cases. Pet owners have reported bilateral deafness to be present upon anesthesia recovery from such procedures and the hearing loss was nearly always permanent. It is difficult to establish whether the reported hearing loss is sensorineural or conductive without the brainstem auditory evoked response (BAER) test, which can distinguish between sensorineural hearing loss (using an air-conducted auditory stimulus) and

VITA

Cathryn Kay Nichols was born in Baton Rouge, Louisiana, graduated from Belaire High School in 1986, and then began studies of biology at Louisiana College in Pineville. She transferred to Louisiana State University, where she completed a Bachelor of Science degree in Zoology in December, 1993. Cathryn entered the Zoology graduate program at LSU in August, 1995, where she taught as a teaching assistant in Comparative Anatomy for four years while performing research on biomedical adaptation of the G-proteins in deep sea fish. Cathryn graduated from LSU in May, 1999, with a Master of Science degree in Zoology. She worked as a research associate in the Dietary Obesity Laboratory at Pennington Biomedical Research Center from 2001 to 2004 when she left to pursue her desire to teach anatomy. She was hired as a teaching associate in gross anatomy at the LSU School of Veterinary Medicine in 2004 enrolled as a part-time graduate student in 2005 under the direction of Dr. George Strain, while still employed full-time as a gross anatomy teaching associate. She was appointed to the faculty as an instructor in September, 2011 and continues to teach in gross anatomy and neuroscience in the professional curriculum. Cathryn also teaches human anatomy and physiology as an adjunct instructor at Our Lady of the Lake College in Baton Rouge. Cathryn Stevens-Sparks plans to graduate with her PhD in Veterinary Medical Sciences in August, 2012 and continue teaching at the LSU School of Veterinary Medicine.