Visualizing Cochlear Specializations that Enhance Protection of Hearing Function in Bats

by
Jamie Lynn Peterson

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ABSTRACT

The cochlea is a fluid-filled coil of the inner ear that transforms sound waves into electrical signals for processing in the brain. The mammalian auditory system is most commonly studied using the mouse, *Mus musculus*, due to anatomical similarities of the cochlea between auditory generalists, such as mice and humans. Auditory specialists, such as bats, exhibit unique resistance to age-related hearing loss, or presbycusis. This adaptation enables bats to navigate while flying with echolocation throughout their lifetime. Studying comparative cochlea anatomy can aid in understanding specializations of the mammalian auditory system and hearing loss among species. There is a significant gap in available educational resources for comparative cochlea anatomy focusing on bats and mice.

The purpose of this project was to develop an interactive educational resource for comparative cochlea anatomy of the big brown bat, *Eptesicus fuscus* and *Mus musculus* with a 3D overview animation depicting labeled cochlea models. Segmentations of histological and micro-CT data were modified and sculpted to build idealized anatomical models suitable for teaching purposes. A separate section of the interactive allows the user to explore comparative cochlear anatomy of bats and mice as related to hearing loss. The user interface and interactivity were coded to allow exploration of bat and mouse cochlea regions and intuitive navigation between sections about specific anatomical structures and bat hearing loss research. The results of this project provide a didactic and accessible visualization for auditory researchers, graduate students, and lay audiences to review basic cochlear anatomy, compare cochlear anatomy of bats and mice, and strengthen their understanding of human age-related and noise-induced hearing loss.

Jamie Lynn Peterson

CHAIRPERSONS OF THE SUPERVISORY COMMITTEE

Amanda Lauer, **MS**, **PhD**, Preceptor
Associate Professor, Otolaryngology Research, Department of Otolaryngology-Head and Neck Surgery
The Johns Hopkins University School of Medicine

Lydia Gregg, **MA**, **CMI**, **FAMI**, Department Advisor
Associate Professor, Department of Art as Applied to Medicine and Division of Interventional Neuroradiology, Department of Radiology and Radiological Science
The Johns Hopkins University School of Medicine

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INTRODUCTION

Cochlea Overview

The cochlea is a fluid-filled coil of the inner ear located in the petrous portion of the temporal bone. The apex of the cochlear spiral points rostrally towards the mandible, while the base points towards the external ear to receive sound vibrations. The role of the cochlea is to transform sound waves from the middle ear into electrical signals for processing in the auditory system of the brain. The cochlea is surrounded by the bony labyrinth and otic capsule, within which the membranous labyrinth houses the cellular structures that detect incoming sound frequencies.

The interior of the otic capsule is sectioned into three fluid-filled chambers or scalae (**Figure 1**). The uppermost chamber is the scala vestibuli, the middle chamber is the scala media, and the lower chamber is the scala tympani. At the base of the otic capsule are two membrane-covered windows that are open to the middle ear ossicles. The oval window is in contact with the foot of the stapes at the base of the scala vestibuli while the round window is at the base of the scala tympani (Robles, 2001). The scala vestibuli and scala tympani both contain perilymph fluid and join together at the helicotrema of the apex of the cochlea. The scala media is filled with endolymph fluid (Slepecky, 1996). The scala media is separated from the scala vestibuli superiorly by Reissner's membrane and separated from the scala tympani inferiorly by the basilar membrane. The lateral wall of the scala media is formed by the stria vascularis, which is attached to the spiral ligament. The scala tympani ends at the round window, while scala media and vestibuli continue beyond the cochlea base to the vestibular system of the inner ear (Slepecky, 1996).

The membranous labyrinth contains the cellular structures of the organ of Corti. The organ of Corti is housed within the scala media and spirals along the basilar membrane from apex to base. The organ of Corti's cellular structures consist of the receptor cells, inner hair cells, outer hair cells, and supporting cells. Inner hair cells are flask-shaped cells that are aligned in a single row and topped with stereocilia (Kössl and Vater, 1995). Outer hair cells are cylindrical-shaped cells that are arranged in rows of three and are topped with three rows of stereocilia. Outer hair cells are thought to enhance the sensitivity and selectivity of the cochlea and participate in efferent pathways that protect the cochlea from sound-induced trauma (Raphael, 2003, and Kössl and Vater, 1995; Fuchs and Lauer 2019).

At the base of the inner hair cells are numerous afferent nerve endings from type I spiral ganglion neurons. These nerve fibers carry signals to the brain for processing. Approximately 80-90% of afferent endings connect at the inner hair cells via the spiral ganglion, while the remaining percent end at the outer hair cells as type II spiral ganglion neurons (Kössl and Vater,1995, Vater, 2000). At the base of outer hair cells, efferent nerve fibers connect to a single large terminal (Kössl and Vater,1995). The supporting cells of the organ of Cori are non-sensory cells that consist of Henson, Claudius, Pillar, Deiters, and Bottcher cells that create a rigid scaffolding for the outer hair cells (Kössl and Vater,1995).

The organ of Corti rests on top of the basilar membrane (**Figure 2**). The basilar membrane moves in response to the arrival of sound waves, causing the hair cells' stereocilia to brush against the tectorial membrane (Vater, 2000). The movement of the sensory hair cells creates electrical signals that travel to the brain for processing via the auditory nerves. Hair cells along the basilar membrane are stimulated by different

frequencies. Low-frequency sounds stimulate hairs cells in the apex of the cochlea, while high-frequency sounds stimulate hair cells at the base (Vater, 2000). Damage to the cochlea plays an essential role in the onset of presbycusis or age-related hearing loss. The loss of sensory hair cells and damage to the nerve fibers over time causes age-related hearing loss in most mammals.

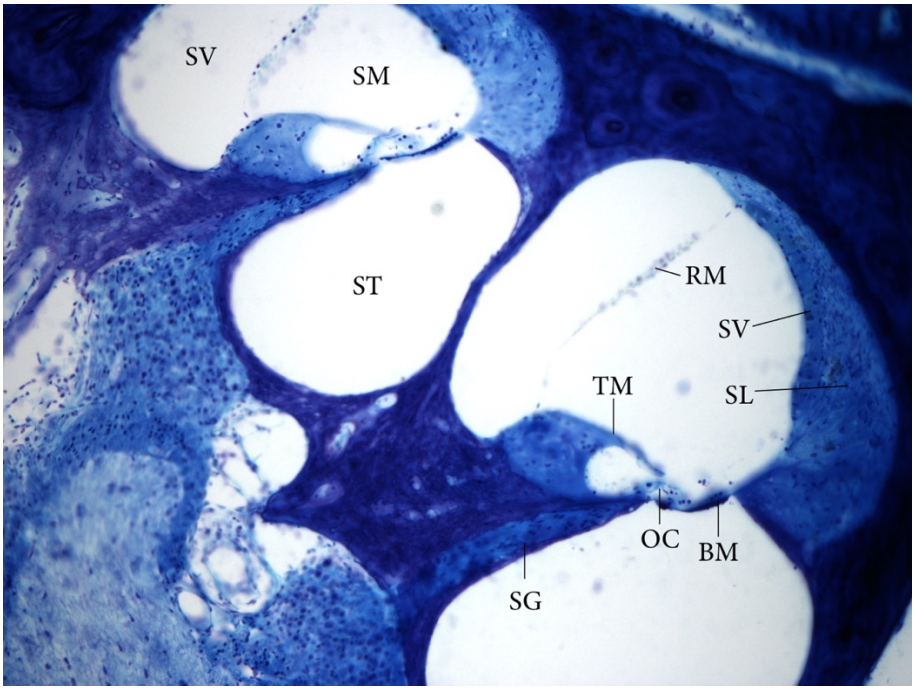


Figure 1. Membranous cochlea structures of the big brown bat, *Eptesicus fuscus*. Histological slide of specimen G16_L (**Table 1**). SV, scala vestibuli; SM, scala media; ST, scala tympani; RM, Reissner's membrane; SV, stria vascularis; SL, spiral ligament; TM, tectorial membrane; OC, organ of Corti; BM, basilar membrane; SG, spiral ganglion.

Bat and mouse cochlea anatomy

The mammalian auditory system is commonly studied using the mouse, *Mus musculus*, as a model organism for understanding noise-induced and age-related hearing loss in humans due to anatomical similarities of the cochlea. The goal of this research is to develop and assess animal models for both normal and pathological features of hearing in

order to make comparisons with human hearing. This approach is called “comparative hearing research”, which studies the structures, physiological functions, and hearing abilities of various species in order to determine the essential principles of how structures determine function, and aid in clarifying the evolution of hearing among animals (Fay and Popper, 1993).

While mice are frequently used as the animal model for researching noise-induced and age-related hearing loss, bats are rarely used as models for studying hearing loss, despite their presumed resistance to noise and age-related damage. The bat cochlea is comprised of the common mammalian set of sensory and supporting structures, however, it has adapted mechanisms for sound transmission and frequency evaluation to accommodate for a frequency range far above humans (Vater, 2000). Unlike mice and humans, bats exhibit a unique resistance to noise-induced and age-related hearing loss. A specially adapted auditory system allows bats to navigate using echolocation for flying, hunting, and roosting throughout their lifetime (Vater, 2000). To understand prevention of acquired hearing loss in humans, the science community is beginning to look to bats as model organisms for the mammalian peripheral auditory system.

It has long been assumed that bats are unaffected by loud noise exposures and hearing loss. However, new research shows that the big brown bat, *Eptesicus fuscus*, can develop hearing loss due to a combination of stressors including malnourishment, housing stress, inbreeding, and exposure to noise pollution in captivity (Retta et al., 2019). Comparing cochlear morphological differences between the big brown bat and mouse is useful for studying protections against hearing loss (**Figure 3**). The big brown bat is an auditory specialist that has a higher and broader range of hearing compared to the house

mouse, *Mus musculus*, an auditory generalist. There are remarkable variations in cochlea structure between these two species.

Comparing skull dimensions helps to envision the size difference of the two species. Skull size of *Eptesicus fuscus* is ~18.5mm in length and ~12.5mm in zygomatic width (Albrecht, 2003) compared to the *Mus musculus* skull length of ~21.0mm and width of ~11.0mm (Cory, 1912). Comparing cochlea size to the dimension of the skull, the *Eptesicus fuscus* cochlea is proportionality much larger.

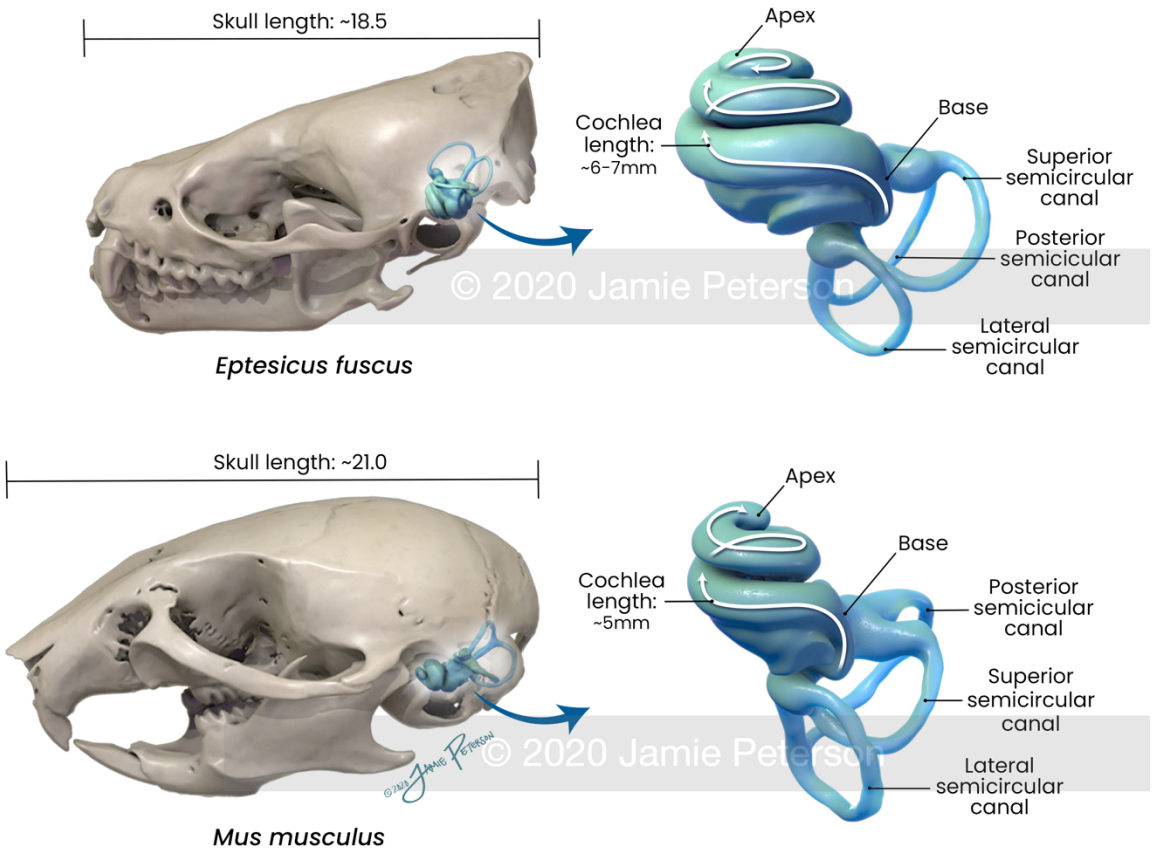


Figure 2. Skull and cochlea coil measurements.

Unpublished data from the Lauer Lab in the Center for Hearing and Balance at Johns Hopkins University School of Medicine show that the coil length of *Eptesicus fuscus* is approximately 6-7mm from base to apex compared to *Mus musculus* coil length of approximately 5mm. Cochleae can also be measured by the number of turns, *Eptesicus fuscus* cochlea has ~2.5 turns (Vater, 2000) compared to ~2.0 turns in *Mus musculus* (Slepecky, 1996). The extra half turn of the big brown bat cochlea supports a broader hearing range. Based on observations in the Lauer Lab, the axis of the big brown bat's cochlea is more ventrally oriented compared to the mouse.

There are also membranous and cellular structural differences between the cochleae of *Eptesicus fuscus* and *Mus musculus*. The basilar membrane of *Eptesicus fuscus* is overall longer, narrower, and thicker compared to *Mus musculus* (Kössl and Vater, 1995). This increases the stiffness of the membrane, allowing for sensitivity to higher frequency signals (Kössl and Vater, 1995). Sensory hair cell adaptations of *Eptesicus fuscus* include shorter outer hair cells and stereocilia compared to *Mus musculus*, a specialization thought to increase sensitivity to higher frequency sounds (Kössl and Vater, 1995 and Mao et al., 2017). Studying comparative cochlea anatomy can aid in understanding specializations of the mammalian auditory system and differences in susceptibility to hearing loss across species.

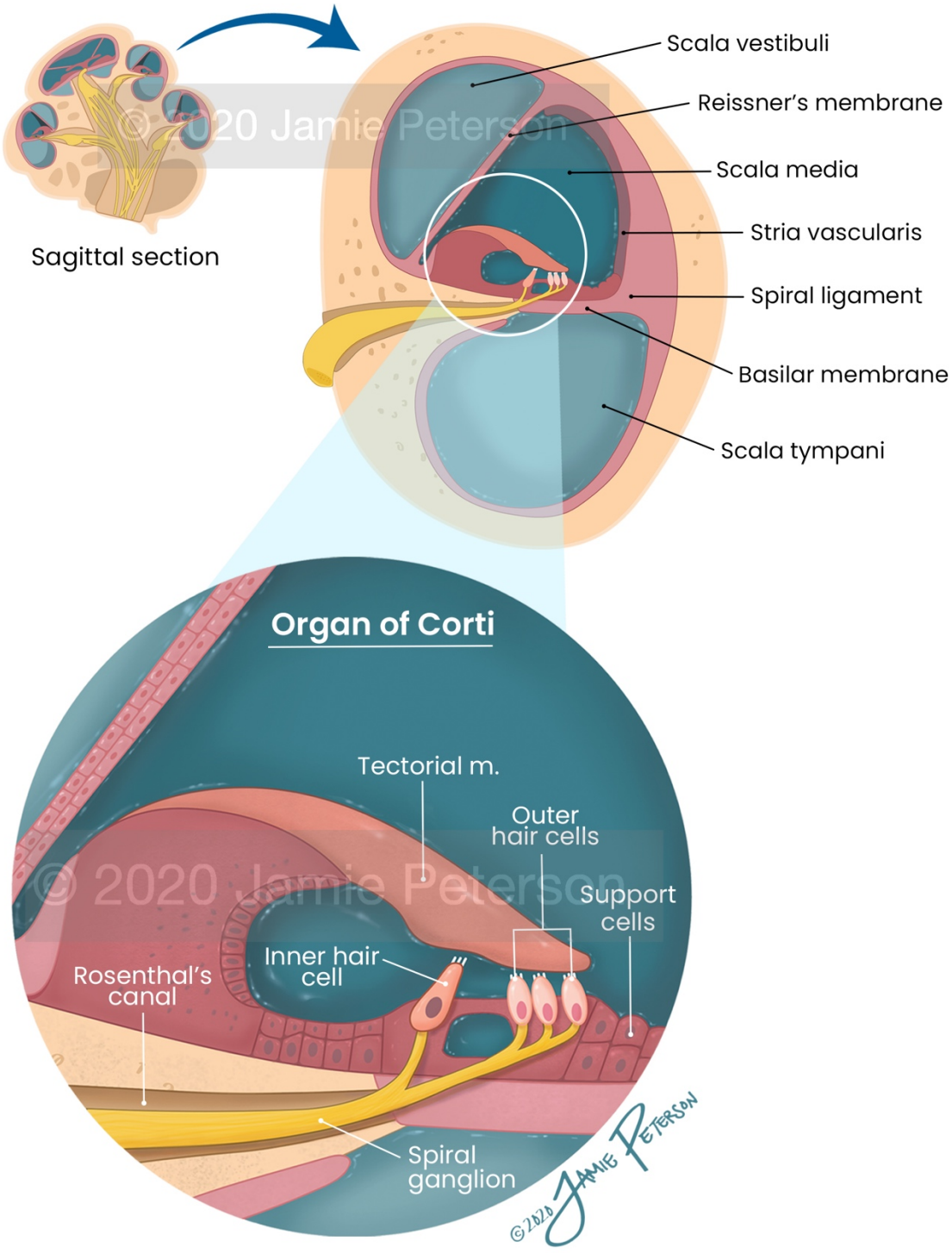


Figure 3. A visual summary of the cochlea membranous structures of *Eptesicus fuscus*. The illustration shows the cochlea regions discussed in this project.

Interactive comparative anatomy in education

It is challenging to visualize the three-dimensional structure of the cochlea using two-dimensional images and histology slides alone. Available interactive educational resources are limited to human cochlea anatomy, such as "The Interactive Ear: A Guide to Human Hearing" (Amphilon) and "Anatomy.tv" (Informa UK Limited). There are no interactive cochlea educational resources available on mice or other species despite the frequent use of animal models in mammalian auditory system research. Discussions with graduate students and researchers at Johns Hopkins University revealed a need for an interactive resource that would offer depictions of cochlea anatomy comparing bats and mice.

Depictions of cochlea anatomy of bats and mice are found in the literature and include supporting images, histological sections, and illustrations. Textbooks such as *Hearing by Bats* (Popper et al., 1995), *Comparative Hearing: Mammals* (Fay et al., 1994) and *The Cochlea* (Dallos et al., 1996) present descriptions, illustrations, photographs, histological sections, and detailed information on the mammalian cochlea. However, these resources lack interactivity for the learner. Some written resources compare the cochleae of the horseshoe bat (*Rhinolophus*), frog-eating bat (*Trachops cirrhosis*), little brown bat (*Myotis lucifugus*), and short-tailed fruit bat (*Carollia perspicillata*) to other mammals including mice, rats, gerbils, Guinea pigs, cats, and humans (Popper et al., 1995). There is a gap in available educational resources for comparative cochlea anatomy and visual side-by-side comparisons of *Eptesicus fuscus* and *Mus musculus*.

Computer-based interactive imagery, also known as interactive educational modules, can help students to develop mental images and understand spatial relationships of anatomical structures (Khalil, 2005). Interactive images are dynamic and allow students to control the influx of information at their own pace. Students can self-test, self-question, and self-evaluate their learning through the use of interactive labels, images, and models. Interactive educational modules that implement the use of 3D models, images, or animations depicted along with 2D images help the student to visualize structures in 3D (Yammine, 2014). Although formal reports on the efficacy of interactive imagery in facilitating learning are limited in number, interactive media is thought to help users develop new learning skills, allow students to practice recall and self-regulation, and may support and promote independent life-long learning (Khalil, 2005).

Learning theories of instructional design can provide valuable insight into how educational resources can best aid learners in understanding complex material like comparative anatomy. During this project several learning theories were taken into consideration when creating the interactive and animated material. Mayer's Cognitive Theory of Multimedia Learning (Mayer, 2003 and Mayer, 2014), Bruner's Constructivist Theory (Clark, 2018), and the learning concept of Interactive Imagery Strategy by Khalil (Khalil, 2004) are learning theories and strategies that were utilized in the design of this project.

Objectives

Three main objectives directed the development of this project:

1) Communicate cochlea anatomy of *Eptesicus fuscus* and *Mus musculus* by creating an interactive educational resource and 3D cochlea overview animation. Ensure accessibility of the interactive to allow diverse audiences to review basic cochlear anatomy, as well as compare cochlear anatomy of bats and mice.
2) Develop 3D cochlear models to enable accurate mapping and high-quality data visualization of cochlear damage and hearing loss in bats due to inbreeding, malnourishment, and environmental noise pollution.
3) Promote the significance of bat age-related and noise-induced hearing loss research and its potential impact on strengthening the understanding of human age-related and noise-induced hearing loss.

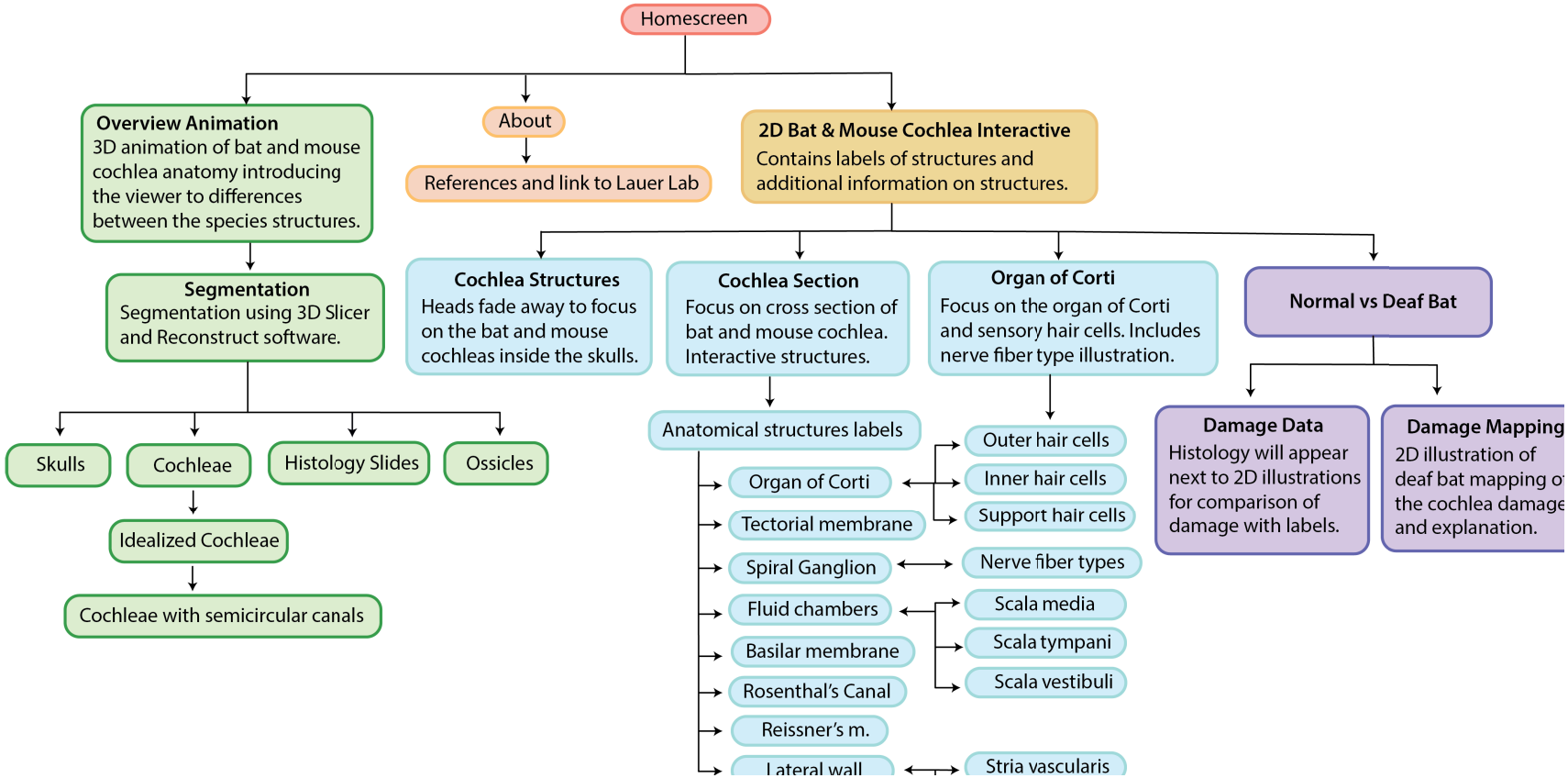
Audience

The primary audience is auditory researchers, neuropathologists, and graduate students studying the mammalian auditory system. The secondary audience is an educated lay audience interested in mammalian cochlear anatomy and age-related and noise-induced hearing loss.

MATERIALS AND METHODS

User interface design and 2D assets

Grayscale wireframes were developed in Adobe Illustrator and Photoshop during the early planning of the interactivity and content organization of the educational interactive, **Appendix A**. The preliminary wireframes helped to determine the layout, design, page navigation, content, and assets needed for the interactive. The 2D vector assets were designed referencing histological slides and 3D micro-CT data and then created in Adobe Illustrator for Adobe Animate. The vector assets were imported into Adobe Animate 2019 for the creation of the interactivity. The developed 2D interactive contains three main sections: Overview Animation section, Cochlea Anatomy section, and Normal vs Deaf Bat section. Secondary pages in the dropdown menus consist of Cochlea Location, Cochlea Structures, Cochlea Section, Organ of Corti, Damage Data, and Damage Mapping.


Homescreen
Overview Animation
3D animation of bat and mouse cochlea anatomy introducing the viewer to differences between the species structures.
About
References and link to Lauer Lab
2D Bat & Mouse Cochlea Interactive
Contains labels of structures and additional information on structures.
Segmentation
Segmentation using 3D Slicer and Reconstruct software.
Cochlea Structures
Heads fade away to focus on the bat and mouse cochleas inside the skulls.
Cochlea Section
Focus on cross section of bat and mouse cochlea. Interactive structures.
Organ of Corti
Focus on the organ of Corti and sensory hair cells. Includes nerve fiber type illustration.
Normal vs Deaf Bat
Skulls
Cochleae
Histology Slides
Ossicles
Idealized Cochleae
Cochleae with semicircular canals
Anatomical structures labels
Organ of Corti
Tectorial membrane
Spiral Ganglion
Fluid chambers
Basilar membrane
Rosenthal's Canal
Reissner's m.
Lateral wall
Outer hair cells
Inner hair cells
Support hair cells
Nerve fiber types
Scala media
Scala tympani
Scala vestibuli
Stria vascularis
Damage Data
Histology will appear next to 2D illustrations for comparison of damage with labels.
Damage Mapping
2D illustration of deaf bat mapping o
the cochlea damage
and explanation.

