Brief Anatomical Guide to the Amphibian Inner-Ear Dissection

Joshua D. Salvi

August 20, 2014

These notes briefly review the dissection of the inner ear of the American bullfrog, *Rana catesbeiana*. The purpose here is to outline anatomical landmarks associated with both the macro- and micro-dissection stages. This dissection requires knowledge of these landmarks and a general understanding of inner-ear anatomy in order to best approach and isolate each structure.

An anesthetized bullfrog is doubly pithed and its head removed with scissors. Pithing is a procedure by which the frog's brain is destroyed with a pithing rod inserted through the foramen magnum. Pithing is achieved by first holding the frog, dorsal side up and rostral end facing away from the experimenter, with the lower extremities extended. The frog is then grasped with the first finger atop the nose and the second finger below the jaw. Its head is then rotated forward. At the frog's midline at the level of the occipital process (a small bump near the tympanum), there is a region of increased compliance. This is the location of the foramen magnum. During this process, one must take care to not apply high pressure to the frog, as its skin is sensitive to pain. The pithing rod is then rapidly plunged through the foramen magnum into the cranial vault. Moving the probe within the vault achieves brain destruction. At this point, the frog is dead. The pithing rod is then slowly withdrawn and rotated away from the experimenter (reversing its orientation while remaining in the midline plane). Once the rod's tip departs the cranial vault through the foramen magnum but is still under the soft tissue, it is then forced caudally through the vertebral foramina in order to destroy the spinal cord. This procedure is termed double pithing. Note that during this process, the frog may jump or make noise. These are motor reflexes and are not higher brain functions. After the procedure, the legs become stiff and finally limp due to flaccid paralysis. With the frog now deceased, the experimenter should test for corneal and withdrawal reflexes.

The frog's head may now be removed. The frog's head is held with the thumb atop the nose and first finger grasping its vomarine teeth (the two teeth at the rostral end of the frog's upper palate) for stability. To remove the lower jaw, scissors are used to sever the temporomandibular joint bilaterally. An orthogonal cut caudal to the tympanum severs the head. This requires that the scissors cut through the vertebrae at the midline. If the frog was appropriately doubly pithed, no reflexes will emerge and there should be no propulsion of

blood from the frog's body. If this does occur, it may serve as a reminder to hone one's skills in the second stage of pithing. After the head is removed, it may be kept moist with standard saline, Ringer's solution, or artificial perilymph, all of which should be appropriately oxygenated throughout the remainder of the experiment. For the dissection of the inner ear, it is most common to use standard saline or artificial perilymph.

The severed head is placed under a dissection microscope for lumination and precision (Figure 1). The frog has two sets of teeth, which include two vomarine teeth and rows of maxillary teeth along the lateral perimeters of the head. An ivory-colored upper palate conceals structures below. At the lateral end of the palate, near the level of the tympanum, one may find a hole of less than 0.5 cm in diameter known as the Eustachian tube. Within this cavern is a long cylindrical bone, the columella auris. The columella in amphibians, reptiles, and birds is a bony and cartilaginous rod that couples the tympanic membrane to the inner ear. Associated structures include the extracolumella and ascending process. The palatal tissue is then cut and reflected to expose structures below. Here one can see, from rostral (anterior) to caudal (posterior), the back of the bulbous oculi and its appendages, a region of bone and cartilage within which lies the inner ear, and tan-colored muscle.

The muscle and top layer of cartilage is then removed to expose the inner ear (Figure 2). Within this region, a lollipop-shaped structure emerges. Close examination reveals a smaller white hemispherical structure. This is the otolith of the sacculus, a mass of calcium carbonate crystals. Additionally, one side of this bulbous structure may contain dark patches of melanocytes. These act as landmarks for the basal (neural) side of the sacculus. Opposite that side is the otolith, with the saccular macula in the middle.

Close inspection of the inner under a dissection microscope reveals additional structures (Figure 3). Here the head has been rotated, with the anterior (rostral) end to the right and the medial side at the top of the image. Cranial nerve VIII enters and exits the spinal cord at the midline. Near the spinal cord along the VIIIth cranial nerve sits Scarpa's ganglion, a collection of neuronal cell bodies associated with vestibular end organs. Moving laterally, the nerve branches in multiple directions. One branch extends to the amphibian papilla and the basilar papilla, the two primary hearing organs in the bullfrog. Another branch extends to the vestibular organs, including the utriculus, sacculus, and semicircular cristae. Finally, a small branch enters the lagena, a mysterious organ whose function remains one of legend. Note the lacunar shape of the lagena. This provides a prominent landmark in the dissection procedure. One can also more readily spot the melanocytes dotting the tissues.