3D assets

Sources of data

Segmentations of micro-computed tomography (micro-CT) datasets of two *Eptesicus fuscus* specimens were obtained through a free digital repository, MorphoSource.org. Specimens included one female skull dataset with 462 images and a separate mandible set with 313 images each with a z-axis slice interval thickness (distance between images) of ~20μm. One male full body dataset with 200 images with a slice thickness of ~40μm was also segmented (**Table 1**). MorphoSource is hosted by Duke University Research Computing server to allow universities and research institutions to upload and organize unpublished data projects for research and public use. The *Eptesicus fuscus* datasets were uploaded to MorphoSource by researchers at the University of Michigan Museum of Zoology (Project: Digitizing Extant Bat Diversity).

The segmentation of a micro-CT dataset of a female *Mus musculus* skull scanned at ~40μm was obtained from another free digital repository, Digital Morphology, hosted by The University of Texas at Austin (digimorph.org). Additional *Eptesicus fuscus* and *Mus musculus* micro-CT datasets were provided by the Department of Engineering, Whiting School of Engineering at Johns Hopkins University and Pound Human Identification Laboratory at Johns Hopkins University. Segmentations of these datasets were modified to create the idealized *Eptesicus fuscus* and *Mus musculus* skulls and cochlea models for educational purposes (**Table 1**).

Sequential 30μm histological slides of *Eptesicus fuscus* and *Mus musculus* were provided by the Lauer Lab in the Center for Hearing and Balance at Johns Hopkins University School of Medicine. These sagittal histological slides consist of one deaf

Eptesicus fuscus specimen, one normal *Eptesicus fuscus* specimen, *and* one normal *Mus musculus* specimen for structural comparison. Segmentation of the histological slides were used for reference in the creation of the interactive assets (**Table 2**).

Data Type	Identification Name/Number	Source	# of Slices	Source Location	Purpose
Eptesicus fuscus micro-CT, Skull	M18423	MorphoSource.org	462	University of Michigan Museum of Zoology	Segmentation
Eptesicus fuscus micro-CT, mandible	M18424	MorphoSource.org	313	University of Michigan Museum of Zoology	Segmentation
Eptesicus fuscus micro-CT, full body	iDigBio: 110759 MorphoSource: M53150	iDigBio.org MorphoSource.org	2000	University of Michigan Museum of Zoology	Segmentation Skull
Eptesicus fuscus micro-CT, head	Eptesicus Inner Ears	Department of Mechanical Engineering	970	Johns Hopkins University, Whiting School of Engineering	Segmentation Cochlea and semicircular canals
Mus musculus micro-CT	MorphoSource: M13514	MorphoSource.org	685	Pennsylvania State University	Segmentation Cochlea and semicircular canals
Mus musculus micro-CT	1903_F	Pound Human Identification Laboratory	822	Johns Hopkins University, SOM Identification Laboratory	Segmentation Cochlea, semicircular canals, and skull
Eptesicus fuscus STL, Skull	M18423	MorphoSource.org	-	University of Michigan Museum of Zoology	Reference
Eptesicus fuscus STL, mandible	M18424	MorphoSource.org	-	University of Michigan Museum of Zoology	Reference
Mus Musculus STL, skull	TMM M-3196	Digimorph.org	-	The University of Texas at Austin	Reference

Table 1. Skull and Cochlea datasets. Compiled list of data including type, species, location, and intended purpose of the data collected.

Histological slides	Identification Name/Number	Source	# of Slices	Source of Location	Purpose
Eptesicus fuscus, Normal	G16_L	Lauer Lab, JHU, SOM	48	Johns Hopkins University, SOM	Segmentation membranous structures
Eptesicus fuscus, Deaf	Brown24_L	Lauer Lab, JHU, SOM	39	Johns Hopkins University, SOM	Segmentation membranous structures
Mus musculus, Normal	P20599_L	Lauer Lab, JHU, SOM	28	Johns Hopkins University, SOM	Segmentation membranous structures

Table 2. Histology data collection. Compiled list of data collected including species, location, and intended purpose of the data collected.

Overview of software

Numerous software packages were used throughout this project to segment and manipulate the data, create 2D and 3D assets, and develop the 2D interactive and 3D animation. 3D Slicer (version 4.10.2, Fedorov et al., 2012) was used to view volumetric micro-CT datasets of *Eptesicus fuscus* and *Mus musculus* and create segmentations of selected cochlear structures. Reconstruct (version 1.1.0.0, 2007) was used for histological slide stacking, alignment, and structure segmentation. Pixologic ZBrush 2019 was used to modify, sculpt, and idealize bat and mouse skulls, cochleae, and cochlea structures. Maxon Cinema 4D (C4D) (version R20) was used to manipulate and repair the 3D models further. MeshLab (v.2016.12) was used to optimize the 3D models for ZBrush and 3D animation in C4D.

Information architecture diagrams for the 2D interactive were created with Adobe Illustrator and Photoshop 2020. Wireframes for the 2D interactive (**Appendix A**) and storyboards for the 3D animation (**Appendix B**) were created in Adobe Illustrator and Adobe Photoshop 2020. Illustrator and Photoshop were also used for creating the 2D illustration assets used for the interactive module for Adobe Animate 2019.

Segmentation in 3D Slicer

Volumetric datasets were imported into 3D Slicer for segmentation. 3D Slicer is an open-source software program for medical image processing and three-dimensional visualization of volumetric datasets (Kikinis, 2014). Segmentations were added by clicking the **Add Data** widget in the upper left corner or by clicking **File > Add Data**. Within the **Add data into the scene** window, the dataset was added by selecting **Choose File(s) to Add**. Before importing the data, **Show Options** was clicked, and **Single File** was unchecked.

Segmentation was performed using the **Segment Editor** module of 3D Slicer after importing the volumetric data. The data can be viewed in three windows: (A) coronal, (B) sagittal, (C) axial. Manual segmentation of the cochlea from *Eptesicus fuscus* and *Mus musculus* cranium was preformed instead of using the automated process. After loading the data, the Segment Editor of Slicer was selected in the top menu bar, and the **Add (+)** button was clicked to create a new empty segmentation layer. The coronal view was used to create the initial segmentation of the cochlea. The **Paint tool**, shortcut 1, was used to paint the desired area of the cochlea (**Figure 5**). The cochlea was painted every ten slices by navigating through the slices with the view slider. A live 3D view of the segmented area was turned on periodically to check the resulting surface by clicking **Show 3D**. The 3D view was turned off while segmenting to avoid slowing of the software operations. To clean up the segmentation slices, the **Eraser tool**, shortcut 3, was used to remove undesired and overpainted areas.

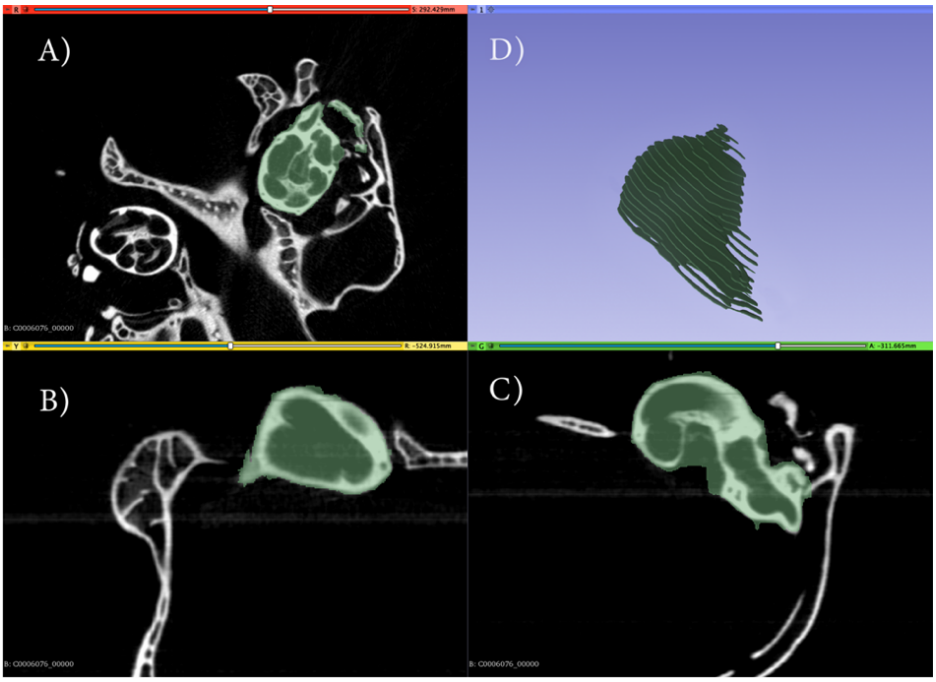


Figure 5. Left cochlea segmentation of *Eptesicus fuscus* in 3D Slicer. (A) Axial, **(B)** Sagittal, **(C)** Coronal, **(D)** live 3D views. The green painted area is shown in the orthogonal views as slices. *Text within image not intended to be read.*

The **Fill between slices** tool was used to connect the segment slices and create a 3D surface of the segmentation. The **Initialize** button, under **Fill between slices** displayed a preview of the calculated 3D segmentation. Clicking **Apply** turned the segmentation into a 3D surface. To create a complete segmentation of the exterior cochlea and the semicircular canals, painting in multiple views was required.

Segmentations of the axial and sagittal views were created using the same painting method and **Fill between slices** to create 3D segmentations. Painting in multiple views ensured that the whole cochlea was segmented, however, all three segmentations needed to be painted and filled separately and then combined to create a complete cochlea representation. The **Add** operation under **Logical operators** was used to combine the three segmentation views into one complete external cochlea shape (**Figure 6**).

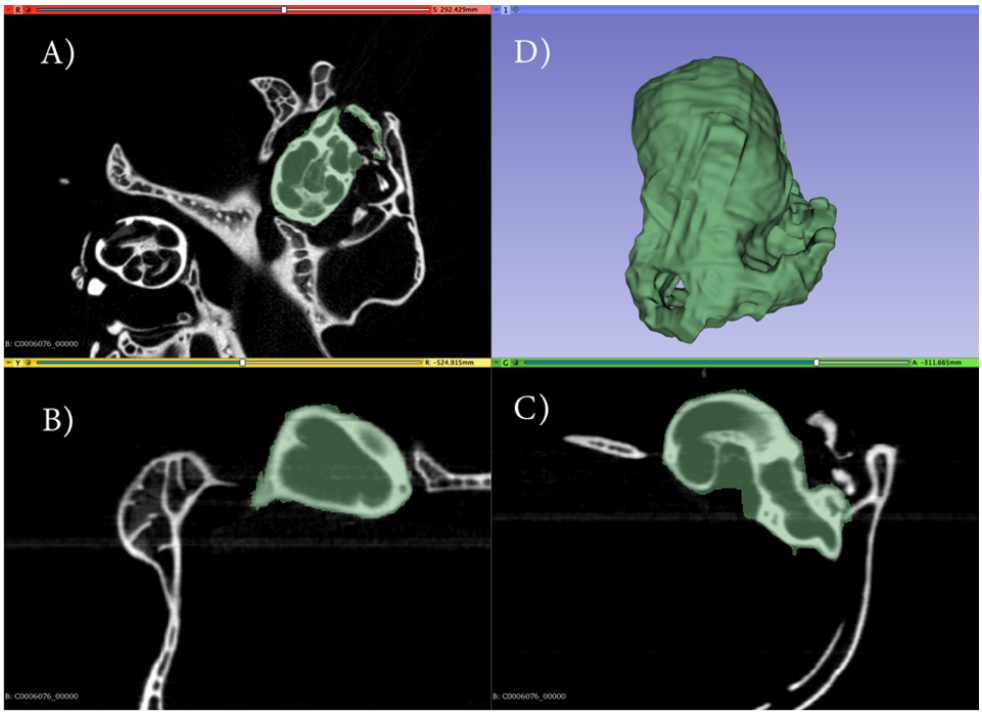


Figure 6. Left cochlea segmentation after Fill between slices and Logical operators. The Paint tool and live 3D view are active, this model is the completed external cochlea segmentation after Fill between slices and combining segments from all three views. *Text within image not intended to be read.*

Threshold was used to generate a clean surface model of the cochlea and semicircular canals from the initial segmentation. To segment out the cochlea and semicircular canals, a new segment was created in the Segment Editor. **Threshold** was set to **auto-> maximum** and the threshold range slide bar was used to fine tune the selection targeting the fluid-filled chambers of the cochlea and semicircular canals. The **Editable area** was restricted to the original segmentation before applying the threshold to create a complete cochlea and semicircular canal model of the fluid-filled chambers.

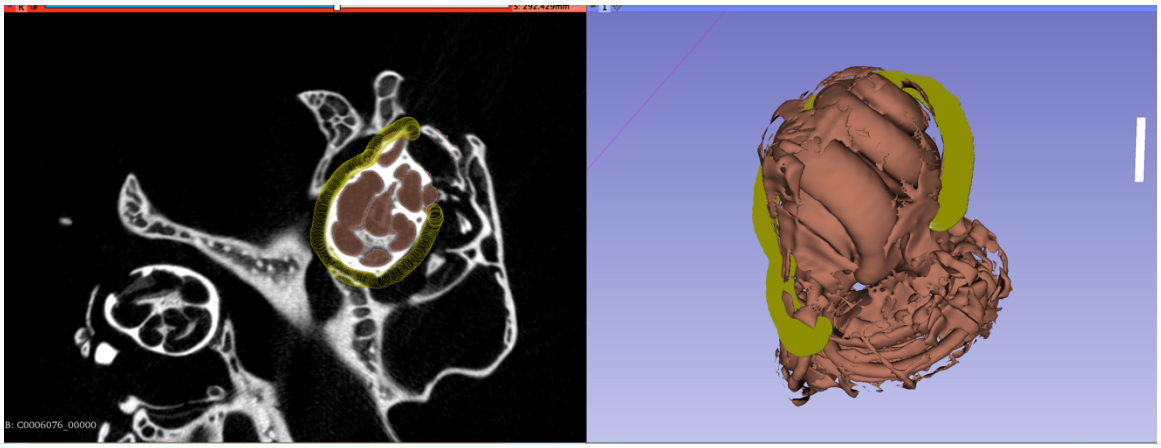


Figure 7. Left cochlea segmentation threshold of inner cochlea chambers. Results of thresholding inside the cochlea in axial and 3D views. Unwanted surface fragments were removed using the Eraser tool, shown in yellow. *Text within image not intended to be read.*

The cochlea surface model needed additional cleaning and repair after Thresholding. Undesired small surface fragments around the model were removed automatically using **Islands** > **Remove small islands**. Larger fragments connected to the cochlea surface were removed using the **Eraser** and **Scissor tools** on the axial, sagittal, coronal, and 3D views using the Sphere brush (**Figure 7**). Additional segmentations needed to repair the model were created using the **Paint tool** and combined with the cochlea surface model using **Logical operators** to fill holes and missing semicircular canal sections (**Figure 8**).

Cochlea segmentations completed in 3D Slicer were exported as STL files by clicking the drop-down menu arrow beside the **Segmentations…** button and selecting **Export to files…**. Each segmented layer was saved as an STL. The cochlea STL file was imported into C4D and ZBrush for further sculpting and repair.

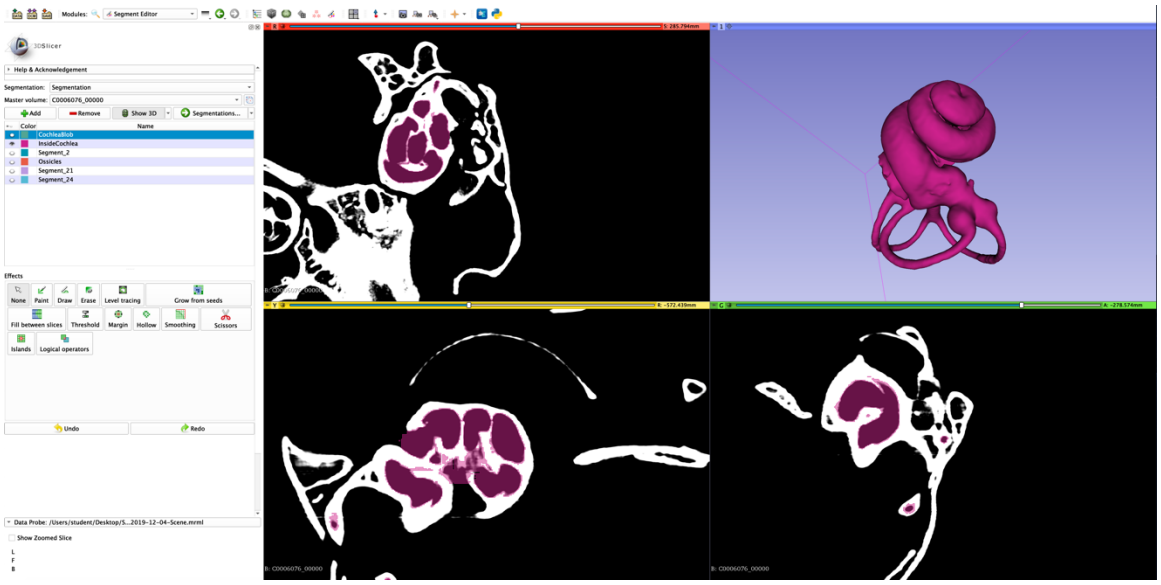


Figure 8. Left cochlea segmentation of inner cochlea chambers. Results after cleaning the surface of specimen, Brown24_L. The whole cochlea and semicircular canals shown in pink. *Text within image not intended to be read.*

Segmentation of *Eptesicus fuscus* cochlea from the Whiting Engineering Department involved extra preparation for 3D Slicer segmentation. The micro-CT dataset needed recalibration to include z-axis slice interval thickness and image resolution data. The dataset was stacked by importing the files in Adobe Photoshop under **File > Scripts > Load Files into a Stack**. The images were sorted by name to ensure that the files were in order before stacking. Once the images were stacked, they were saved as a DICOM file and imported into Horos for recalibration.

In Horos, the file was opened in **3D Volume Rendering**. The calibration numbers were entered into the dialog box for **Pixel X** and **Y resolution** and **Slice interval**. The slice interval thickness was calculated by taking the z-axis coordinate of the first image of the sequence and subtracting it from the last z-axis coordinate in the stack. This number was divided by the total number of images to get the slice interval thickness in micrometers. The Pixel X and Y resolution numbers were found in the log document from the scans and

were converted to micrometers (**Figure 9**). The calibration allowed a 3D volume reconstruction. The calibrated data was exported as a DICOM file and was added to 3D Slicer for segmentation of the cochlea.

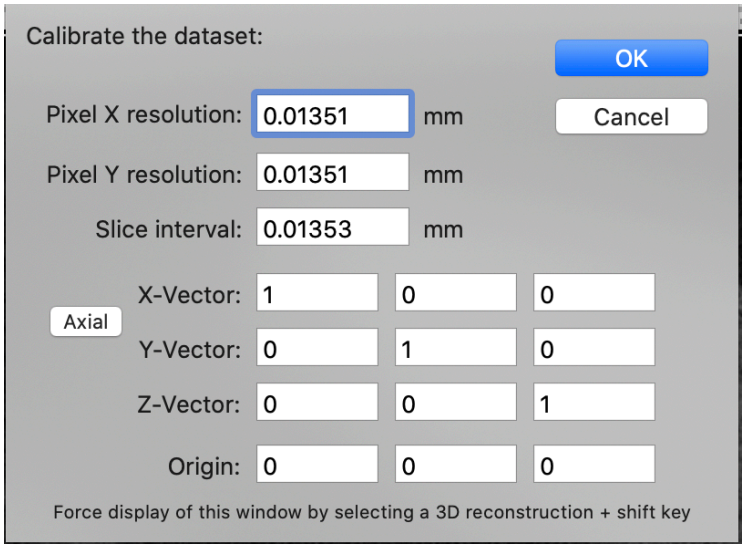


Figure 9. Recalibration window in Horos. The calculated calibration numbers used to prepare the Johns Hopkins *Eptesicus fuscus* specimen for segmentation in 3D Slicer.

Segmentation of histological slides in Reconstruct

Reconstruct is a free editor for Windows PCs designed to assist with montaging, aligning, analyzing, transforming, and displaying data of histological slides, also known as serial sections (Fiala, 2005). Before segmentation of cochlea structures, the serial sections needed to be imported, aligned, and locked into place following the instructions from the Reconstruct User Manual (Fiala, 2009) (**Figure 10**).

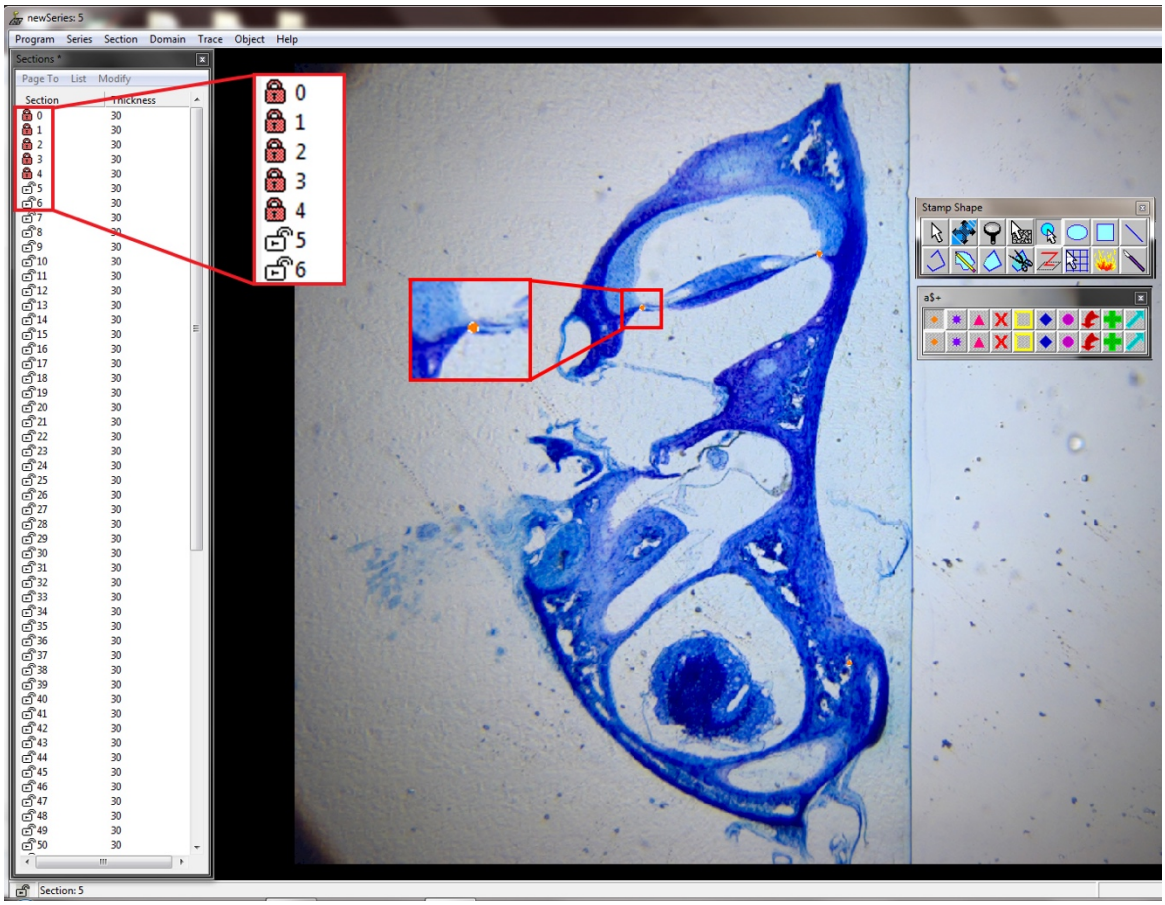


Figure 10. Reconstruct tools work screen. A slide is aligned using the orange dots as alignment markers. The sections panel to the left shows the slides that have been aligned and locked using the red lock icon. The tools palette and the stamp shape palette are shown to the right. *Text within image not intended to be read.*

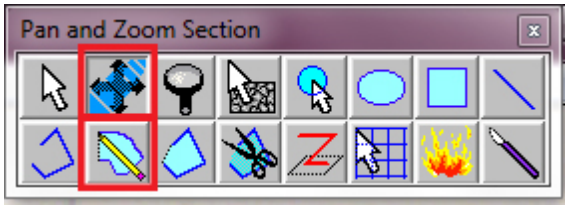


Figure 11. Reconstruct tools palette. Highlighted in red are the Drawing Freehand tool and Pan and Zoom tool. Draw Freehand tool was used to trace the cochlea structures. Pan and Zoom tool aided in navigation and zooming into the histology slide for tracing.

Segmentations of the cochlea structures were created using the **Draw Freehand** tool in the Reconstruct tool palette (**Figure 11**). This tool allowed for controlled tracing around the desired structure using a mouse or tablet stylus to fill the shapes with assigned colors. Segmentation of the cochlea structures included: sensory cells of the organ of Corti, tectorial membrane, spiral ligament, basilar membrane, Reissner's membrane, stria vascularis, and the spiral ganglion (**Figure 12**). The middle mouse scroll button was used to navigate up and down through the serial sections for segmentation.

The **Zoom and Pan** tool was used to zoom into structures by holding the right mouse button and dragging the mouse up and down (**Figure 11**). The structures were traced individually using the **Draw Freehand** tool on each serial section. Each structure was assigned a different color and name under the **Series > Series Options > Names/Colors**, for segmentation organization (**Table 3**). The process of segmentation was completed for three specimens, one deaf and one normal *Eptesicus fuscus,* and one normal *Mus musculus* separately.

Structure	Sensory cells	Tectorial membrane	Spiral ganglion	Basilar membrane	Reissner's membrane	Stria vascularis	Spiral ligament
Color Code	Yellow	Orange	Purple	Green	Teal	Red	Pink

Table 3: Color coding table for Reconstruct segmentation. Each structure was assigned a color for organization and recognition between the specimen.

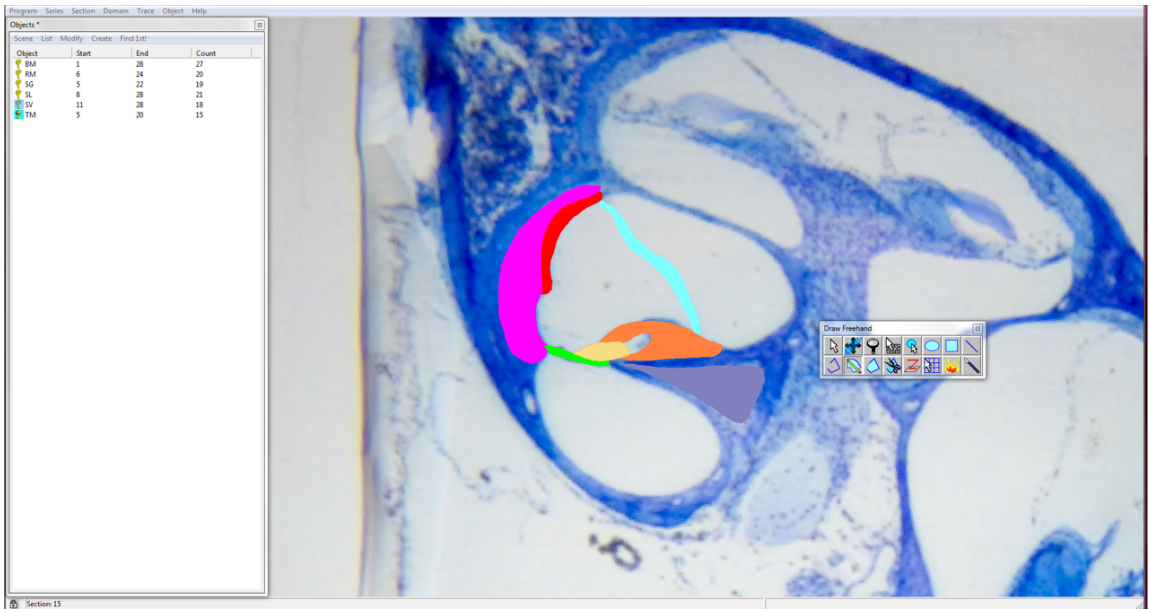


Figure 12. Reconstruct segmentation of the cochlea. Finished result of segmentation tracing of all the structures from specimen P20599_L, normal mouse. Object panel on the left lists the structures and the number of slides traced to each structure. *Text within image not intended to be read.*

After segmentation was completed, a 3D scene was generated to view the 3D representation of the structures (**Figure 13**). The structures were added to a 3D scene to be manipulated by clicking **Object** to open the object window and selecting **Add to Scene** under **List Objects > select the structure name > Scene**. The surface model was adjusted under preferences **Series > Options > 3D**. The model settings were changed to **Boissonnat surface**. The Biossonnat setting gave the 3D representations a smoother surface. Repeating the 3D scene process created representations of the structures listed. Selecting all the structures and refreshing the 3D Scene window displayed all the structures together and showed the anatomical relationships between them.

Complete cochlear structures were exported through the 3D scene window by selecting **Scene > Export** and selecting **VRML 2.0**. The Virtual Reality Modeling Language (VRML 2.0) file extension allowed the surface model to be imported into C4D for further model manipulation.

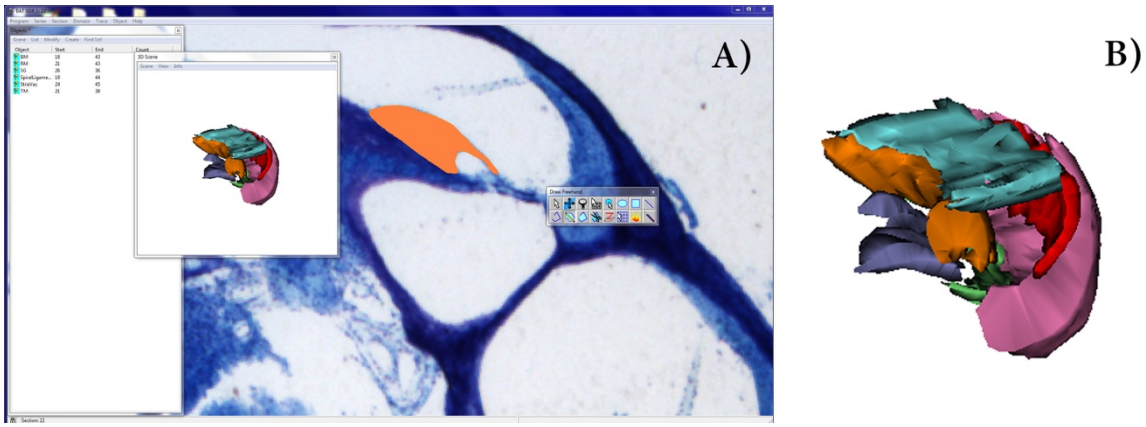


Figure 13. Reconstruct segmentation and 3D Scene. The 3D Scene shows specimen, G16_L. Normal bat structures are highlighted as 3D representations of the tracings. **(A)** shows Reconstruct screen with the result of the segmentation in the small windows **(B)** Close up of the 3D representation of the traced structures. *Text within image not intended to be read.*

Creation of 3D models

1. Optimizing surface exports

Before sculpting in ZBrush, the models derived from micro-CT data were optimized to remove segmentation arifacts in MeshLab using **Quadratic Edge Collapse Decimation** under **Filters > Remeshing, Simplification, and Reconstruction > Simplification: Quadratic Edge Collapse Decimation** (**Figure 14**). Optimizing the models in MeshLab removed mesh errors and decreased the number of faces significantly. The optimized models were ready for sculpting and repair in ZBrush.

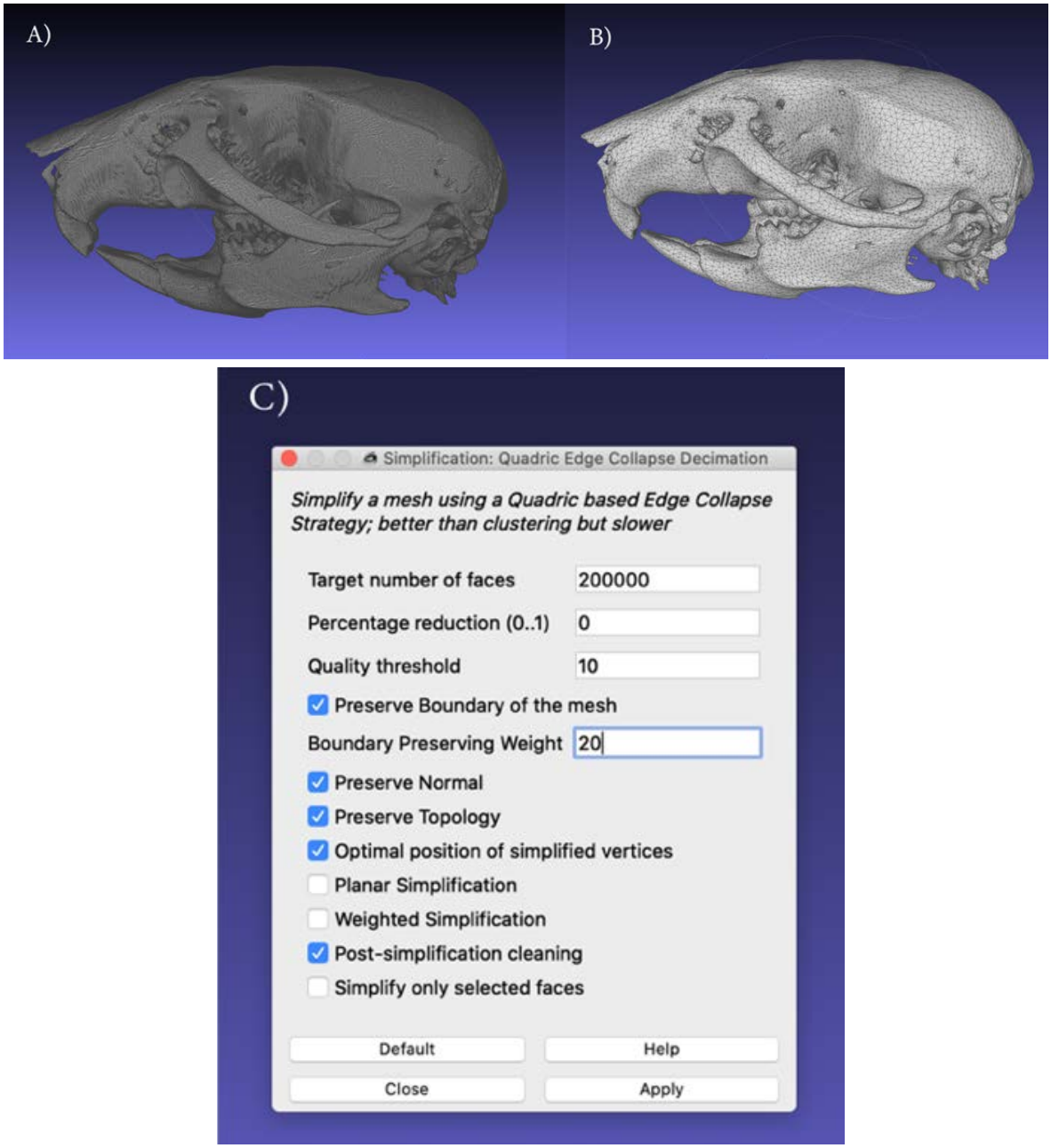


Figure 14. Optimizing model meshes in MeshLab. Quadratic Edge Collapse Decimation, (QECD) of the mouse skull. **(A)** before the mouse skull had 3,708,490 faces and was too complex, **(B)** After QECD and the mesh has 200,000 faces, **(C)** the control panel with desired numbers.

2. Importing histological surfaces

The structures in the VRML 2.0 files were imported into C4D as polygon objects. The structures were exported under **File > Export > STL** for ZBrush. The STL file was imported into ZBrush by selecting **Zplugin > 3D Print Hub > Import STL**. Separation of the models in ZBrush was performed using **Polygroups** (**Figure 15**). Under Polygroups, the **Auto Groups** button separated the structures into different Polygroups within one Subtool. The Polygroups were separated into individual Subtools by selecting **Tools > Subtools** > **Group Split**. Each structure could then be duplicated and renamed before sculpting and repair as an independent Subtool.

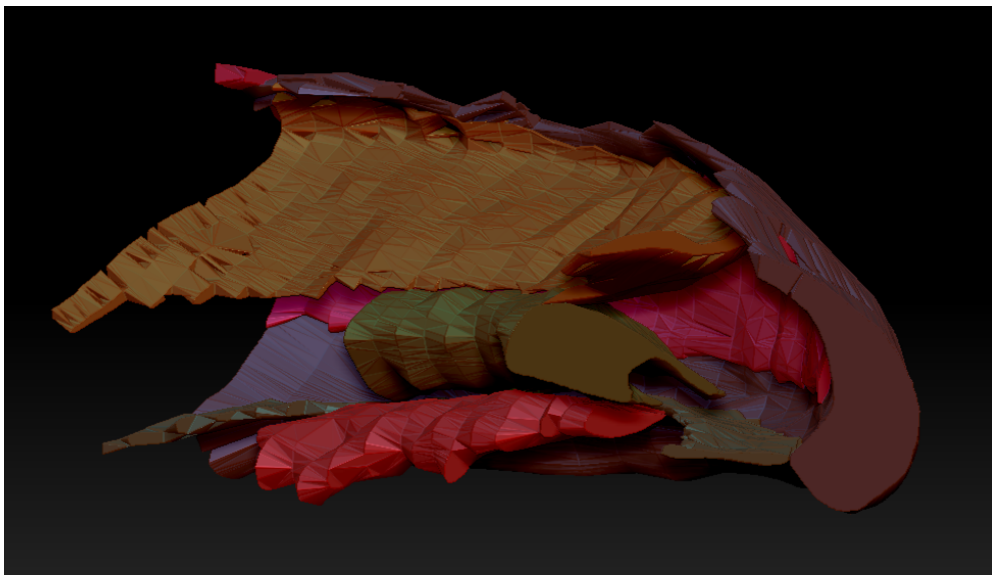


Figure 15. Reconstruct segmentation surface imported into ZBrush, Brown24_L, deaf bat. Results of separating the structures into individual subtools for repair and sculpting. Each subtool is separated, duplicated, and renamed.

3. Sculpting in ZBrush

The optimized segmentation surface models were brought into C4D. The skulls and cochleae came in aligned in anatomical position and as separate objects. In C4D, the project scale was increased by 10x, turning the models from 10cm to 100cm. Increasing the scale of the models helped to prevent sculpting issues in ZBrush due to the original skulls' small dimensions.

The eleven surface models for the big brown bat and ten surface models for the mouse were imported into ZBrush for sculpting, smoothing, repairing of holes, and other modifications to transform the data into idealized models of the craniums and cochleae of both species. The optimized big brown bat and mouse craniums and cochleae were imported into ZBrush using the **GoZ** plugin. Under **Plugins > C4D_PyGoZ-master > Export to ZBrush**. GoZ is a plugin that bridges ZBrush with other 3D packages for model creation. GoZ aided in keeping the same project scale when moving back and forth between C4D to ZBrush. GoZ also helped to keep the skulls and cochleae in anatomical position when moving between the programs (**Figures 16 & 17**).

Before the models were modified in ZBrush, duplicates of each model were created, renamed, and brought into **Dynamesh** mode to prepare the surfaces. The mandible of the bat was scanned separately from the cranium and required manual aligning using the Gizmo 3D tool. The two subtools were then merged to create a complete skull (**Figures 16 & 17**).

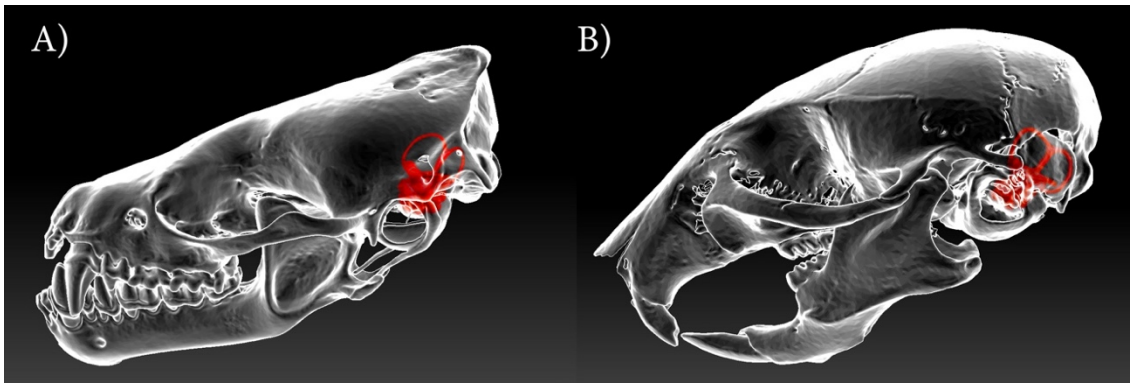


Figure 16. Alignment of cochleae in skulls, lateral view. Cochleae are in anatomical position, shown in red. **(A)** *Eptesicus fuscus* complete with mandible aligned, **(B)** *Mus musculus.*

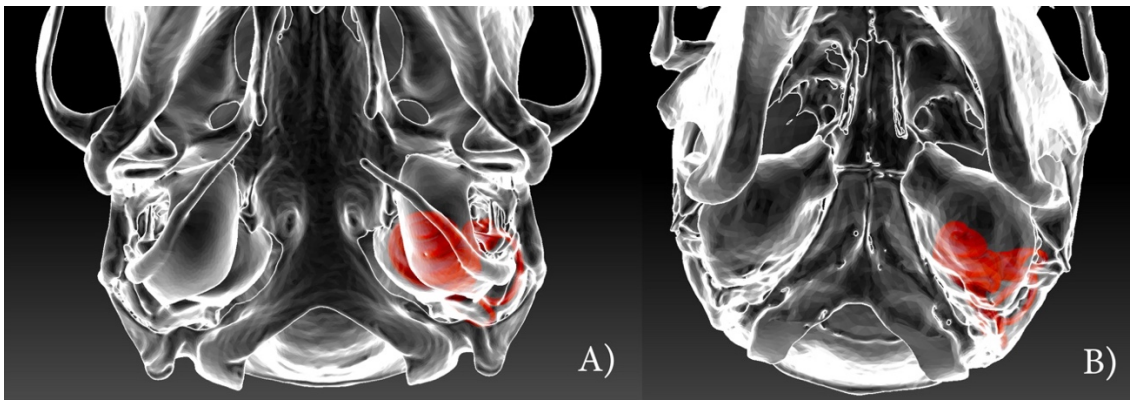


Figure 17. Alignment of cochleae in skulls, inferior view. Cochleae are in anatomical position, shown in red. **(A)** *Eptesicus fuscus*, **(B)** *Mus musculus.*

The skull and cochleae models had artifacts from segmentation that needed to be addressed. The big brown bat skull had holes and missing sinus anatomy. To remove the small fragments of the sinuses, the skull was selected, and **Auto Groups** was clicked to create different Polygroups (**Figure 18**). Holding the **Shift** + **Control** keyboard keys together and clicking on the model hid the fragmented sinus Polygroups. The hidden Polygroups were deleted using the **Delete hidden** feature followed by **Close Holes** button.

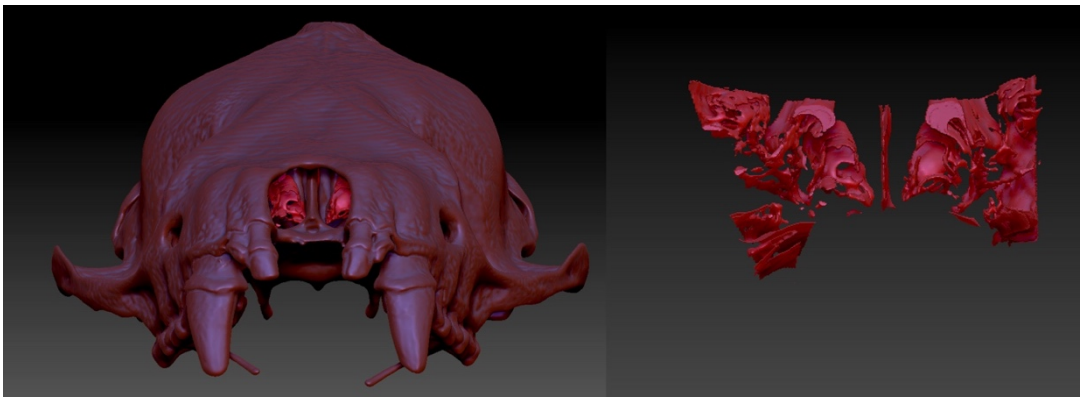


Figure 18. Removal of sinuses using Polygroups. Damaged sinuses, in pink, were selected and turned into a new polygroup for deletion.

The bat skull had hole artifacts from segmentation that needed to be repaired. The geometry around the holes was selected using the **SelectLasso** tool and hidden by holding down **Shift + Control + Option**. The holes were deleted and closed using the **Delete Hidden** and **Close Hole** buttons. The main brushes used to refine the models were the Clay Build Up, Move Topological, Smooth, and Damian Standard brushes to reinforce detail and textures lost with smoothing and closing holes. Move Topological was used over the Move brush because it does not affect the mesh on the unseen side of the model. Large regions missing in the skull were repaired using sphere subtools that were moved, scaled, merged, and Dynameshed to the skull to fill large holes. Repairs to the mouse skull were completed using the same method.

The histological structure models were modified separately from the skull and cochlea models. The models needed numerous modifications including sculpting, smoothing, trimming, and hole repair. After duplicating and Dynameshing the models, the geometry and holes were repaired using the SelectLasso tool, Delete Hidden, and Close holes process. After segmentation, the models were trimmed using the **Trim Rectangle** brush to a create uniform ends for the models (**Figures 19-21**).

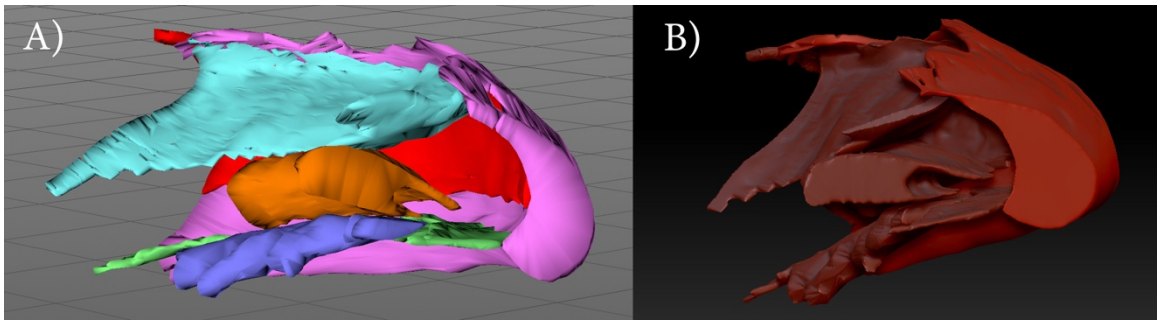


Figure 19. Segmented histological 3D models of Brown24_L, deaf bat. (A) 3D models from Reconstruct imported into C4D, (**B**) Resulting 3D models after clean-up and repair in ZBrush.

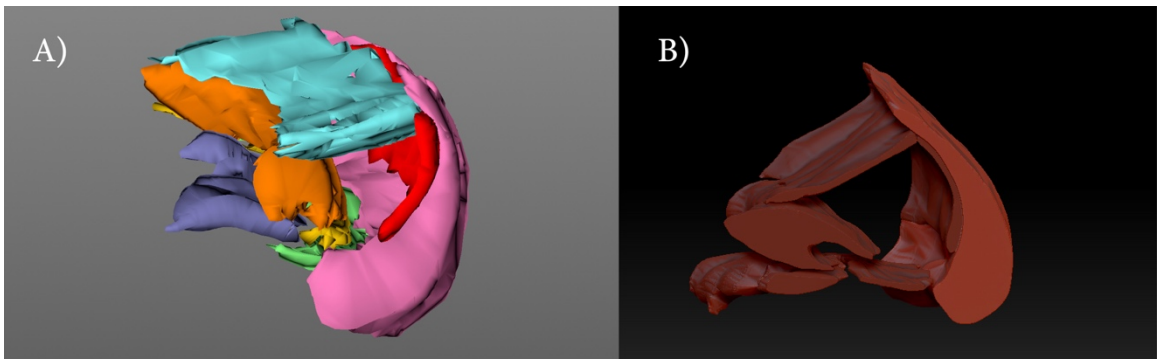


Figure 20. Segmented histological 3D models of G16_L, normal bat. (A) 3D models from Reconstruct imported into C4D, **(B)** Resulting 3D models after clean-up and repair in ZBrush.

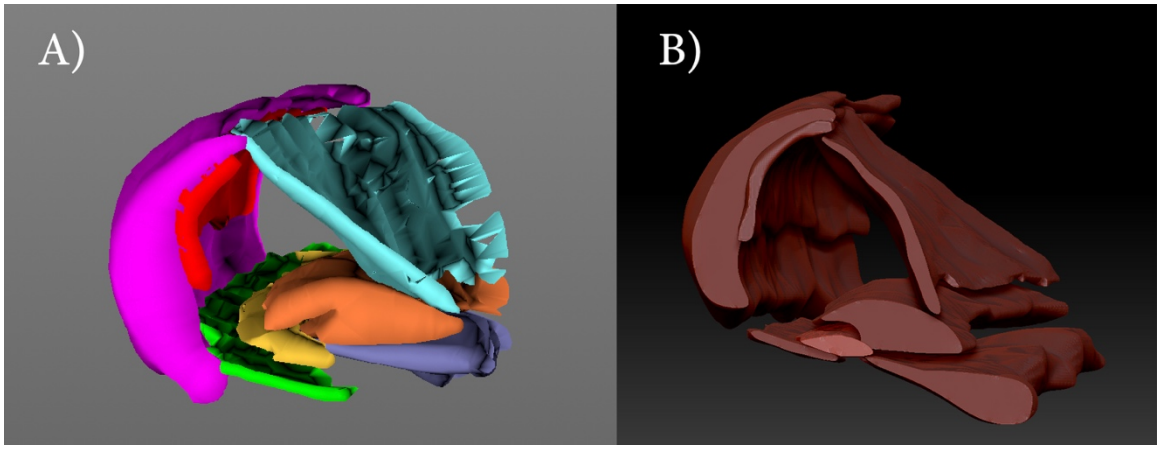


Figure 21. Segmented histological 3D models of P20599_L, normal mouse. (A) 3D models from Reconstruct imported into C4D, **(B)** Resulting 3D models after clean-up and repair in ZBrush.

4. Repair and artifact removal

The big brown bat cochlea model was missing a large section of the internal spiral wall that could not be repaired in ZBrush due to reversed normals. The model was exported to C4D for repair. The normals were reversed by selecting all polygon faces and selecting **Mesh tab > Normals > Reverse Normals**. To locate defects, the outer surface of the cochlea was hidden using the **Hide Selection** tool under the Select menu with **Only Select Visible Elements** enabled. **Mesh Checking** was activated by hitting **Shift+M** on the keyboard and clicking **Enable Mesh Checking** to identify Non-manifold and Boundary edges.

The defective polygons were deleted using the **Live Selection tool.** Deletion of the polygons did not delete the vertices; to ensure the vertices were deleted, **Mesh menu > Commands > Optimize** was selected. The holes were filled using the **Polygon Pen** tool to connect the vertices and create new geometry. The resulting large polygons were subdivided to create more geometry, under the Mesh menu selecting **Commands > Triangulate and then Mesh > Commands > Subdivide**. The model was smoothed using the Sculpt tools in Sculpt mode after the holes were closed. The repaired model was exported back to ZBrush using GoZ for final sculpting and smoothing of the cochlea (**Figures 22 & 23**).

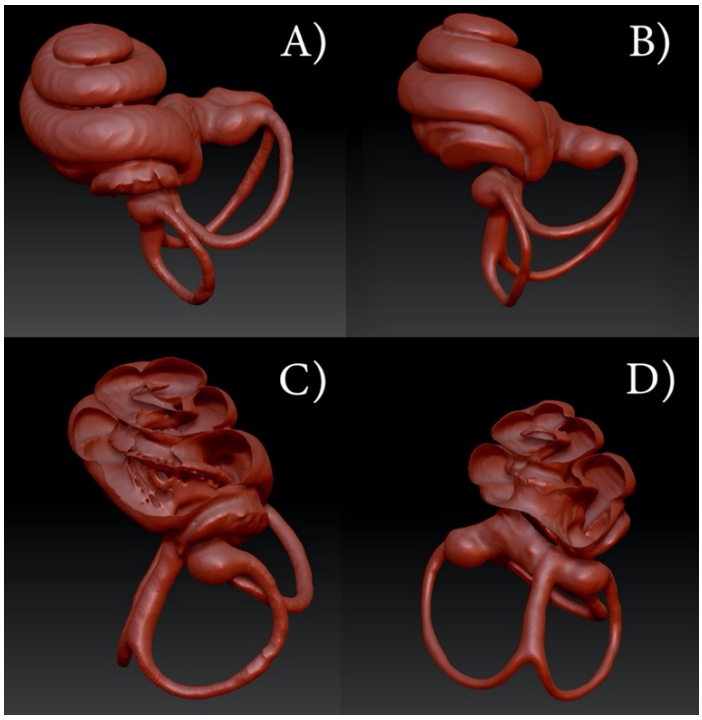


Figure 22. Bat cochlea models before and after repair in ZBrush and C4D. (A) Anterior view before repair, **(B)** anterior view after repair, **(C)** cross section of cochlea with holes, **(D)** repaired cross section.

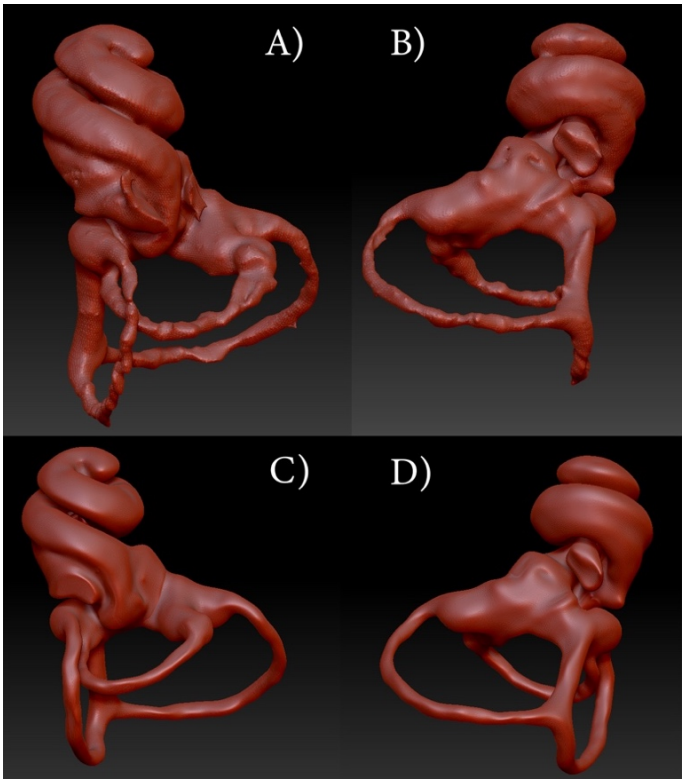


Figure 23. Mouse cochlea models after repair in ZBrush. (A) Anterior view before repair, **(B)** posterior view before repair, **(C)** Anterior view after repair, **(D)** posterior view after repair.