The inner-ear organs may be removed by severing the VIIIth cranial nerve and cutting each of the semicircular canals. Removal of the tissue reveals the otic capsule below (Figure 4). Note the presence of additional holes through which the semicircular ducts pass. These are the approximate positions at which the cuts to the semicircular canals must be made. Since these cuts are relatively blind as they are below the intact tissue, it is beneficial to remember the structure of the otic capsule and approximate locations of each canal.

After removal of the inner-ear organs from each ear, the entire tissue is placed in a bath of oxygenated saline and visualized with the aid of a dissection miscoscope (Figure 5). Each ear is a mirror image of its partner. Starting from CN VIII, one can readily define the lagena and near this organ the sacculus. Opposite the lagenar side of the sacculus lies the utriculus. In these images, it is easy to spot the severed semicircular canals. Not shown in these images are the amphibian papilla and basilar papilla. These can be found on the side of the lagena, underneath the nerve. In the images, these appear as semi-translucent structures between the lagena and cranial nerve and out of the focal plane. To isolate the sacculus, the perilymphatic cistern is first opened. This cistern lies on the neural (basal) side of the sacculus and corresponds to the side shown in the figures. After the perilymphatic cistern is opened and its tissue reflected, the sacculus may be removed by severing its apical side from the endolymphatic cistern. Note that this side of the tissue holds the otolith, and the field of view will be muddied by the presence of otoconia. After the sacculus is removed, the challenge of visualizing each end organ is significantly reduced. The following figures steps through each end organ after isolation from associated structures in order to assist an experimenter in the end-organ hunt.

The isolated sacculus is an egg-shaped structure with its sensory epithelium, the macula, in the center (Figure 6). Prior to dissection, the sensory epithelium is covered by otoconia, a few of which remain in the image. Hair bundles embedded in the macula are tightly coupled to a bean-shaped otolithic membrane. The bundles may be exposed by first digesting the tissue in an enzyme, such as protease XXIV or collagenase IA, for a period of time defined by the enzyme and its concentration. After digestion, an eyelash is used to peel away the otolithic membrane and reveal the hair bundles below.

The lagena is a small structure with a crescent-shaped otolith (Figure 7). While its sensory function is not well-understood, the lagena proves useful as a handle when manipulating the tissue during dissection. Damage to the lagena need not be inevitable, but consequences of destruction are at this time nonexistent.

The basilar papilla can be found by following the deep branch of CN VIII behind the lagena and sacculus (Figure 8). The nerve then divides once more to innervate the basilar papilla and continue toward the posterior vertical canal. This organ has an open tube-like structure that can be easily viewed from one end. Hair cells are innervated by the basilar papilla nerve, which fans outward as it innervates the sensory epithelium. Within the basilar papilla chamber, hair bundles protrude toward the center and are coupled to an overlying tectorium. Pressure gradients within the chamber deflect the tectorium and consequently stimulate sensory hair cells. This organ is one of the two primary auditory organs in amphibians, responding to high-frequency (>1,000 Hz) stimuli.

Hunting for the amphibian papilla may prove to be difficult in a first attempt, as it is embedded deep within a chamber of cartilaginous tissue (Figure 9). Finding the organ becomes simple, however, if one follows CN VIII. After removing the sacculus, the nerve branches are readily observed. One of these branches passes behind the sacculus toward the side opposite the utriculus. The

nerve then branches into two. One branch continues toward the basilar papilla and posterior vertical canal. Another branch abruptly turns backward by about 135 º. This curved branch is the amphibian papilla nerve. Viewing the tissue from the side, two chambers can be seen that togeher resemble a porcine snout. The wall dividing these chambers contains the amphibian papilla. Its sensory epithelium projects into the chamber on the interior of the curvature of the amphibian papilla nerve. The opening of this cavern is blocked by a thin contact membrane. Pressure onto the membrane deflects it, which in turn causes a vibration of the tectorial membrane of the amphibian papilla. Motion of the tectorial membrane subsequently excites hair cells whose hair bundles are tightly coupled to it. One may then remove the bluish cartilage around the amphibian papilla to expose this sensory organ (Figure 10). The sensory epithelium of the amphibian papilla resembles a hatchet, with its low-frequency triangular patch at the rostral end and an extension rostrally along a tonotopic axis. Near the midpoint of the epithelium lies a tectorial curtain that is attached to the tectorial membrane coupled to hair bundles below. The entire tissue is embedded in cartilage, rendering dissection of the intact sensory epithelium of the amphibian papilla more difficult than that of the sacculus. Note the relative positions of the sacculus, amphibian papilla nerve, and round window in this figure.

A dissection of the amphibian inner ear requires both knowledge of the anatomy and technical skill to parse through this anatomy. Hopefully, the information in this brief note is adequate as an introduction to the anatomy. The technical skill, however, cannot be honed in such a document.