3D Animation

The bat and mouse skulls, whole cochleae with semicircular canals, and idealized cochleae were optimized for C4D in ZBrush. Each model's polygon count was reduced using the **Decimation Master** in the Zplugin menu. Each model was pre-processed before decimation. The percent of the decimation was adjusted using the **% of decimation** slider. A decimation percent of forty was chosen to reduce the number of polygons down and still keep detail. The models were imported to C4D using the GoZ plugin to ensure the models stayed in anatomical position.

In C4D, the materials were created and applied, lighting was set up, and movement of models and cameras were keyframed. Model animation sequences were rendered separately for each model and saved as PNG files. The rendered sequences were imported into Adobe After Effects for compositing. Translucency effects and transitions were created by animating masks and opacities of the different layers. Leader lines and labels were created in After Effects. Other 2D assets were created in Adobe Illustrator and Photoshop and imported into After Effects as individual assets for the animation.

The animation was rendered from Adobe Media Encoder 2020 and uploaded to YouTube for Closed Captioning (CC). CC was added automatically under **Subtitles/Closed Captioning**. Editing options for CC can be found in the Video Manager. **Add new subtitles or CC button and change language > create new subtitles or CC** was selected. This generated CC can be edited by adding new text boxes to break up sentences by hitting the **+ button**. Text can be changed to correct any auto subtitle mistakes, punctuation, and timing issues.

2D interactive educational module

Summary of interactivity

This web-based interactive resource was designed to have three main sections: a section containing a pre-rendered Overview Animation, Cochlea Anatomy, and Normal vs Deaf bat research pages (**Figures 44-53**). Under the Cochlea Anatomy section are secondary pages for Cochlea Location, Cochlea Structures, Cochlea Section, and organ of Corti that contain comparative cochlea anatomy information. Dropdown pages under the Normal vs Deaf section, Damage Data and Damage Mapping, are for learning about bat hearing loss research and the purpose and process of cochlea damage mapping in research.

Interactivity in Animate

1. **Coding in Animate**

The 2D interactivity was created in Adobe Animate 2019. Adobe Animate is a vector-based computer animation program used to make interactive websites, web applications, and video games published using the HTML5 (Hypertext Markup Language 5) platform. The project was created as a HTML5 file for manual coding of buttons and elements. Publish Settings were set up before the module could be exported. Animate settings were changed by selecting **File > Publish Settings > Basic**, and unchecking **Loop Timeline.** Under Image Settings, **Combine image into spritesheet** was checked to help speed up publishing.

The assets were imported to the stage and converted into symbols by selecting the art, right-clicking, and selecting **Convert to Symbol** to name the symbol (**Figure 24**). Each symbol also needs an **Instance** name for identification in the code. All the assets were imported, converted to Symbols and given Instance names with specific capitalization for

the code, for example “MouseHead”. The **Instance** name is the calling name for the HTML code. The Instance name does not have to match the Symbol name, but the same name helps for organization of the assets and code calling.

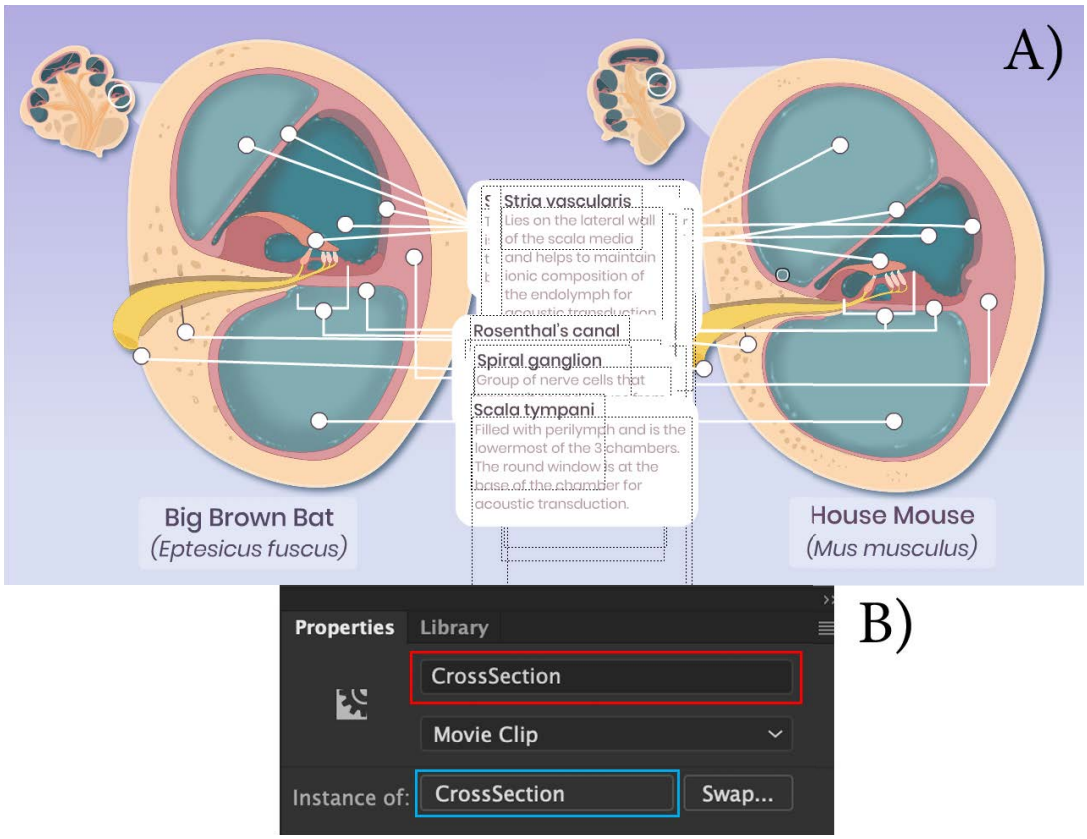


Figure 24. Symbol naming and artwork layering in Animate. (A) Example of the symbol CrossSection, within the symbol are symbols and instances of artwork and annotations, **(B)** The Symbol name is highlighted in blue and the Instance name is highlighted in red. *Text in pop-ups not intended to be read.*

Each page button was created with the following layers: a white rounded rectangle background, colored text, an underline, and a wrapper. The wrapper is an invisible rectangle large enough to cover the text to avoid horizontal flickering when the module is published and viewed in a web browser. The button elements are nested within the button symbol. Each page button was assigned artwork that is visible, hidden or plays an

animation when clicked. By default, all the assets in the file will show at the same time when published and need to be coded in the **ActionScript** menu as **alpha=1** and **visible= true;** to show the art or **alpha=0** and **visible = false;** to ensure the unwanted art is hidden when the module is opened. Each page button is assigned the desired artwork to show when clicked and the artwork that remains hidden. Calling the correct page button within the "navBar" symbol to target the correct artwork, requires layered coding as shown in **Appendix C, Buttons**. Inside the curly brackets {}, the code for the art was placed for calling or keeping the art hidden when the button is clicked.

Each button and annotation cue was coded to be a pointer. This turns the mouse arrow icon into a hand icon indicating the button is active and clickable; refer to **Appendix C, Buttons** for coding cursors. All the buttons were coded for MouseOver and MouseOut demands as well. This fades the buttons to 50% when the cursor hovers over them and changes them back to 100% when the cursor moves off of them.

The anatomical structures in each section were assigned small circular annotation cueing elements for the user to click on for more information on the structures. A total of 34 annotations were coded for the 17 key structures of the cochlea. Each cue and annotation was turned into a Symbol and Instance and named after its structure for organization. Each cueing element was coded to call up one annotation and to turn off all other annotations when clicked, refer to **Appendix C, Annotations**. Two sets of cueing elements were needed for the bat and mouse cochleae to allow the user to click on either set and view the annotations.

2. Creating animated elements

Creating animations in Adobe Animate requires nesting of all the artwork within one large symbol for organization and coding purposes. Within the large symbol "MouseHead", for example, the artwork was separated into multiple symbols and instances of the bat and mouse skulls, cochleae, and structure labels for keyframing and **Tweening**. New frames are added to the animation using the **F5** button, frames help to organize and control the elements of the animation (**Figure 25**). Keyframes can be added using the **F6** button, keyframes indicate where a new symbol instance will appear on the timeline. The artwork can be keyed to scale, rotate, move, or fade on and off between keyframes.

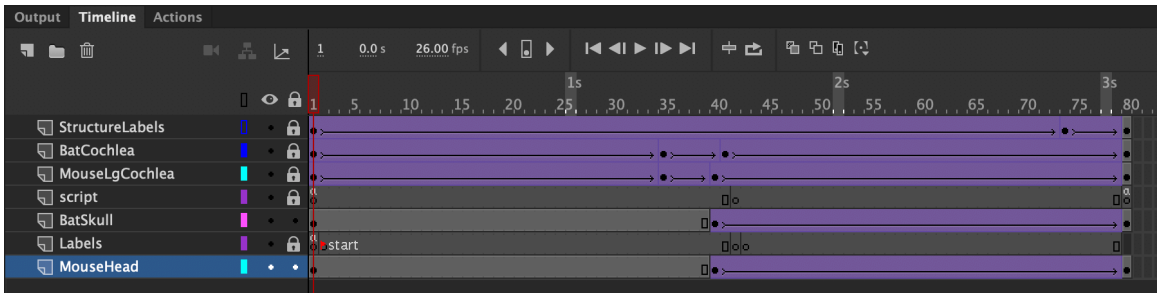


Figure 25. Keyframes and Tweening in Animate. Example of keyframes and Tweening in Adobe Animate. Purple bars are the added Tweens between the keyframes for smooth animation. *Text within image not intended to be read.*

The bat and mouse cochleae were animated to rotate and enlarge while the skull artwork disappeared. Tweens were created to allow the symbol properties to be constrained between the keyframes and play over time, a **Classic Tween** was used for all the animated elements. Easing the animation smoothed the movements between keyframes. With a frame within the Tween selected, **Properties menu > Tweening > Classic Ease** was selected to change the ease.

In Adobe Animate, the animations play on a continuous loop automatically when published. Adding a script layer and coding keyframes within the animation will stop the animation from looping. The first keyframe was selected and the code **this.stop();** was added in the ActionScript menu to prevent the animation from playing automatically when published and allow for coding of a button to play the animation. In order for the animation to be activated, a **Start** label was added to the first keyframe of the animation for the code to the animation. The cochlea animation was coded to be played when the Cochlea Structure button under the Cochlea Anatomy dropdown menu is clicked, refer to **Appendix C Buttons** for animation coding.

Hosting for Web

The interactive module was hosted on **jpetersonillustration.com**. A link was added to the Lauer Lab website, **www.lauerlab.com**. The interactive folder containing the FLA, HTML, and JS files was added to the WordPress server. In the WordPress administration website, a page was built to house the interactive. After the files were uploaded, the module could be loaded in a web browser by targeting the module (e.g. sitename.com/name_of_app/index.html).

RESULTS

2D Assets

A total of 72 bat and mouse assets were created and imported into Adobe Animate, including portraits, skulls and silhouettes, whole cochleae, half cochleae, cross sections, organ of Corti, damage data and mapping illustrations, buttons, icons, and annotations (**Figures 26-28**). The interface consisted of 13 buttons containing the assets. A total of 33 interactive annotations were made for the 17 essential cochlea structures the interactive highlights. Refer to **Appendix C** for examples of Adobe Animate code.

Figure 26. *Eptesicus fuscus* and *Mus musculus* heads. A) Portraits *of Eptesicus fuscus* and *Mus musculus* showing external ear, B) Silhouettes of the heads with skulls and cochlea.

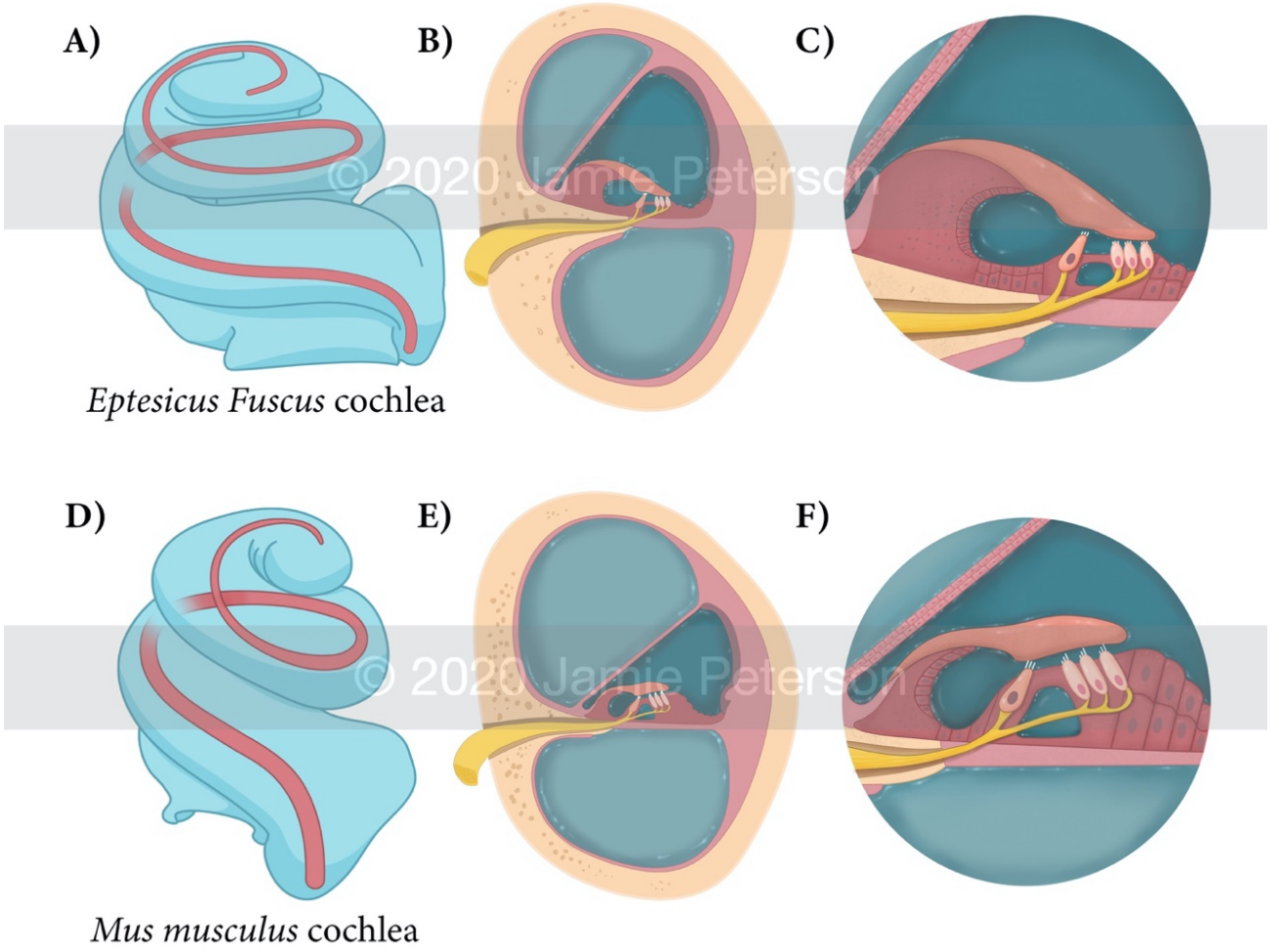


Figure 27. *Eptesicus fuscus* and *Mus musculus* cochlea assets. *Eptesicus fuscus* cochlea A-C and *Mus musculus* cochlea D-F. A & D Whole cochlea with basilar membrane, **B & E** Cross section of cochlea, **C & F** organ of Corti.

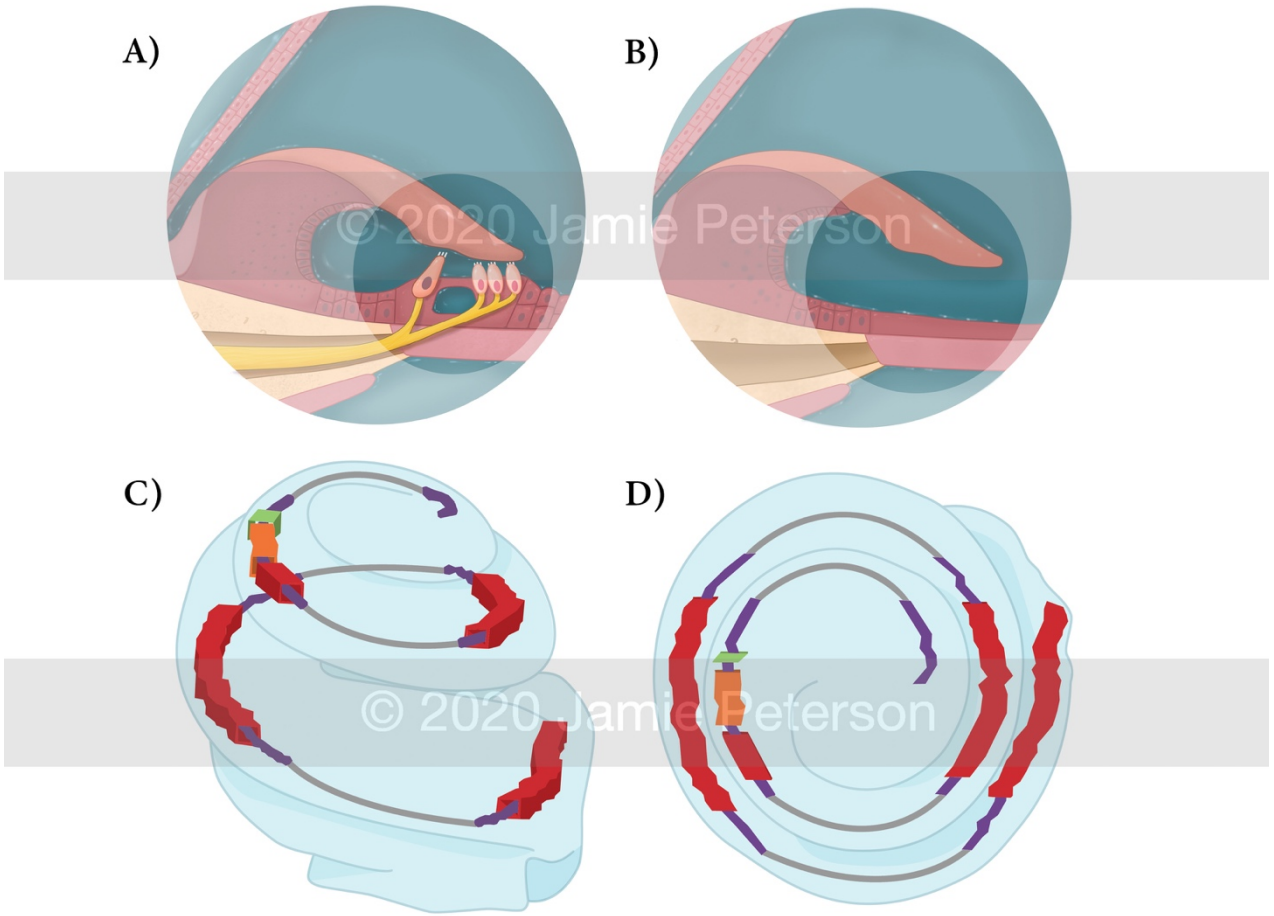


Figure 28. ***Eptesicus fuscus*** **cochlea normal vs deaf assets.** ***Eptesicus fuscus*** **cochlea A)** normal bat organ of Corti, **B)** deaf bat asset, **C)** damage mapping oblique lateral view, **D)** damage mapping superior view. Color coding for damage mapping: red is total loss, orange is partial loss, green is no loss, purple is basilar membrane, gray is lack of data.

3D Assets

Completed 3D models include skulls of *Eptesicus fuscus* and *Mus musculus* (**Figures 29 & 30**), cochleae with semicircular canals (**Figures 31 & 32**), cochleae without semicircular canals (**Figures 33 & 34**), and three models containing seven membranous structures from three sets of serial sections (**Figures 19-21**).

The complete *Eptesicus fuscus* and *Mus musculus* skulls, cochleae with and without semicircular canals were created as educational sculpted models based a segmented micro-CT data. These sculptures were incorporated into the Overview Animation and used as reference material for the 2D assets of the educational module. The surface segmentations of serial sections created from one deaf *Eptesicus fuscus*, one normal *Eptesicus fuscus*, and one normal *Mus musculus* served as reference material for designing the 2D assets for the membranous structures of the cochleae.

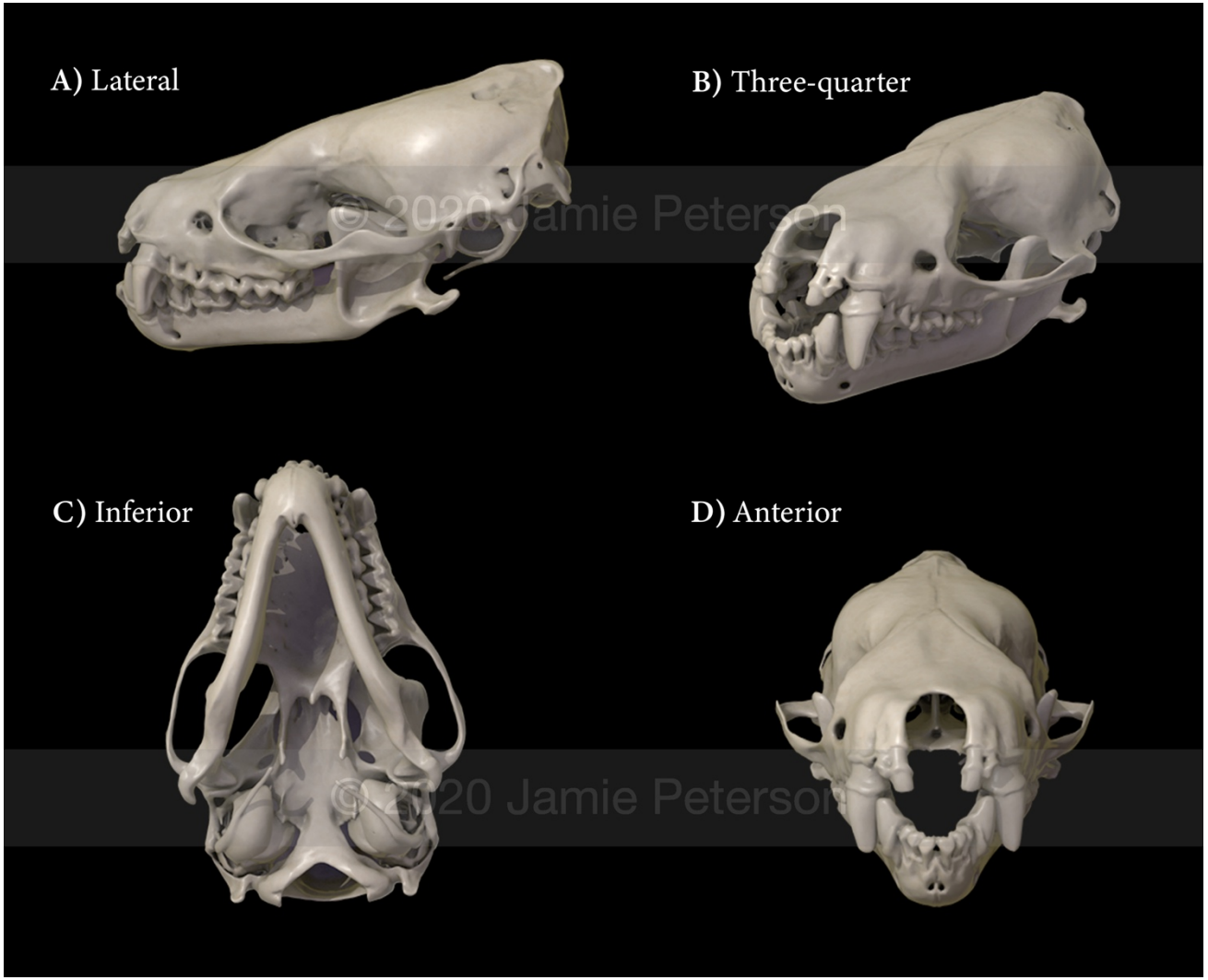


Figure 29. Rendered model of *Eptesicus fuscus* skull. A) Lateral, B) Three-quarter, C) Inferior, D) Anterior views.

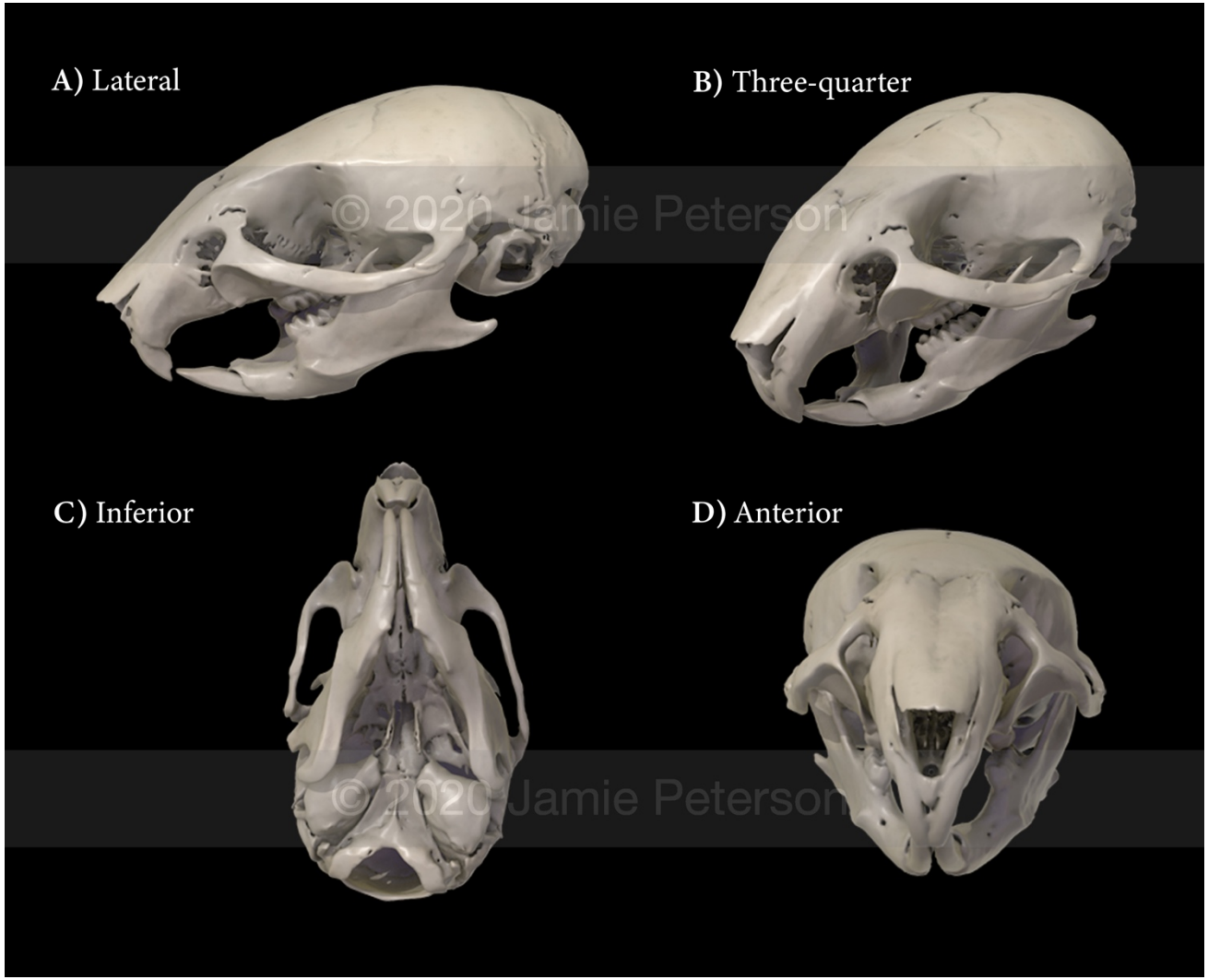


Figure 30. Rendered model of *Mus musculus* skull. A) Lateral, B) Three-quarter, C) Inferior, D) Anterior views.

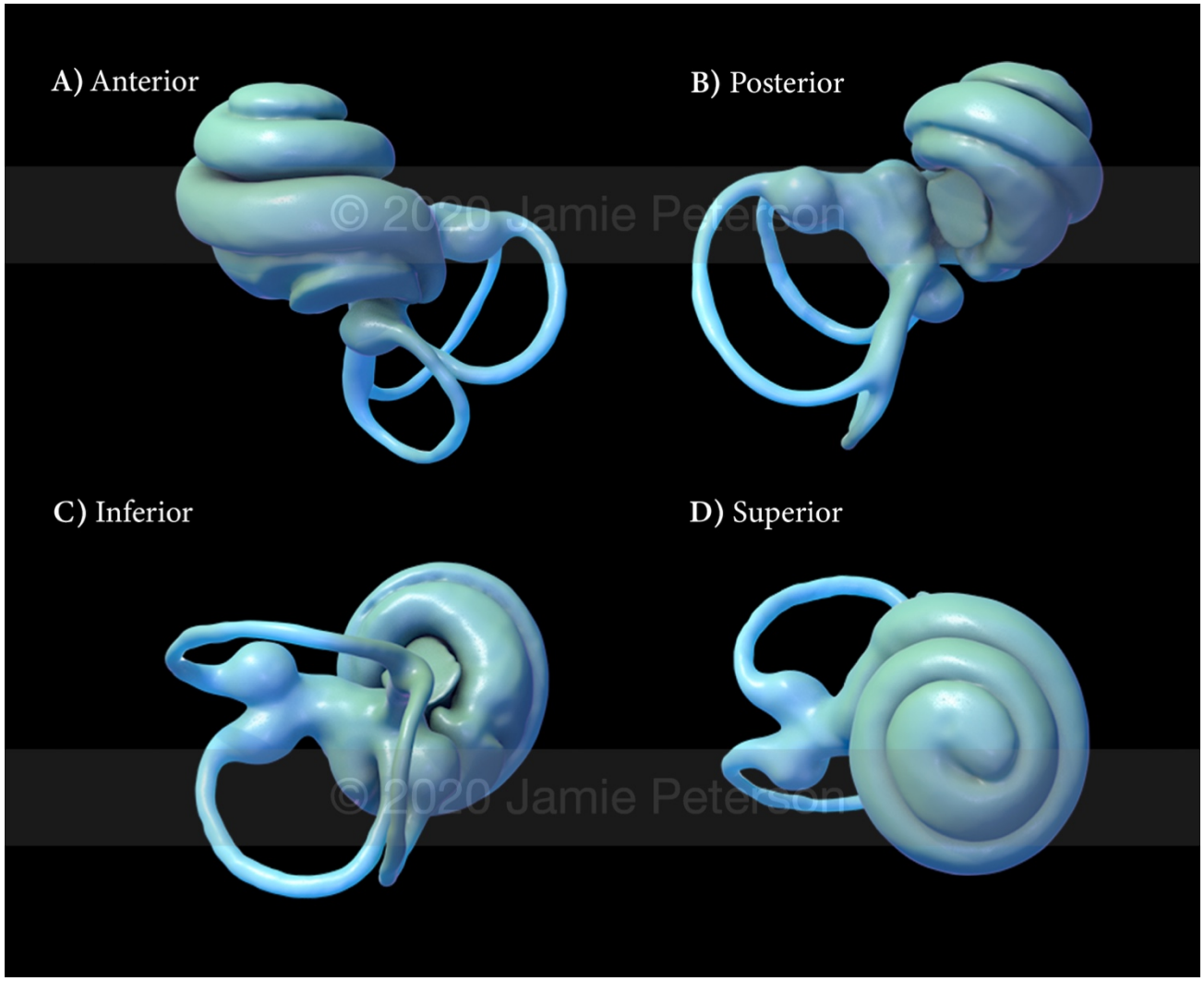


Figure 31. Rendered model of *Eptesicus fuscus* whole cochlea. A) Anterior, B) Posterior, C) Inferior, D) Superior views.

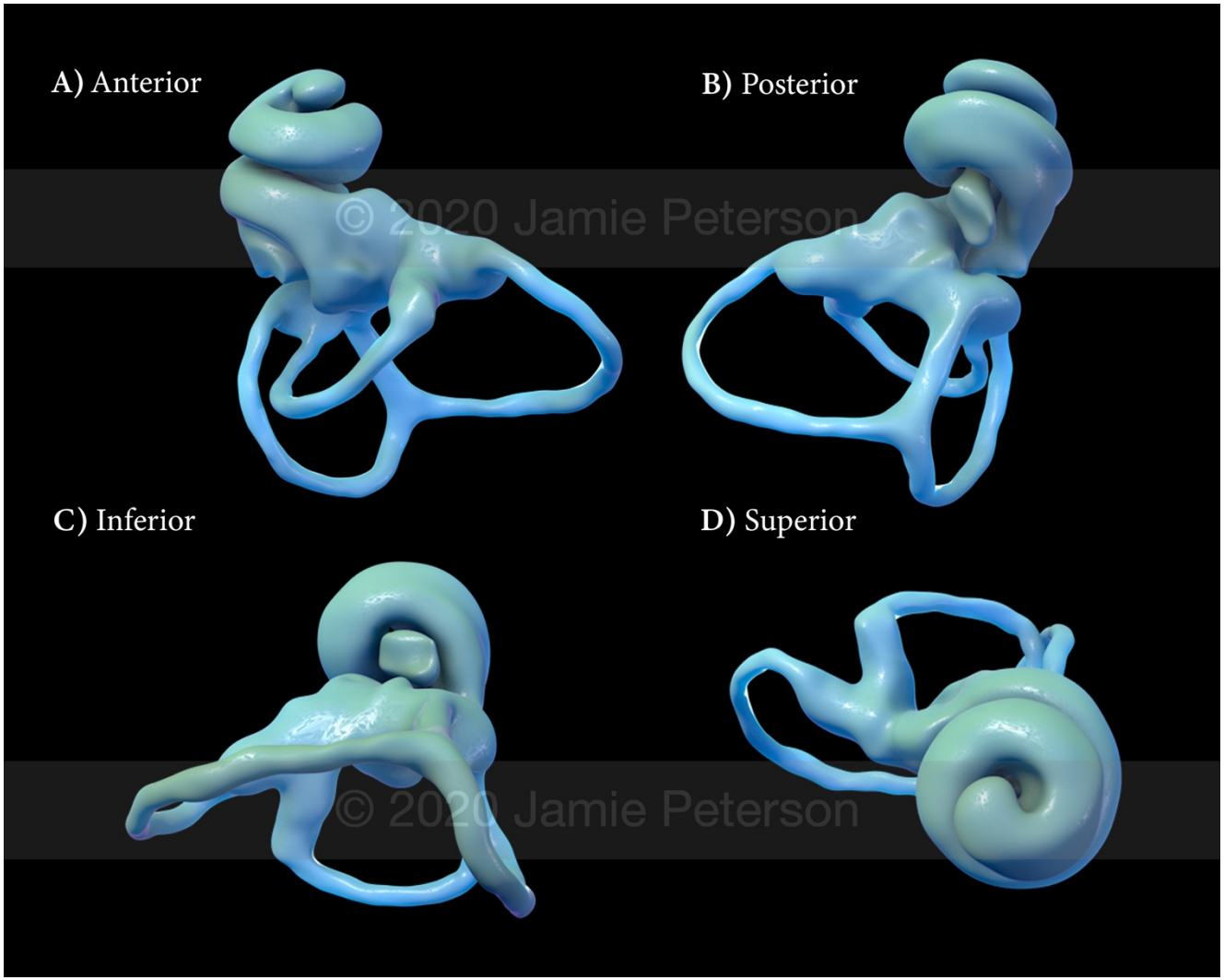


Figure 32. Rendered model of *Mus musculus* whole cochlea. A) Anterior, B) Posterior, C) Inferior, D) Superior views.

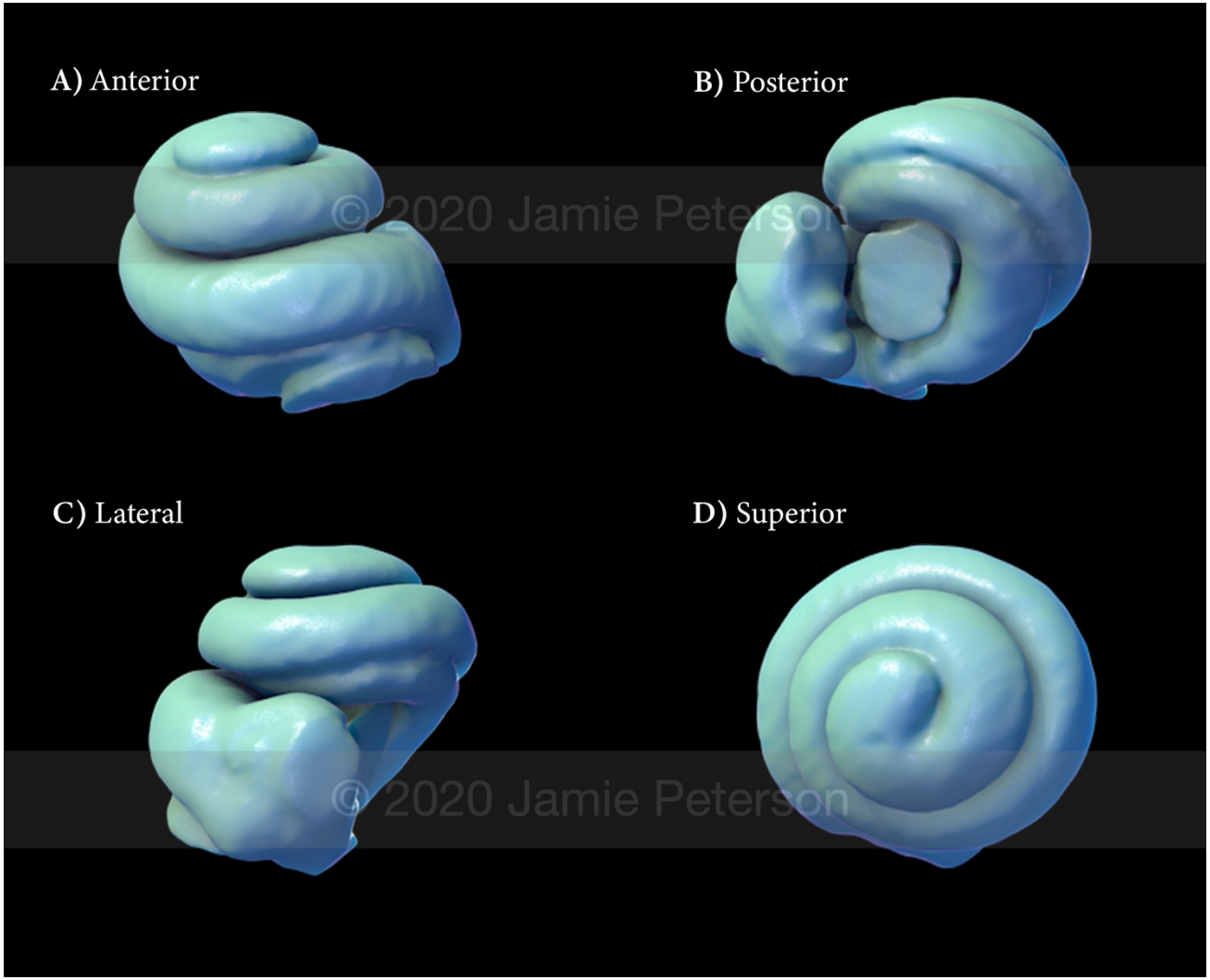


Figure 33. Rendered idealized model of *Eptesicus fuscus* ideal cochlea. A) Anterior, B) Posterior, C) Lateral, D) Superior views.

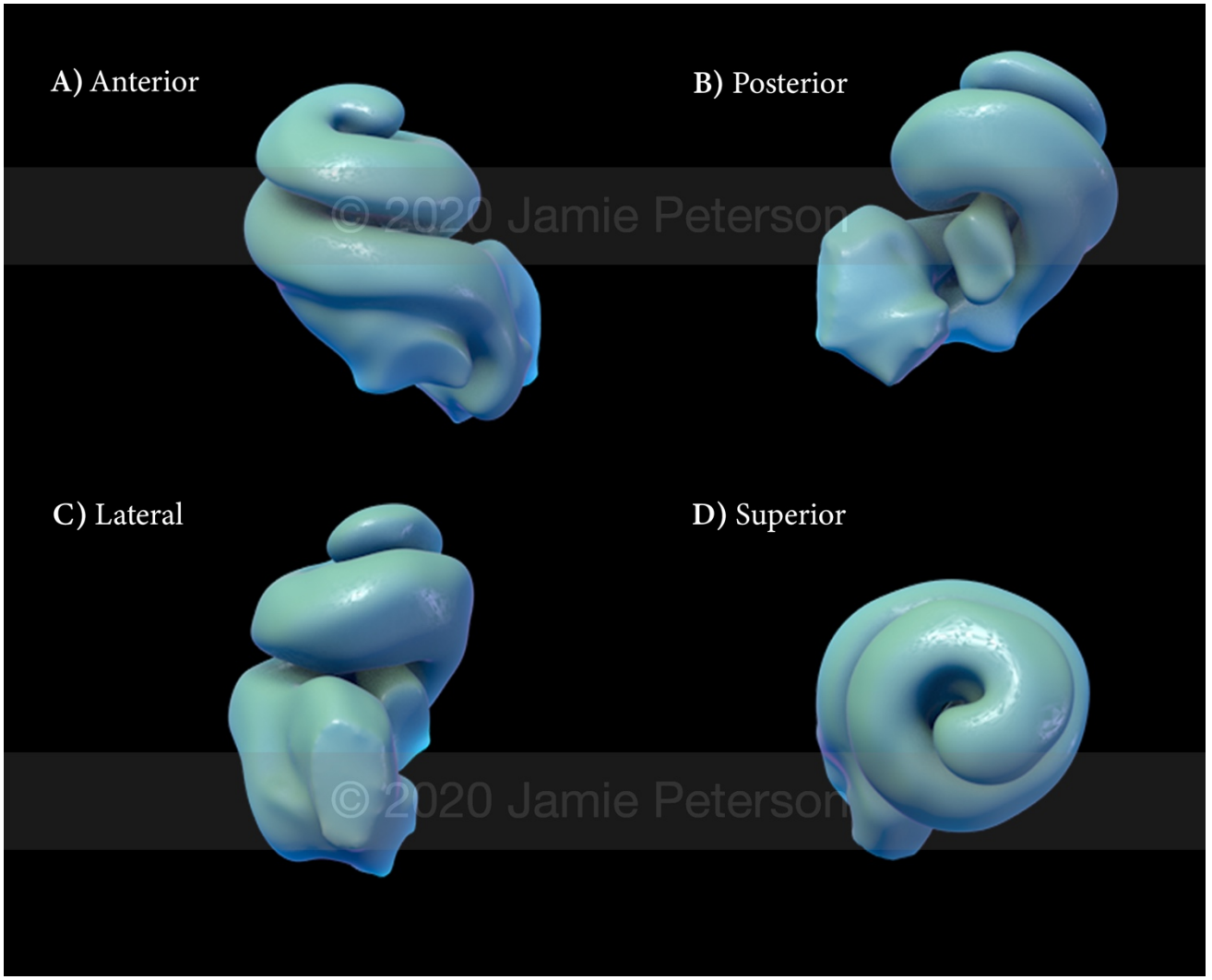


Figure 34. Rendered idealized model of *Mus musculus* whole cochlea. A) Anterior, B) Posterior, C) Lateral, D) Superior views.

Animation stills

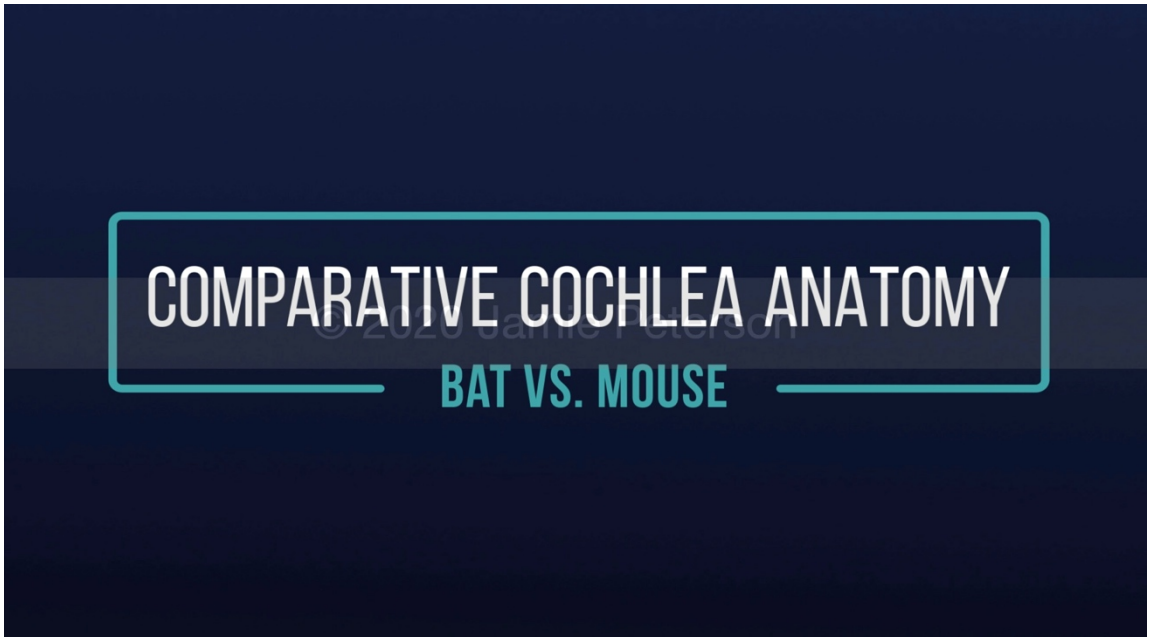


Figure 35. Animation still, Opening title.

Figure 36. Animation Still. Audio: The big brown bat, or *Eptesicus fuscus*, is an auditory specialist with a broad hearing range and high sensitivity to ultrasonic signals used for echolocation.

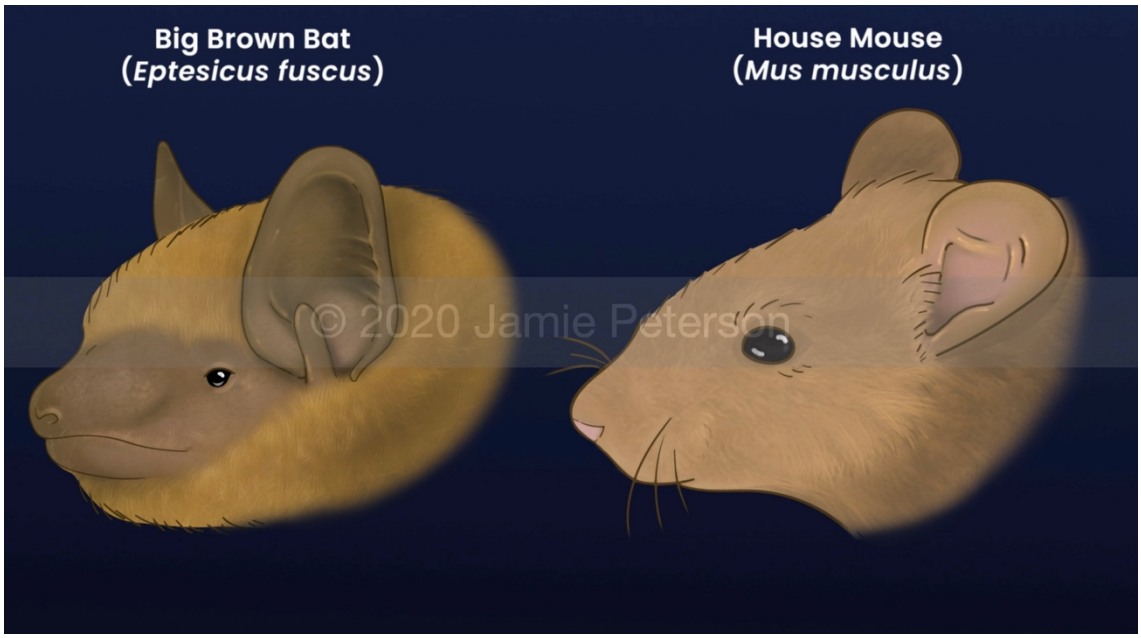


Figure 37. Animation Still. Audio: The house mouse, or *Mus musculus*, is an auditory generalist with a comparatively limited hearing range.

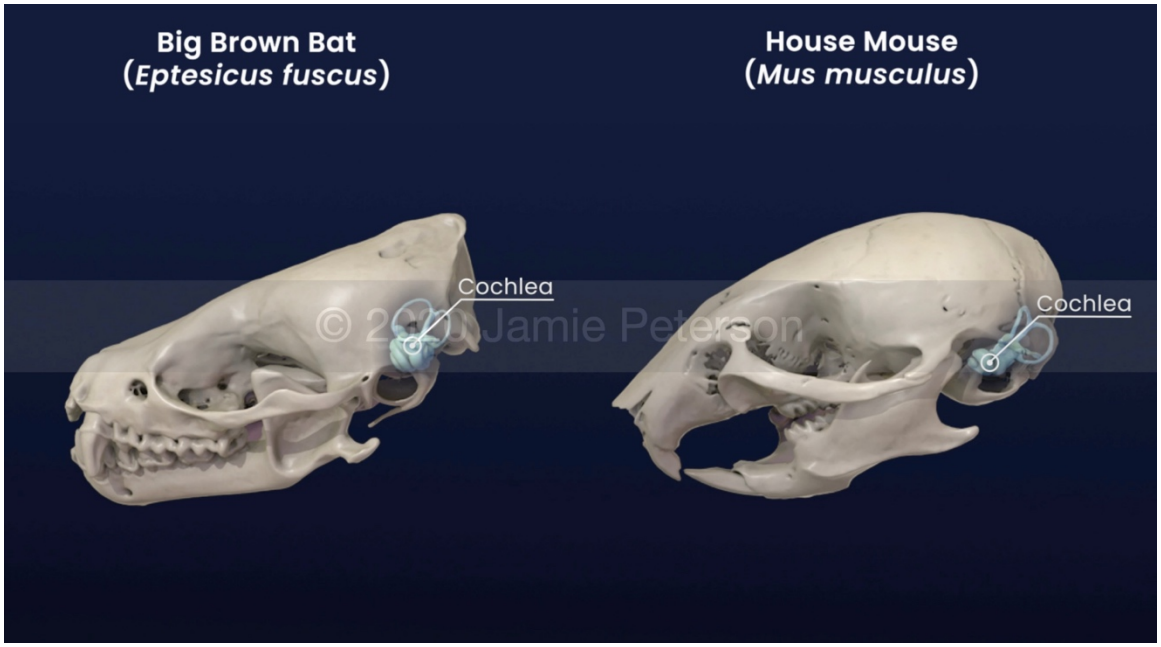


Figure 38. Animation Still. Audio: There are remarkable variations in cochlear structure between these two species. The cochlea is a fluid-filled coil of the inner ear located in the petrous portion of the temporal bone.

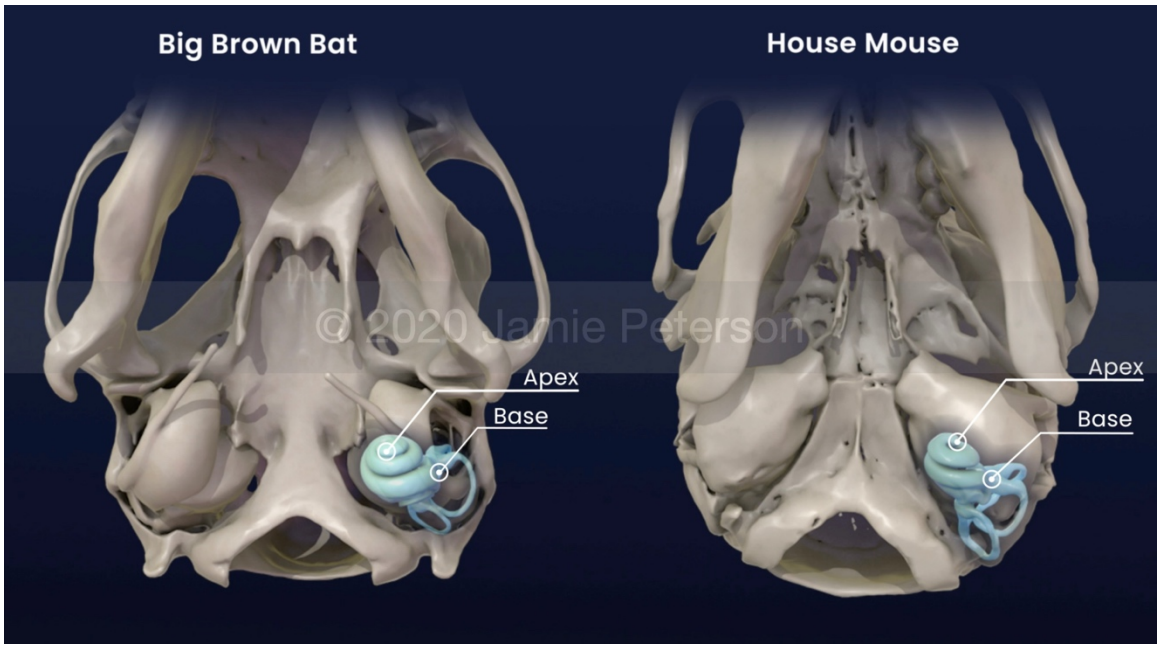


Figure 39. Animation Still. Audio: The apex of the cochlear spiral points rostrally towards the mandible, while the base points towards the external ear to receive sound vibrations. The axis of the big brown bat's cochlea is more ventrally oriented compared to the mouse.

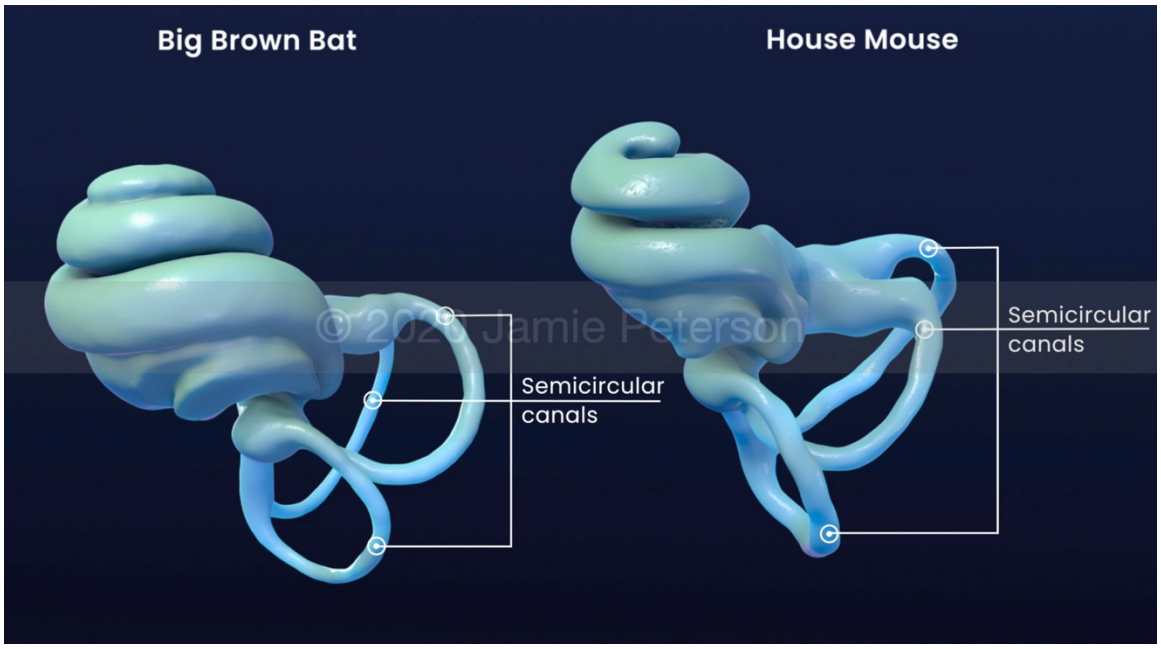


Figure 40. Animation still. Audio: The cochlea transforms sound waves into electrical signals for processing in the brain. It is attached to the semicircular canals, which record the angular velocity of head movements to maintain balance.

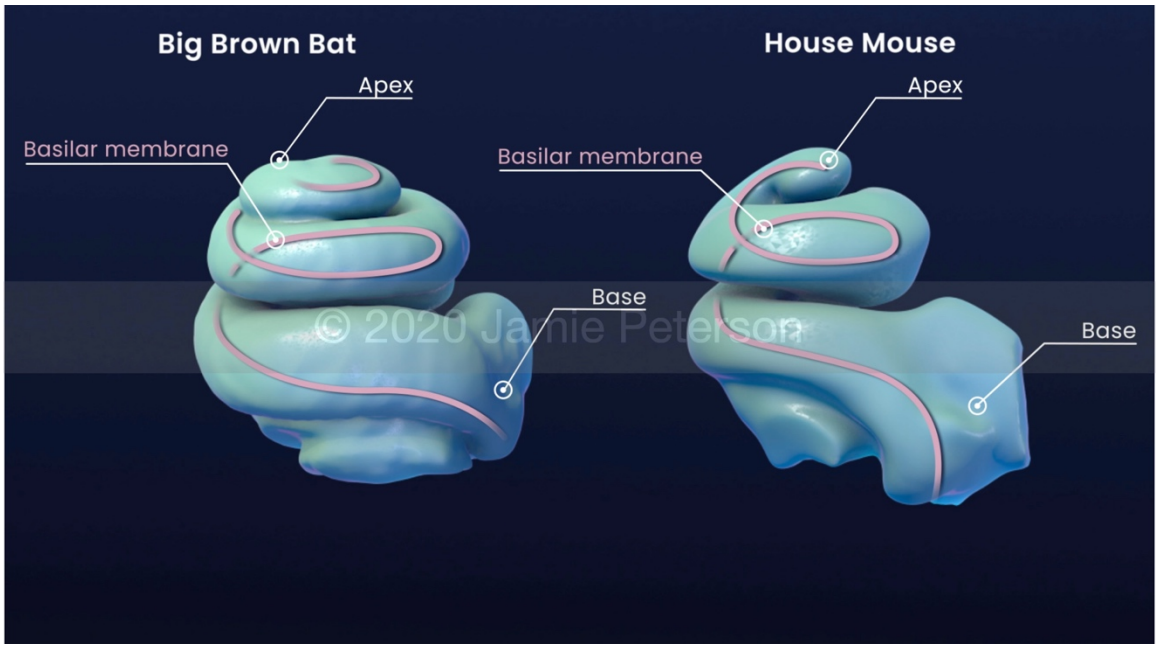


Figure 41. Animation still. Inside the cochlea, different sound frequencies stimulate sensory hair cells along the basilar membrane. Lower-frequency sounds stimulate hair cells in the apex and higher-frequency sounds stimulate hair cells in the base.

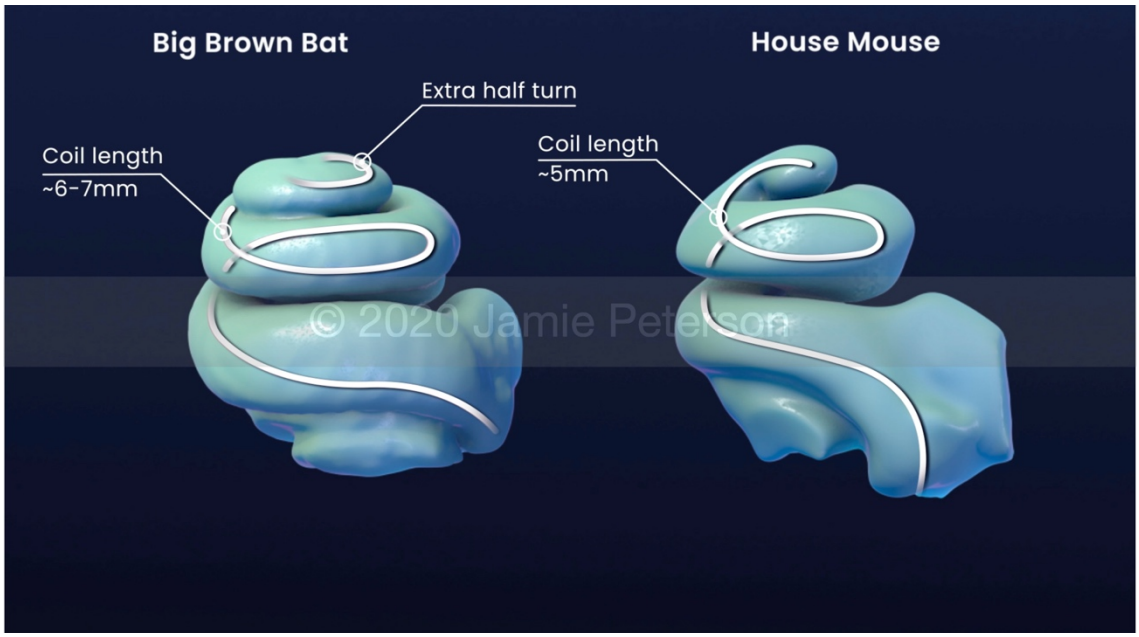


Figure 42 Animation still. Audio: The length of the coil of the big brown bat is approximately 6-7mm compared to that of the mouse, which is approximately 5mm. The extra half turn of the big brown bat cochlea supports a broader hearing range.

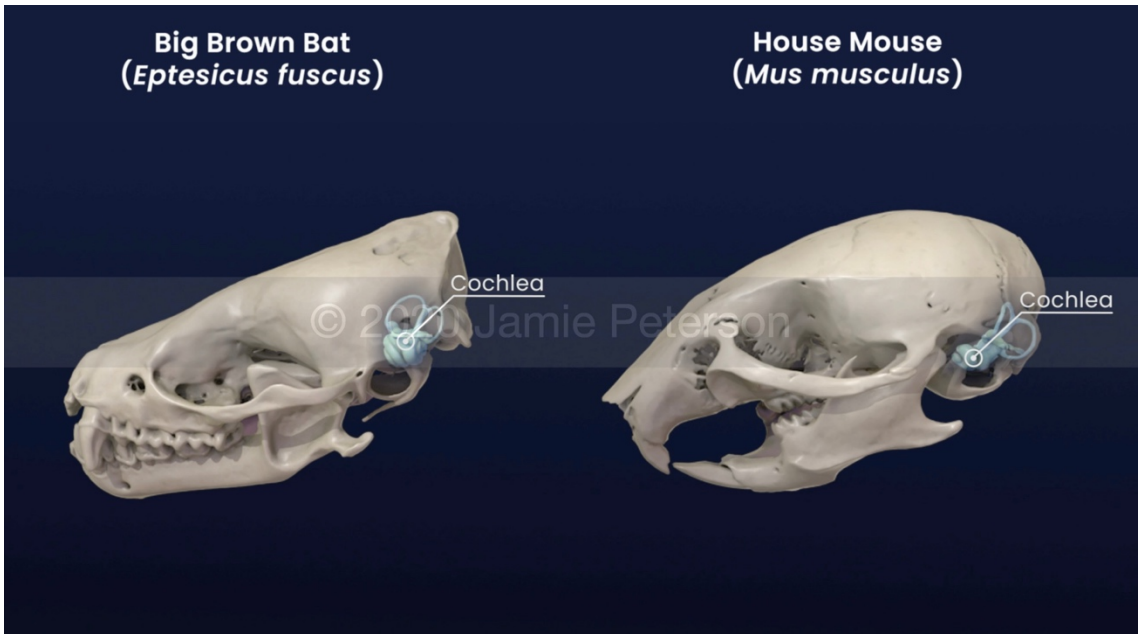


Figure 43. Animation still. Studying comparative cochlear anatomy can aid in understanding auditory specializations and hearing loss in mammals.

Figure 44. Animation still. End Credits. *Text within image not intended to be read.*

Web-based comparative interactive design and deployment

The user can view the animation and 3D models by clicking on the Overview Animation button on the home screen. The complete interactive and animation was uploaded onto a website containing all the necessary Adobe Animate files, HTML/CSS files, Javascript files, and image files, except the pre-rendered animation, which was hosted on YouTube.com. The animation was created to provide a 3D visualization of the anatomical differences between *Eptesicus fuscus* and *Mus musculus* cochleae and provide three-dimensional context to the familiar two-dimensional imagery within in the 2D interactive module and histological slides.

The Overview Animation page contains the data-driven 3D models with didactic 2D elements. The interactive assets found in the education module are contained under the pages accessed via the Cochlea Anatomy button. The dropdown pages include the Cochlea Location button with the cochlea inside the skull and labels, the Cochlea Structures button, which starts the cochlea animation with labels, and the Cochlea Sections button, which depicts structures within one-half turn of the cochlea and the Organ of Corti with interactive annotations (**Figures 50 & 51**). The Damage Data button under the Normal vs Deaf dropdown menu, accesses histological slides with accompanying didactic representations of the slides (**Figure 52**). The Damage Mapping button accesses information on the process and purpose of mapping cochlea damage of deaf big brown bats (**Figure 53**). The Cochlea Section and Organ of Corti interactive annotations allow the user to click on the membranous structures of the cochlea to discover the structures' name, location, function, and allow for future self-testing.

Comparative interactive design stills

Figure 45. Interactivity Cochlea Home Screen page. *Text within image not intended to be read.*

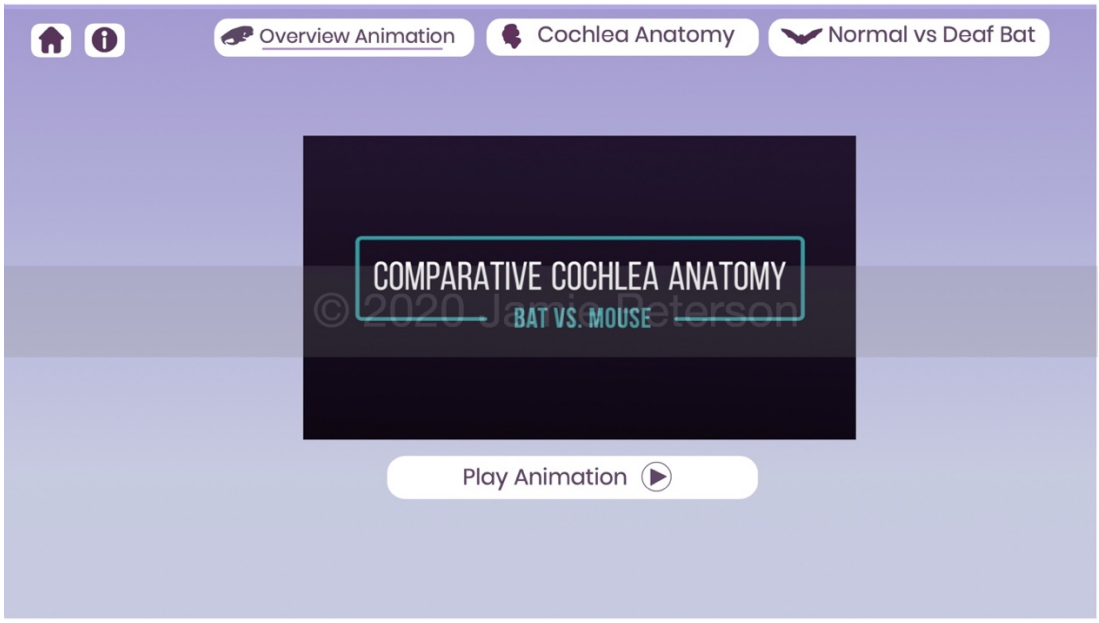


Figure 46. Interactivity Overview Animation page. User can choose to start the overview animation; new window will open for YouTube.

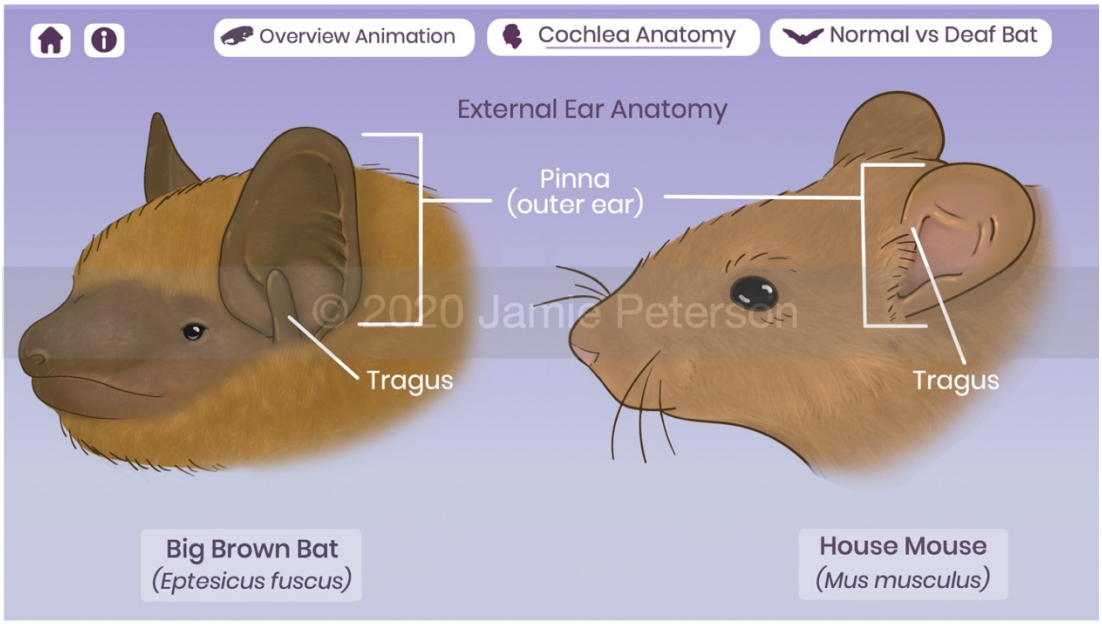


Figure 47. Interactivity Cochlea Anatomy page. Cochlea Anatomy page shows the external ear anatomy for reference of location.

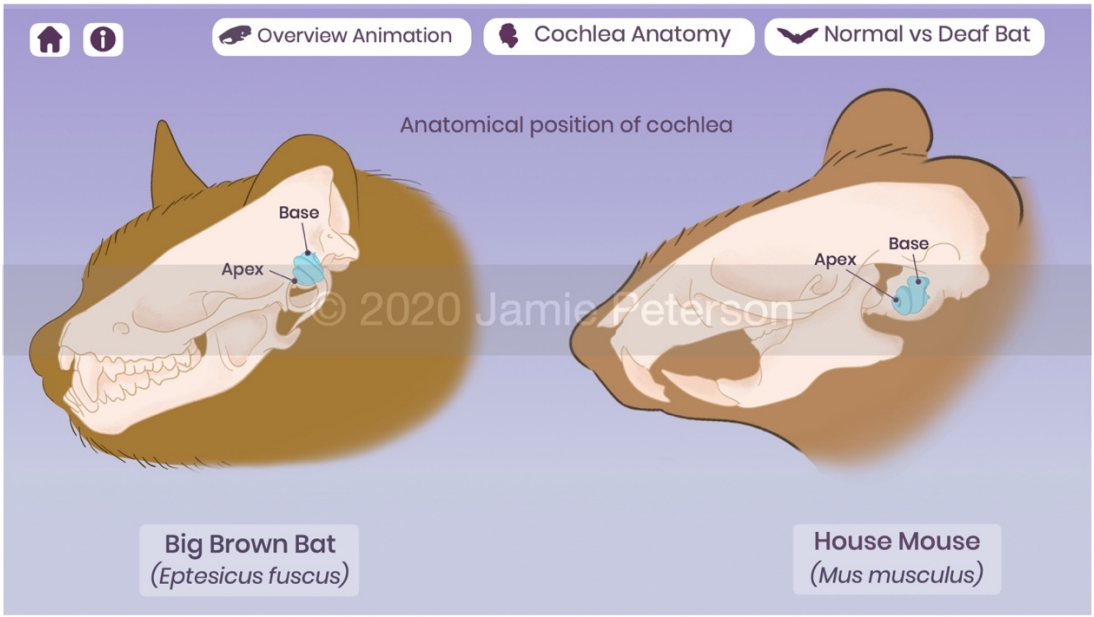


Figure 48. **Interactivity Cochlea Location page.** User can observe the cochlea in anatomical position. The drop-down menu for Cochlea Anatomy is shown.

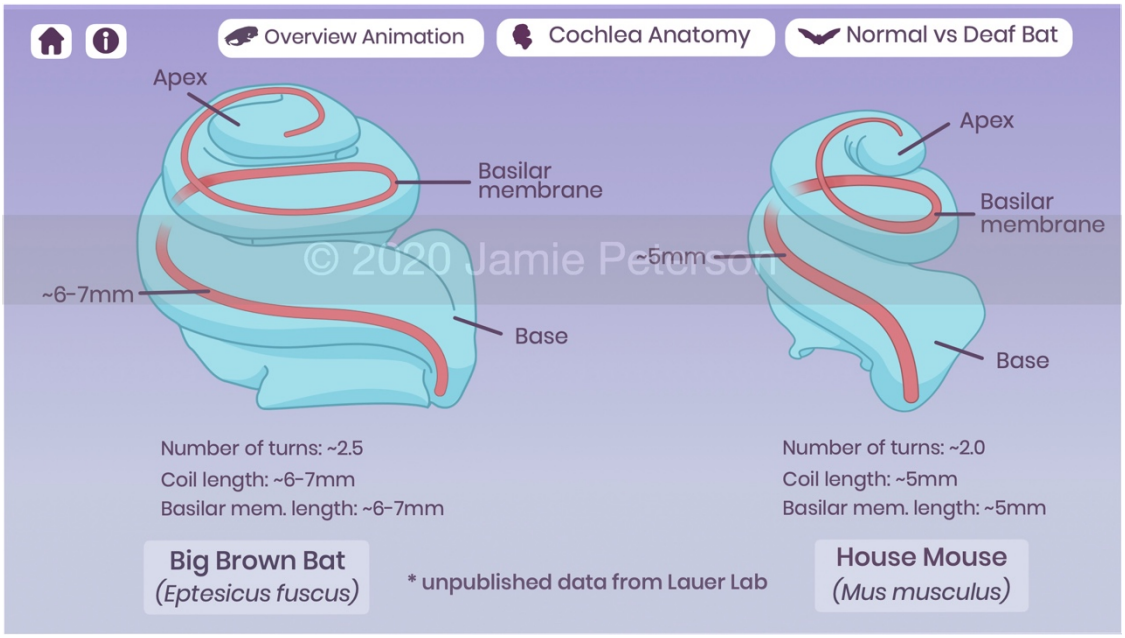


Figure 49. Interactivity Cochlea Structures page. Cochleae animated from the skull to the scientific view with labels and measurements.

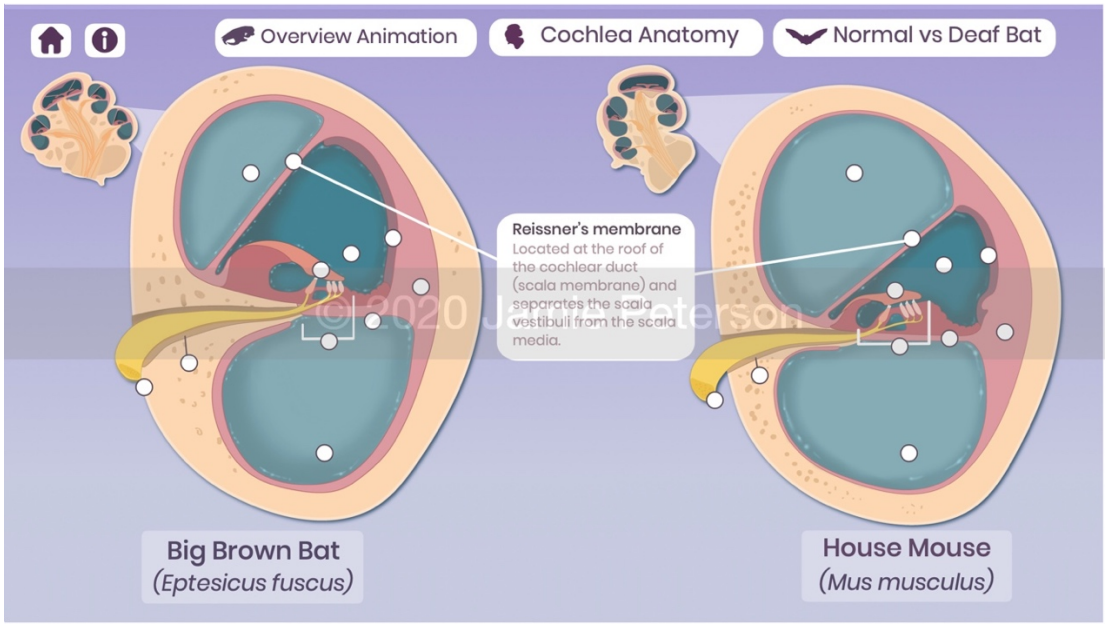


Figure 50. Interactivity Cochlea Section page. A section of the cochlea is enlarged to observe the membranous structures. Each white circle is a cuing element with an annotation. This screen shot shows the annotation for Reissner's membrane. *Text in image not intended to be read.*

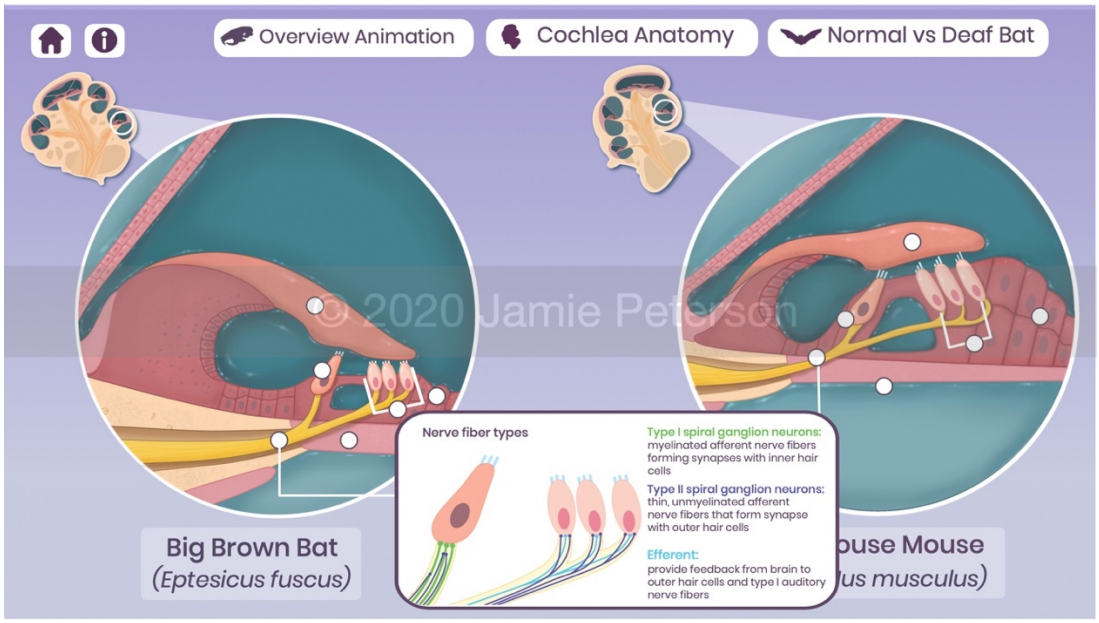


Figure 51. Interactivity Organ of Corti page. The organ of Corti is enlarged from the cross section and the nerve fiber annotation has been clicked. *Text in image not intended to be read.*

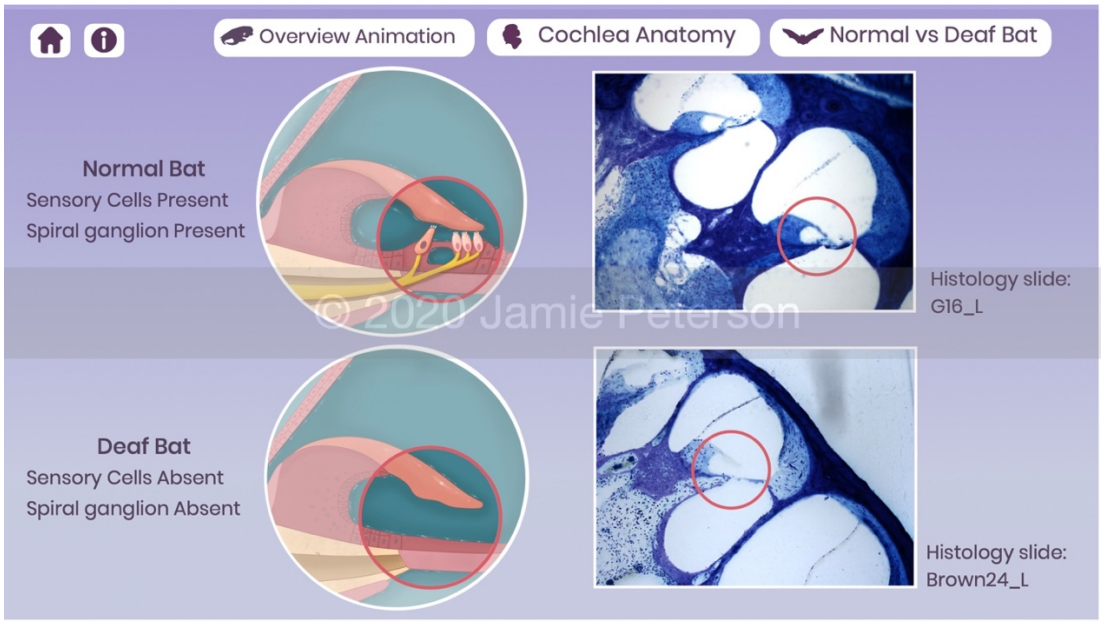


Figure 52. Interactivity Damage Data page. Illustrations of normal and deaf bat accompanied by histological slides from the Lauer Lab depicting bat hearing loss research.

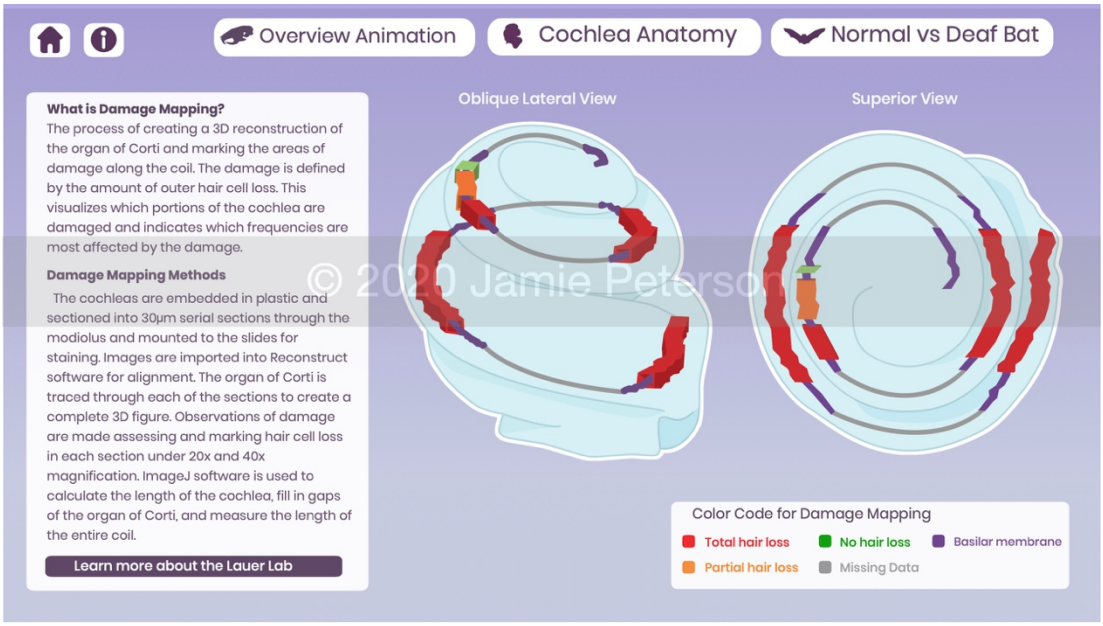


Figure 53. Interactivity Damage Mapping page. Illustrations depict damage mapping based on 3D reconstructions of histological slides. A description of damage mapping and methods is shown to the left with a link to the Lauer Lab for access to more research. *Text in image not intended to be read.*

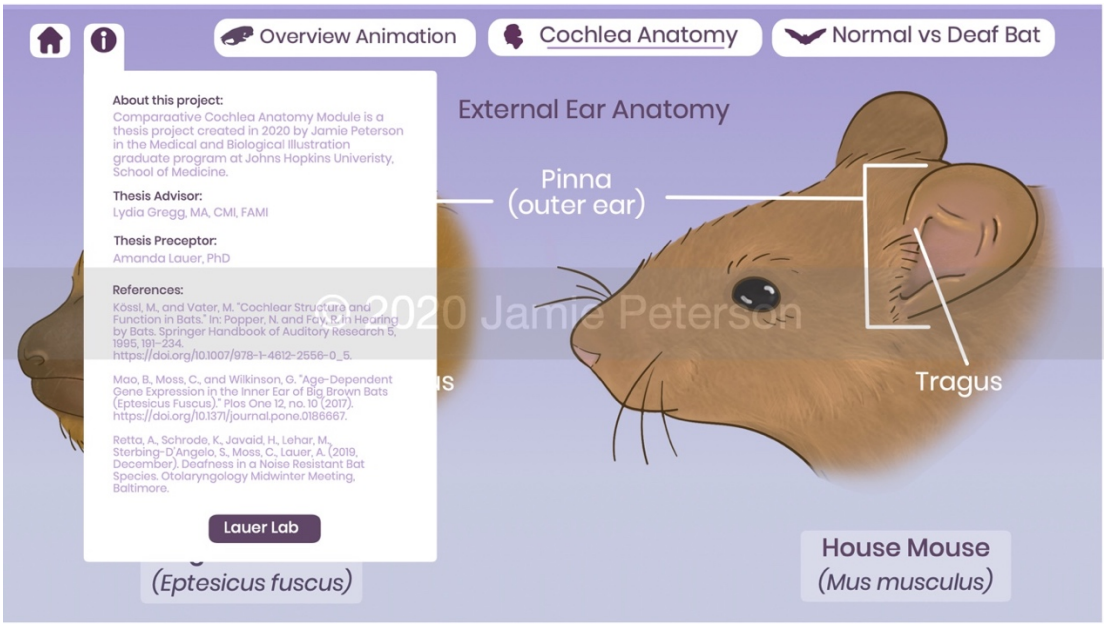


Figure 54. Interactivity Information button. Information about this project, resources and a link to the Lauer Lab for more information on bat hearing research. *Text in image not intended to be read.*

Access to assets resulting from this thesis

The assets resulting from the work of this thesis can be accessed in part at **jpetersonillustration.com** or by contacting the author through the website's contact form. The author may also be reached through the Department of Art as Applied to Medicine graduate program via the website **medicalart.johnshopkins.edu**.

DISCUSSION

Project goals

The primary goal of this project was to fill a gap in the educational material on comparative cochlea anatomy by creating an interactive web-based resource. 3D models developed from segmentation of micro-CT data were employed in an animated overview of the interactive content. The animation introduces *Eptesicus fuscus* and *Mus musculus* cochleae to serve as a guide for studying the anatomy, spatial relationships, and physiology of sound. The interactive content that follows the animation allows the user to explore comparative cochlear anatomy of bats and mice as related to hearing loss. The results of this project provide graduate students, researchers, and lay audiences with a novel didactic tool for visualizing and understanding cochlea anatomy and learning about bat hearing loss research.

Segmentation and 3D model creation

Reconstruct is a free software package for serial stacking and segmentation. Two methods of segmenting histological slides in Reconstruct were investigated, each of which yielded different results. While the Draw Freehand tool method was chosen, the second method for segmenting is the **Trace palette** tool. For this method, a shape stamp is selected and used as a marker for structures. Clicking on a region of interest in several areas through the serial sections will leave markers that are mapped and calculated in Reconstruct to create a 3D representation of the structure. This method is useful for alignment of histological slides and damage mapping, but the **Trace palette** tool does not accurately trace the boundaries of structures.

The results of histology segmentations were useful for understanding the relationship of the cochlea's structures and viewing them in three-dimensions. However, the resulting 3D models yielded no details of smaller structures, such as the sensory hair and supporting cells, and were missing a significant amount of surface geometry. In the future, exploring other programs for histological serial section reconstruction may yield higher quality surface models that could be used in 3D animation or interactive media.

Three-dimensional data that provided the basis of the idealized models included a full surface segmentation of one *Eptesicus fuscus* and one *Mus musculus* dataset. Lower resolution of the *Mus musculus* dataset yielded imprecise semicircular canals that needed significant repair and sculpting. The resolution of the *Eptesicus fuscus* dataset yielded higher quality cochlea and semicircular canal surfaces. In the future, segmentation of multiple specimens with consistent resolutions would allow for identification of anatomical variations and reduce the amount of sculpting needed to repair models.

2D interactive comparative module

Adobe Animate uses vector art to create interactives and websites. Importing vector assets from Adobe Illustrator has a few limitations. If directly importing from Adobe Illustrator, assets must not have transparencies, blurs, drop shadows, gradients, and all line work needs to be converted into shapes. Importing assets from Illustrator to Animate also caused a color shift, desaturating the original artwork. Saving each asset as a PNG file solved these issues and allowed transparencies, drop shadows, gradients, linework, and colors to remain the same as designed. Turning the assets into PNGs also helped to maintain a smaller file size in Animate, which allowed a faster publishing time.

Organization of Symbols and Instances is important to proper HTML code calling. In order to keep track of the numerous Instance names and Symbols for coding of the assets in Animate, a chart of Instance names for all assets, buttons, and annotations was created to refer to during coding in the ActionScript panel. In the future, investigating alternative programs with plugin platforms for building 2D interactives would be more efficient than coding all elements from scratch in Adobe Animate.

3D Overview animation

The introductory Overview Animation was created to provide a concise, narrated comparison of labelled *Eptesicus fuscus* and *Mus musculus* cochlea models. These didactic explanations aim to allow viewers to make stronger spatial connections between the 3D models and information they have learned from 2D images in the available mammalian cochleae literature. Showing the cochlea models from numerous views conveys their full structure and position within the skulls. The use of animated labels aids in highlighting important cochlea structures and anatomical differences between the two species.

Accessibility

The interactive is accessible in any web browser. The user can revisit the website and access the animation and interactive at any time for cochlea anatomy review. The user interface was designed for easy navigation between sections with large section buttons and dropdown menus for access to other pages through one click by the user. For color blind viewers, use of the color white for cueing anatomical annotations creates high visual contrast against the structure to allow for easy targeting (**Figure 50**). The interface was

tested using a color-blind simulator, Coblis, to ensure that all text and colors of structures were legible. For hearing-impaired users, Closed Captioning transcripts are available for the animation on YouTube.

Implications for education and biocommunication

This project used volumetric and histological data as a basis for sculpting educational cochlea models that can efficiently communicate information on mammalian cochlea anatomy and clarify the artwork in the 2D interactive. The structures were refined for use in a didactic animation with integrated audio. Learning theories and strategies related to instructional design provided guidelines and principles for presentation and organization of the project's content.

Mayer's Cognitive Theory of Multimedia Learning focuses on how learners use words and pictures to process information. In general, people process information more efficiently when images accompany text. The use of dynamic text, such as labeling and color-coding, improve the learner's connection between the image and the text. Multimedia Learning is based on three principles: 1) learners have two channels to process information, auditory and visual; 2) learners have a limit of how much information can be taken in by each channel; 3) meaningful learning is attained if the information is relevant, organized, and connects to prior knowledge (Mayer, 2003). These strategies were implemented in this project to provide the user with information and images without being overwhelming. These principles were considered while creating the 3D overview animation. In the animation, cueing elements such as labels and arrows were timed with the audio and visuals to emphasize the structures and their importance. Cognitive Load is the total amount of

mental effort used by working memory for learning (Paas et al., 2010). The overview animation was created to provide information and visuals before the interactive, to construct prior knowledge about cochlea structures and functions. Labels were placed close to corresponding structures to reduce learner's cognitive load and facilitate learning.

Bruner's Constructivist Theory emphasizes learning as being an active process and based on the student's willingness to learn. Learners are constructing new ideas and concepts as they connect learning material to past knowledge. This theory focuses on the learner playing a central role of controlling their own learning and seeking knowledge independently (Clark, 2018). After viewing the cochlear anatomy animation in this project, the user can explore the interactive educational module, controlling the flow of information at their own pace and self-testing their new knowledge on cochlear anatomy. This project is directed towards learners who are interested in clear and concise information on comparative cochlea anatomy and bat hearing loss research. The users are motivated to learn the content so that they can be involved in comparative research related to the cochlea. Through interaction with the module, the user is actively transforming and exploring information on their own.

Khalil's Interactive Imagery Strategy involves the utilization of web-based, dynamic instructional images such as illustrations, photographs, or models to convey information. Dynamic images are combined with interactive elements, such as labels and color coding. These images allow the user to control their learning. The strategy stipulates that 1) images should be clearly labeled, 2) labels should be interactive to allow students to self-access, 3) color-coding should be employed to help make connections, and 4) multiple views of structures are key to spatial learning (Khalil, 2004). The use of 3D models or providing

models from multiple views helps with spatial learning and structural relationships (Khalil, 2004). The overview animation in this project provides the user with different views of 3D models to help with understanding the relationship of the cochlea within the skull and the structure of the cochlea. The 2D interactive offers dynamic labels for the structures, allowing the user to self-test and promoting independent thinking.

Future Directions

This thesis created a novel workflow for the production of an interactive comparative educational module and the creation of accessible and data-driven 3D cochlea models from segmentation. Future development of the project could focus on additional 3D cochlea models, and full implementation of a 3D interactive educational resource for comparative cochlea anatomy. Future 3D models including the middle ear ossicles, a sagittal cut of the cochlea to reveal the membranous structures, and 3D models of the organ of Corti with interactive structures could be developed.

An additional section to the module that includes an adult human cochlea compared to *Eptesicus fuscus* and *Mus musculus* would be beneficial for auditory and comparative cochlea anatomy research. Making the 3D cochlea and skull models available to the public using a 3D model sharing platform would allow researchers and the public access to manipulate and download models for 3D printing or viewing.

User testing

User testing is a necessary component in developing an interactive module to ensure accessibility, navigation, and success in learning content. The interactive module was shown to graduate students and researchers in the Lauer Lab during the final stage of the project. The team provided feedback on the navigation, accessibility of the content, and organization of the interactive module and overview animation. The user testing ensured that the annotations were presented in an engaging way for the learner.

As a result of the user feedback, changes were made to the project. The largest change was the incorporation of interactive 3D cochlea models. The cochleae with semicircular canals were uploaded to Sketchfab, a 3D model sharing platform. Uploading the models to Sketchfab allows the models to be available for other researchers and the public. Interactive 3D models allow the user to see the models from views chosen by the viewer, aiding with spatial learning and structural relationships. The Sketchfab interactives were added to the introductory page of the project. Further user feedback from target audiences on the interactive will improve the content of the final project in the future.

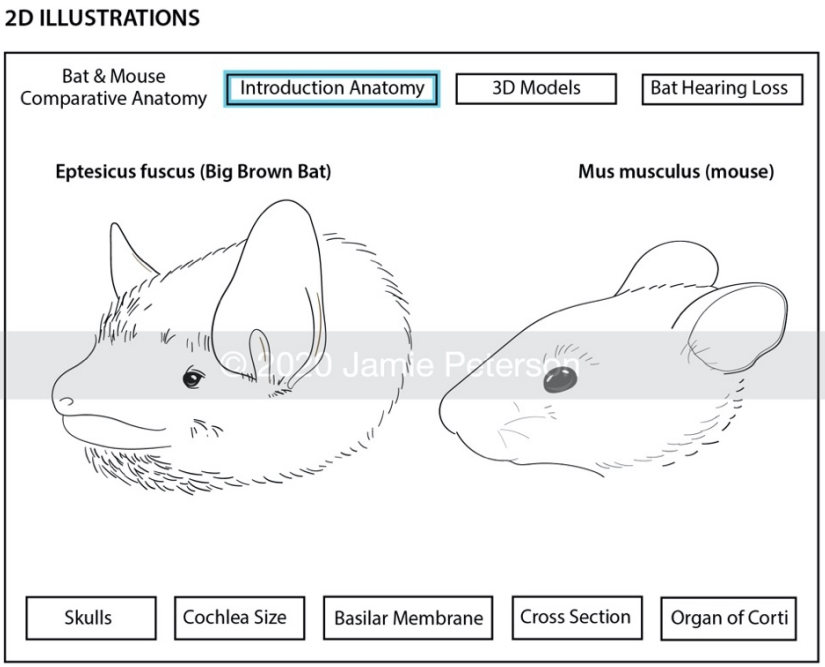
Conclusion

The cochlea is an intricate region of the auditory system that is often studied using animal models, however, there is a lack of clear visuals and educational material for cochlea comparative anatomy. The results of this project offer visual information on comparative mammalian cochlea anatomy and an introduction to hearing loss research in bats. The 3D models for the overview animation were based on novel segmented data and published references for accuracy. The animation and interactive provide users with new visual aids for understanding cochlea variation among species that cannot be easily obtained from raw data, literature, and histological slides. Students, researchers, and clinicians working in hearing research can use the interactive to learn or review anatomy, reinforce their past knowledge, and clarify the structure of the cochlea in three-dimensions.

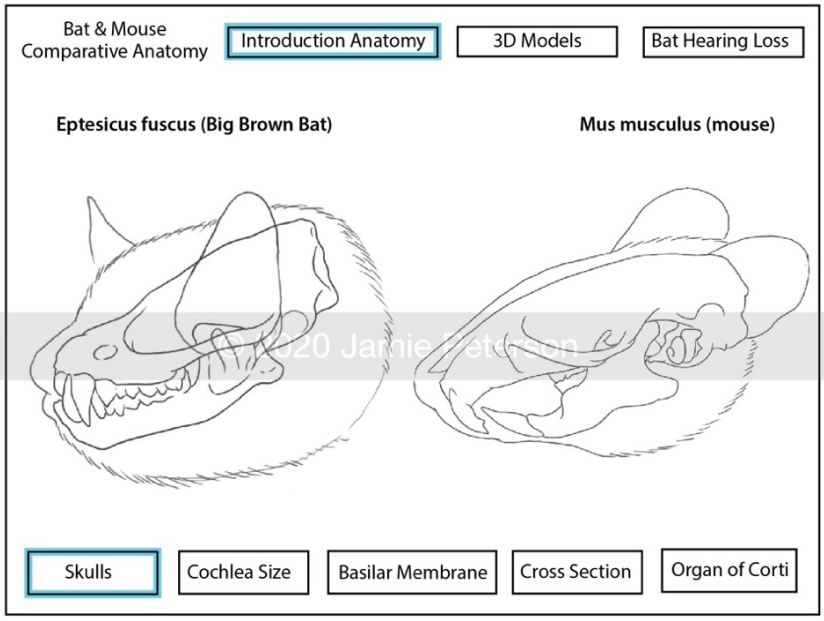
The presented methods and results describe a workflow for future biocommunication undertakings involving animation and interactive educational tools to teach comparative anatomy. The workflow covers micro-CT and histological slide segmentation, 3D model sculpting and repair, and 3D animation and interactive media creation. This novel web resource provides a platform to further enhance the understanding of the mammalian auditory system and reinforce the value of our profession through the transformation of complex data into clear visual communication material.

APPENDICES

Appendix A: Wireframes for interactive

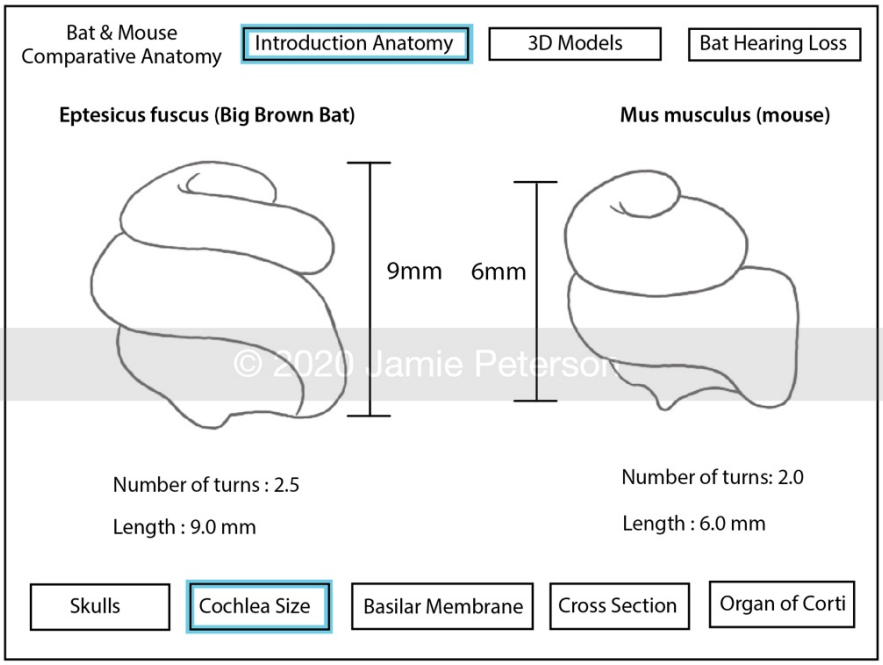


Home Screen. User can choose to start with interactive overview, 3D models, or Bat hearing loss research. Other navigational buttons on the bottom of screen.

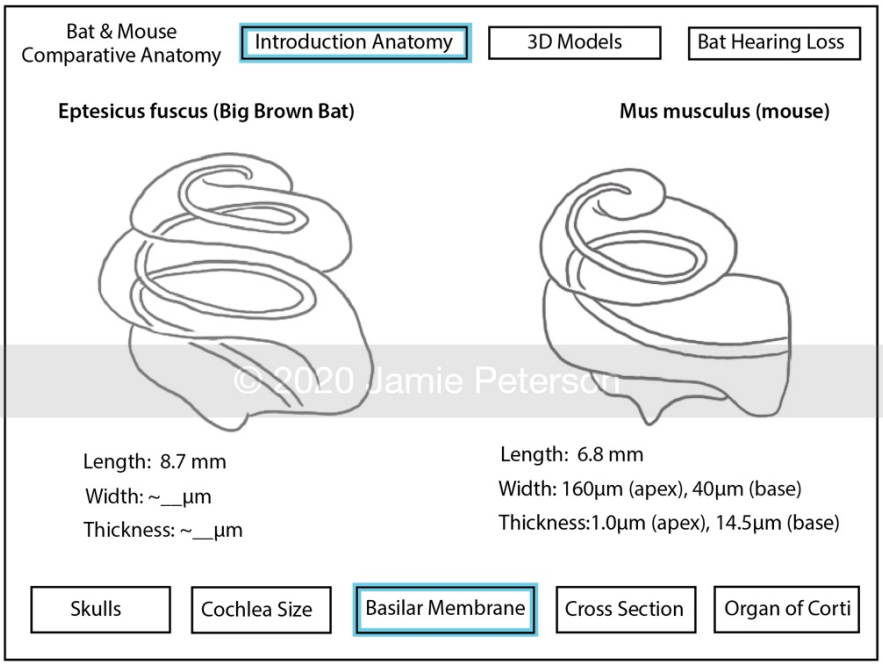


Heads fade to reveal skulls and cochlea within.

Figure 55. Early interactive Wireframes. Preliminary design for interactive education module, 2D illustrations.

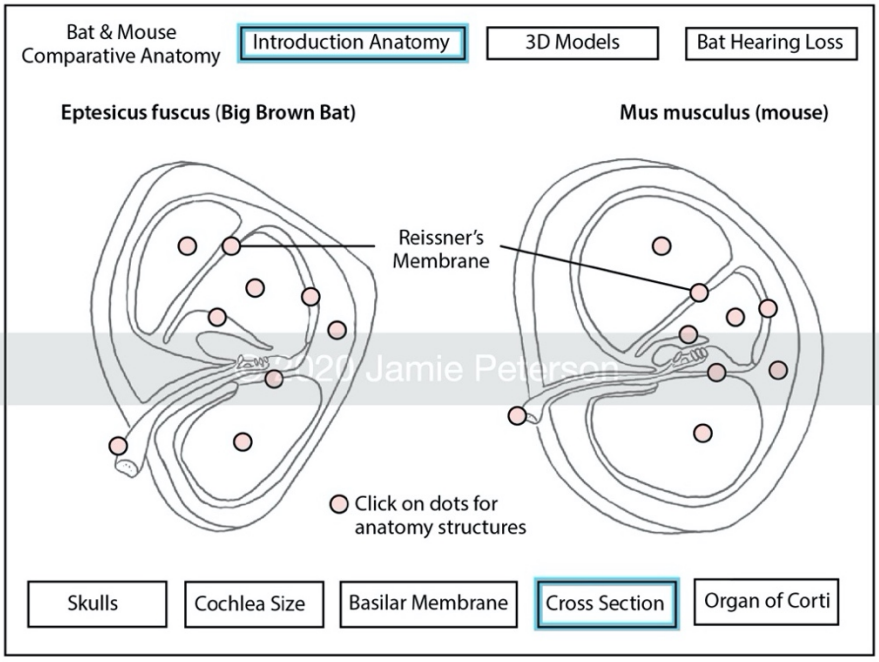


Cochlea lengths and size differences between the two species.

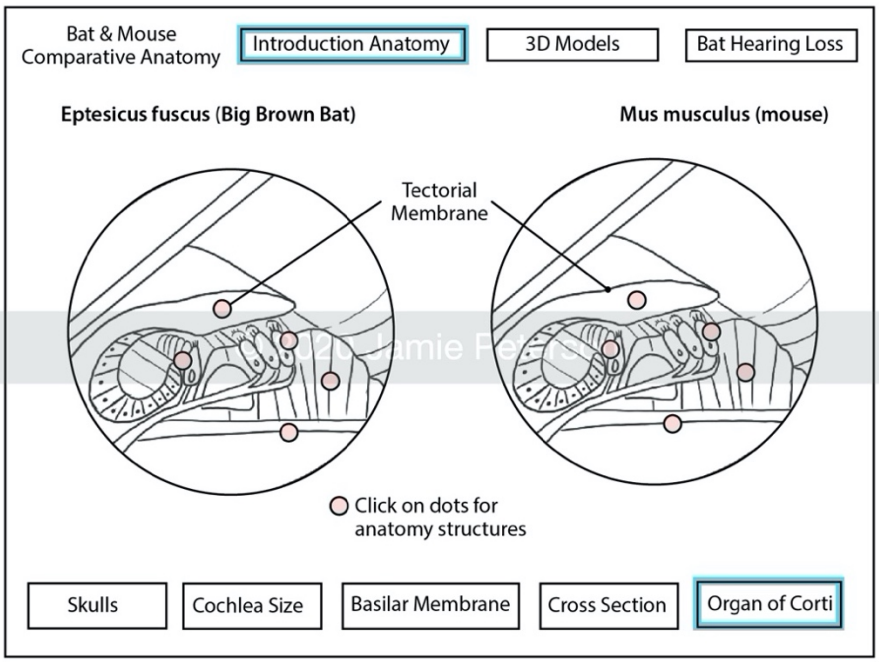


Basilar membrane image and labels.

Figure 56. Early interactive Wireframes. Preliminary design for interactive education module, 2D illustrations.

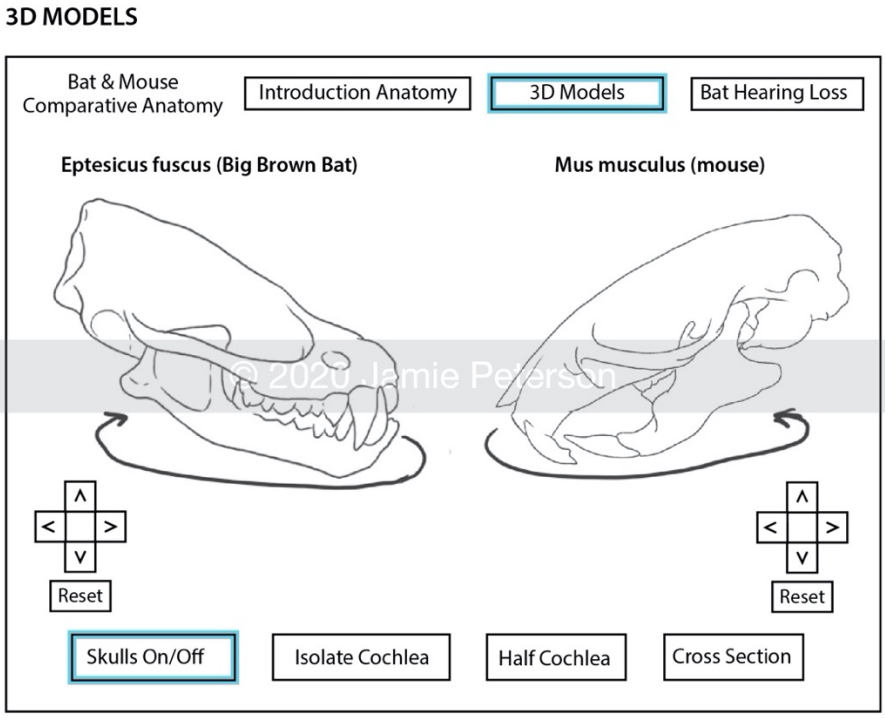


Cross section has interactive dots and when clicked, pop-ups will appear with information.

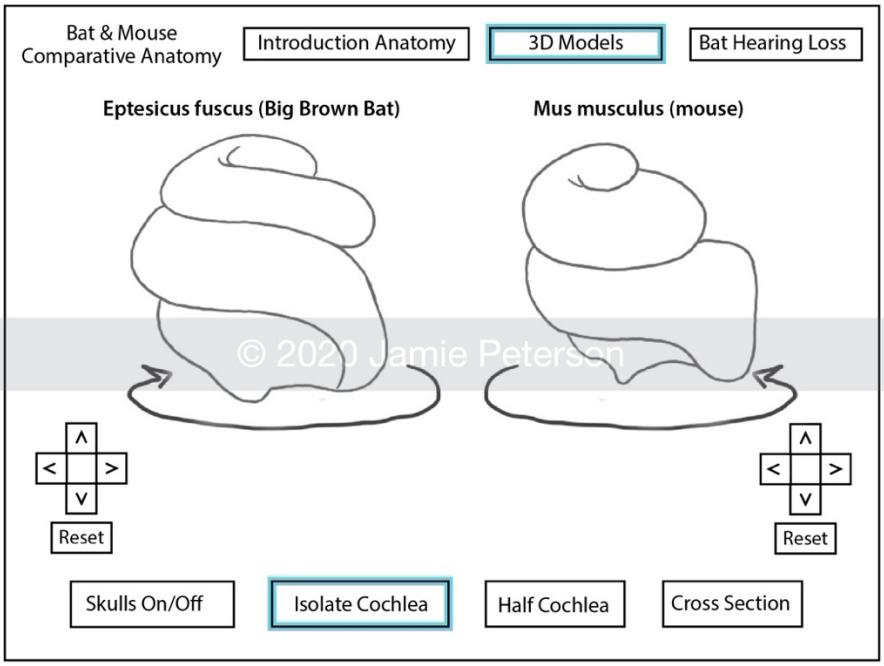


Organ of Corti has interactive dots and when clicked, pop-ups will appear with information.

Figure 57. Early interactive Wireframes. Preliminary design for interactive education module, 2D illustrations.

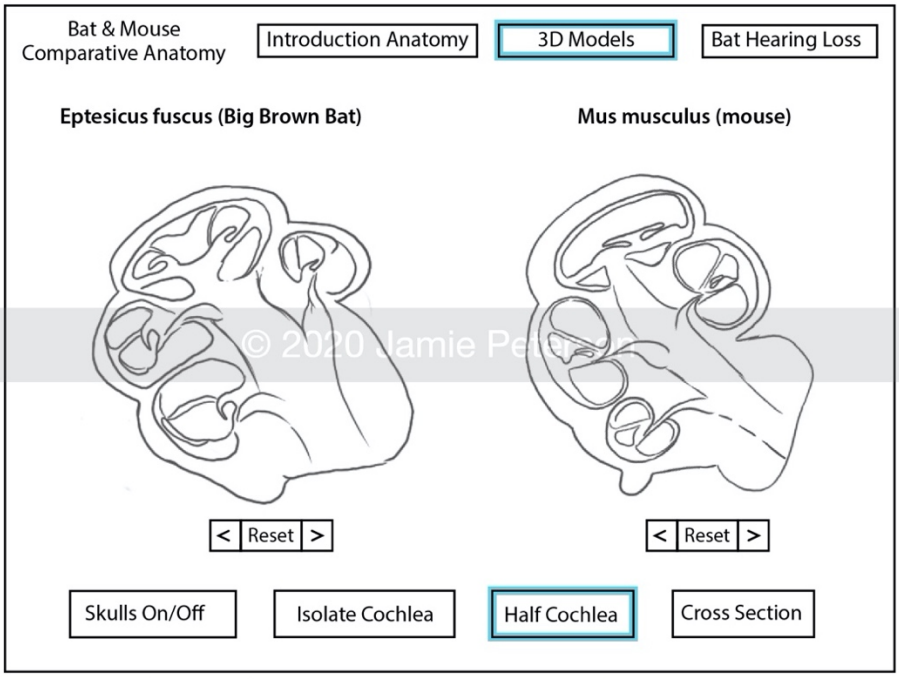


3D skull models of both species are shown side-by-side. Can be moved 360 degrees or snapped to views using arrow buttons.

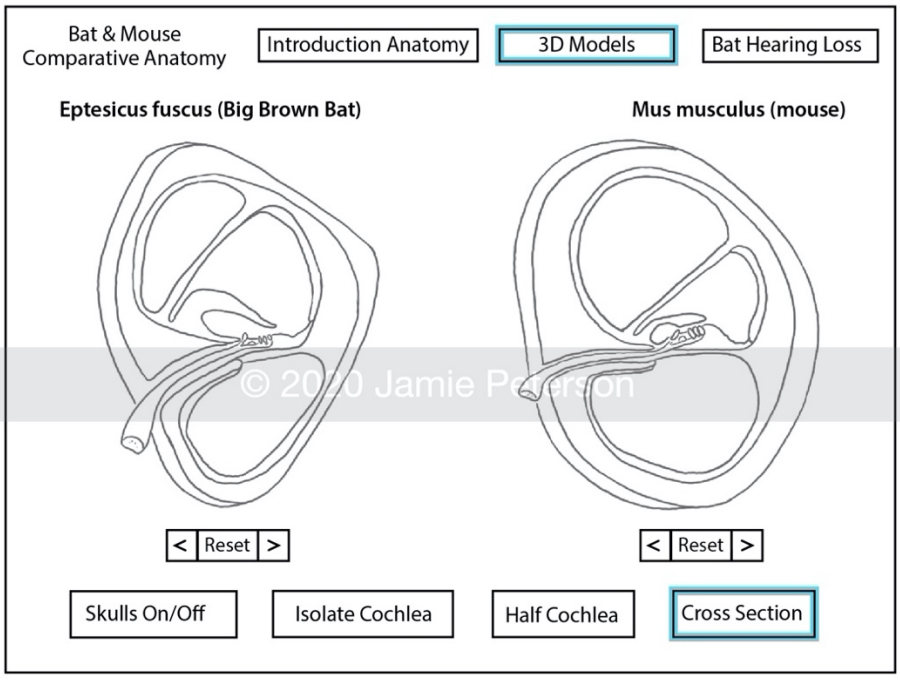


3D cochlea models of both species are shown side-by-side. Can be moved 360 degrees or snapped to views using arrow buttons.

Figure 58. Early interactive Wireframes. Preliminary design for interactive education module, 3D models.

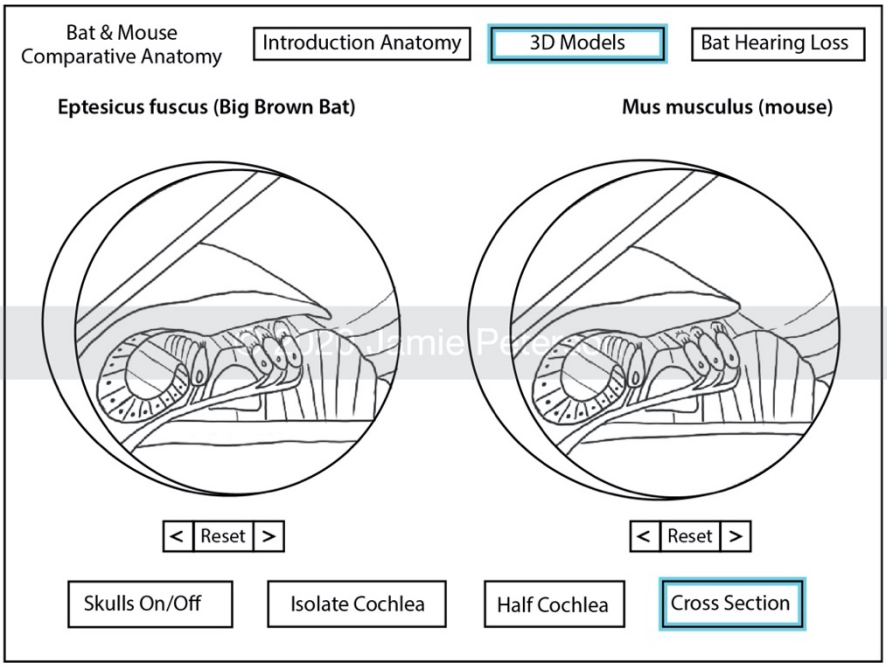


3D half cochlea models of both species are shown side-by-side. Can be moved slightly or snapped to views using arrow buttons.

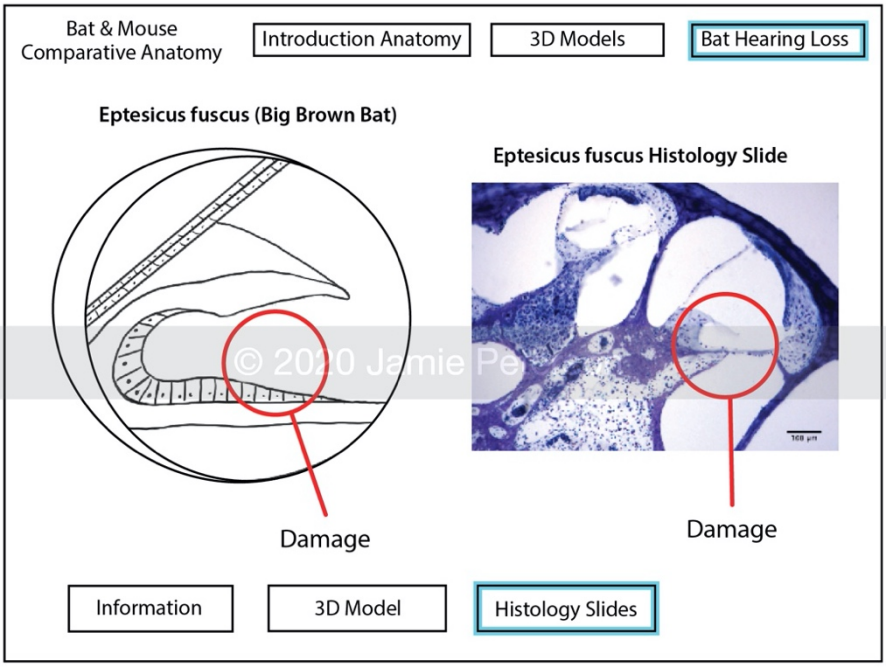


3D cross section models of both species are shown side-by-side. Can be moved slightly or snapped to views using arrow buttons.

Figure 59. Early interactive Wireframes. Preliminary design for interactive education module, 3D models.



3D organ of Corti models of both species are shown side-by-side. Can be moved slightly or snapped to views using arrow buttons.



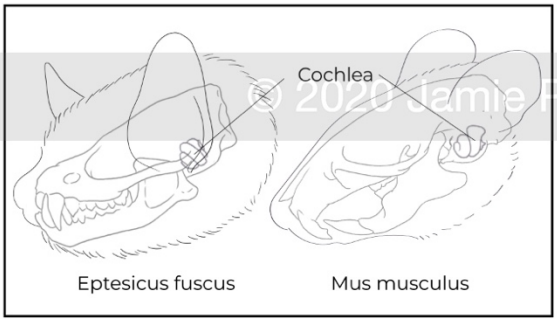
3D models of bat organ of Corti shown next to histology slides.

Figure 60. **Early interactive Wireframes.** Preliminary design for interactive education module, 3D models.

Appendix B: Storyboard for Overview Animation

Project: Thesis Overview Animation
Client:

1

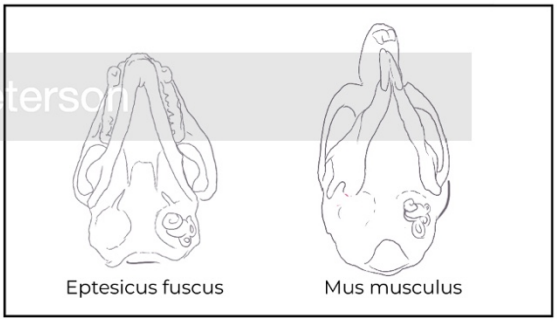


Start: ___.___.___.___ Stop: ___.___.___.___

Video: 2D heads fade to reveal skull in anatomical position. Skulls fade to show cochleae with labels.

Audio: The big brown bat, or Ep-tes-i-cus fus-cus, is an auditory specialist with a broad hearing range and high sensitivity to ultrasonic signals used for echolocation. The house mouse, or Mus musculus, is an auditory generalist with a comparatively limited hearing range. There are remarkable variations in cochlear structure between these two species.

2



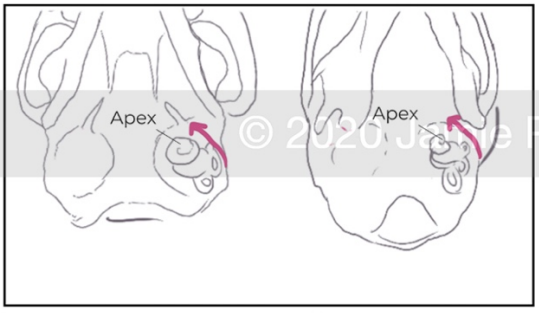
Start: ___.___.___.___ Stop: ___.___.___.___

Video: Skulls rotate to inferior view to show the cochlea angle difference between the two species.

Audio:

Figure 61. Storyboard 1-2. ***Audio text not intended to be read.***

3

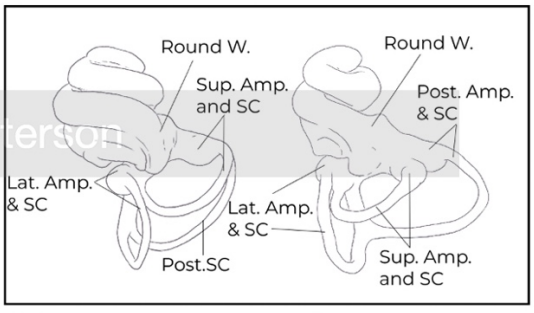


Start: ___.___.___.___ Stop: ___.___.___.___

Video: Close up of inferior view of the skulls to show the cochleae.

Audio: The cochlea is a fluid-filled coil of the inner ear located in the petrous portion of the temporal bone. The apex of the cochlear spiral points rostrally towards the mandible, while the base points towards the external ear to receive sound vibrations. The axis of the big brown bat's cochlea is more ventrally oriented compared to the mouse.

4

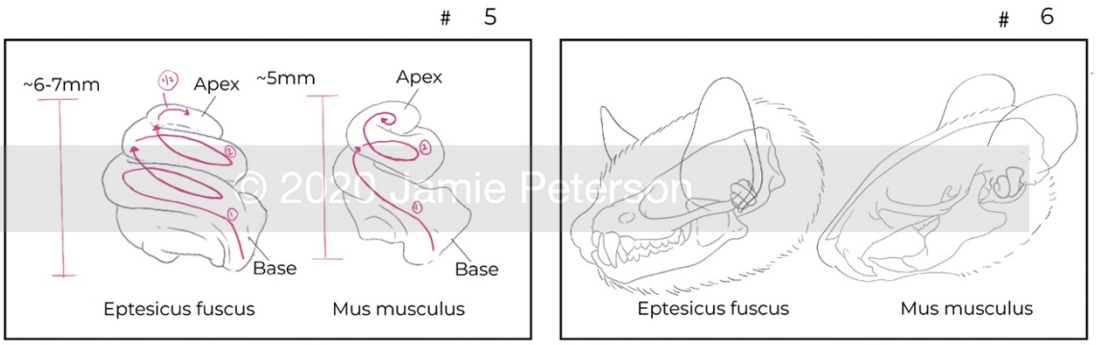


Start: ___.___.___.___ Stop: ___.___.___.___

Video: Skulls fade away and cochleae with semicircular canals enlarge, structures are labeled.

Audio:
The cochlea transforms sound waves into electrical signals for processing in the brain. It is attached to the semicircular canals, which record the angular velocity of head movements to maintain balance.

Figure 62. Storyboard 3-4. ***Audio text not intended to be read.***



Start: ___.___.___.___ Stop: ___.___.___.___

Video: Semicircular canals fade way to reveal ideal cochleae with labels: coil length, turns, apex, and base.

Audio: The length of the coil of the big brown bat is approximately 6-7mm compared to that of the mouse which is approximately 5mm. The extra half turn of the big brown bat cochlea supports a broader hearing range.
Inside the cochlea, different sound frequencies stimulate sensory hair cells along the basilar membrane. Lower-frequency sounds stimulate hair cells in the apex and higher-frequency sounds stimulate hair cells in the base.

Start: ___.___.___.___ Stop: ___.___.___.___

Video: Cochleae rotate back into anatomical position and skulls fade back on.

Audio:
Studying comparative cochlear anatomy can aid in understanding auditory specializations and hearing loss in mammals.

Figure 63. **Storyboard 5-6.** ***Audio text not intended to be read.***

Appendix C: Adobe Animate code

Buttons

```
71  ////////////////////////////////////////////
72  stage.enableMouseOver();
73  ////POINTERS////
74  this.navBar.ExternalBtn.cursor="pointer"
75  this.navBar.IABtn.cursor="pointer"
76  this.navBar.CrossBtn.cursor="pointer"
77  this.navBar.SensoryBtn.cursor="pointer"
78  this.navBar.PhysioBtn.cursor="pointer"
79  this.navBar.CochleasBtn.cursor="pointer"
80  this.navBar.HomeBtn.cursor="pointer"
81  this.navBar.InfoBtn.cursor="pointer"
82  this.navBar.BatBtn.cursor="pointer"
83  this.navBar.DDBtn.cursor="pointer"
84  this.navBar.MDBtn.cursor="pointer"
85  this.navBar.CDBtn.cursor="pointer"
86  this.DMArt.LabBtn.cursor="pointer"
87
88  this.HomeArt.StartBtn.cursor="pointer"
89  this.HomeArt.OABtn.cursor="pointer"
90
91  this.AnimationArt.APBtn.cursor="pointer"
92  this.navBar.AboutPop.LauerLabBtn.cursor="pointer"
93
```

Figure 64. Coding for pointers, Lines 71-89. Each annotation was coded as a pointer, this changes the cursor from an arrow icon to a finger pointing.

```
316 ////////////////////////////////////////////
317 //LAUERLAB BTN///
318 ///Clickable///
319 this.navBar.AboutPop.LauerLabBtn.addEventListener("click", fl_ClickToGoToWebPage);
320 function fl_ClickToGoToWebPage() {
321     window.open("http://www.lauerlab.com/our-research", "_blank");
322 }
323 ///MouseOver///
324 this.navBar.AboutPop.LauerLabBtn.addEventListener("mouseover", mouseOverLauerLabBtn.bind(this));
325 function mouseOverLauerLabBtn(){
326     createjs.Tween.get(this.navBar.AboutPop.LauerLabBtn).to({alpha:.5},500);
327 }
328 ///Mouse Out//
329 this.navBar.AboutPop.LauerLabBtn.addEventListener("mouseout", mouseOutLauerLabBtn.bind(this));
330 function mouseOutLauerLabBtn(){
331     createjs.Tween.get(this.navBar.AboutPop.LauerLabBtn).to({alpha:1},500);
332 }
333
```

```
414 ////////////////////////////////////////////
415 ///ANIMATIONBTN///
416 ///Clickable///
417 this.AnimationArt.APBtn.addEventListener("click", fl_ClickToGoToWebPage_2);
418 function fl_ClickToGoToWebPage_2() {
419     window.open("https://www.youtube.com/watch?v=hE_9mdp69R4/", "_blank");
420 }
421
```

Figure 65. Coding buttons to link to a website, Lines 319-332 and 417-420. These two buttons were coded to link to a different website. Every new link needs an additional underscore and number for the code to call.

```
419  ////////////////////////////////////////////
420  //COCHLEA STRUCTURE BTN///
421  ///Clickable///
422  this.navBar.CochleasBtn.addEventListener("click",clickCochleasBtn.bind(this));
423  function clickCochleasBtn() {
424
425      this.navBar.ExternalBtn.BtnLine.visible=false;
426      this.navBar.IABtn.BtnLine.visible=false;
427      this.navBar.BatBtn.BtnLine.visible=false;
428      this.navBar.CrossBtn.BtnLine.visible=false;
429      this.navBar.SensoryBtn.BtnLine.visible=false;
430      this.navBar.SkullsBtn.BtnLine.visible=false;
431      this.navBar.CochleasBtn.BtnLine.visible=true;
432      this.navBar.CochleasBtn.visible=false;
433      this.navBar.CrossBtn.visible=false;
434      this.navBar.SensoryBtn.visible=false;
435
436      this.MouseHead.gotoAndPlay("start");
437      this.VideoPlayer.visible=false;
438      this.DMArt.alpha=0
439      this.DMArt.visible=false;
440      this.NormalDeafBat.alpha=0
441      this.NormalDeafBat.visible=false;
442      this.navBar.AboutPop.alpha=0
443      this.navBar.AboutPop.visible=false;
444      this.BatMouseLabels.alpha=1
445      this.BatMouseLabels.visible=true;
446      this.MouseHead.alpha=1
447      this.MouseHead.visible=true;
448      this.SkullsArt.alpha=0
449      this.SkullsArt.visible=false;
450      this.FurMouse.alpha=0
451      this.FurMouse.visible=false;
452      this.FurBat.alpha=0
453      this.FurBat.visible=false;
454      this.MouseHead.visible=true;
455      this.CrossSection.alpha=0
456      this.CrossSection.visible=false;
457      this.SensoryCells.alpha=0
458      this.SensoryCells.visible=false;
459      this.HalfCochlea.alpha=0
460      this.HalfCochlea.visible=false;
461      this.SensoryCircle.alpha=0
462      this.SensoryCircle.visible=false;
463  }
464  ///MouseOver///
465  this.navBar.CochleasBtn.addEventListener("mouseover", mouseOverCochleasBtn.bind(this));
466  function mouseOverCochleasBtn(){
467      createjs.Tween.get(this.navBar.CochleasBtn).to({alpha:.5},500);
468  }
469  ///Mouse Out//
470  this.navBar.CochleasBtn.addEventListener("mouseout", mouseOutCochleasBtn.bind(this));
471  function mouseOutCochleasBtn(){
472      createjs.Tween.get(this.navBar.CochleasBtn).to({alpha:1},500);
473  }
474
```

Figure 66. **Coding for Cochlea Structure Button, Lines 422-473.** This button was coded to start the animated element when clicked using the code goToAndPlay("start"); in line 436.

Annotations

```
807  ////////////////////////////////////////////////////////////////////////////////
808
809  ///CROSS SECTION DOTS///
810  this.CrossSection.MainDots.OCBtn.addEventListener("click",clickOCBtn.bind(this));
811  function clickOCBtn() {
812      this.CrossSection.MainPops.OCPop.alpha=1
813      this.CrossSection.MainPops.SVPop.alpha=0
814      this.CrossSection.MainPops.RMPop.alpha=0
815      this.CrossSection.MainPops.STVPop.alpha=0
816      this.CrossSection.MainPops.SMPop.alpha=0
817      this.CrossSection.MainPops.TMPop.alpha=0
818      this.CrossSection.MainPops.SLPop.alpha=0
819      this.CrossSection.MainPops.BMPop.alpha=0
820      this.CrossSection.MainPops.STPop.alpha=0
821      this.CrossSection.MainPops.RCPop.alpha=0
822      this.CrossSection.MainPops.SGPop.alpha=0
823      this.navBar.ClickOnDots.visible=false;
824  }
825  this.CrossSection.MainDots.SVBtn.addEventListener("click",clickSVBtn.bind(this));
826  function clickSVBtn() {
827      this.CrossSection.MainPops.OCPop.alpha=0
828      this.CrossSection.MainPops.SVPop.alpha=1
829      this.CrossSection.MainPops.RMPop.alpha=0
830      this.CrossSection.MainPops.STVPop.alpha=0
831      this.CrossSection.MainPops.SMPop.alpha=0
832      this.CrossSection.MainPops.TMPop.alpha=0
833      this.CrossSection.MainPops.SLPop.alpha=0
834      this.CrossSection.MainPops.BMPop.alpha=0
835      this.CrossSection.MainPops.STPop.alpha=0
836      this.CrossSection.MainPops.RCPop.alpha=0
837      this.CrossSection.MainPops.SGPop.alpha=0
838      this.navBar.ClickOnDots.visible=false;
839  }
840  this.CrossSection.MainDots.RMBtn.addEventListener("click",clickRMBtn.bind(this));
841  function clickRMBtn() {
842      this.CrossSection.MainPops.OCPop.alpha=0
843      this.CrossSection.MainPops.SVPop.alpha=0
844      this.CrossSection.MainPops.RMPop.alpha=1
845      this.CrossSection.MainPops.STVPop.alpha=0
846      this.CrossSection.MainPops.SMPop.alpha=0
847      this.CrossSection.MainPops.TMPop.alpha=0
848      this.CrossSection.MainPops.SLPop.alpha=0
849      this.CrossSection.MainPops.BMPop.alpha=0
850      this.CrossSection.MainPops.STPop.alpha=0
851      this.CrossSection.MainPops.RCPop.alpha=0
852      this.CrossSection.MainPops.SGPop.alpha=0
853      this.navBar.ClickOnDots.visible=false;
854  }
```

Figure 67. **Coding for Annotations, Lines 810-854.** Example of three cueing elements, “dots” and pop-up annotation code. Each cue was coded to call up a specific annotation and hide the rest. A total of 33 annotations were coded.

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VITA

Jamie Peterson was born and raised in San Diego, California. She started her college education at Palomar College, San Marcos, California where she earned two associate degrees; one in illustration, and one in fine arts. There she fell in love with storytelling through illustration. While working as a science tutor for middle school and high school students within San Diego county she discovered an interest in education - routinely drawing her own visuals for biology, chemistry, and human anatomy for her students. A mentor introduced her to the field of medical illustration, she realized that she could combine her interests in art and science into a career. In 2012, she transferred to California State University, Long Beach (CSULB) where she earned her Bachelor of Fine Arts in Illustration and a Biomedical Art Certificate in 2015. Following her graduation from CSULB she continued her science education by earning an additional associate degree from Palomar College in science and mathematics. There, she once again worked as a science tutor and a teaching assistant for illustration and 3D modeling, for college students at Palomar College.

In August 2018, Jamie matriculated to the graduate program in Medical and Biological Illustration at Johns Hopkins University and earned the Ranice W. Crosby Scholarship. At the 2019 Association of Medical Illustrators conference her illustrations “Scent of Life” and “The Lifecycle of the Shield Shrimp” each earned an Award of Merit. Moving forward, she seeks to continue producing precise, accurate, and engaging visual communications for medical and scientific audiences. Jamie is currently a candidate to receive a Master of Arts in Medical and Biological Illustration in May 2020.