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REVA #5: Dissecting and Measuring Cadaveric Human Vagus Nerves

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We use this protocol and it's working

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Abstract

This protocol describes our approach for dissecting and characterizing the gross anatomic features of the vagus nerve in human cadavers. All dissection is performed while the body is in the supine position to minimize movement of the nerve and anatomic landmarks during dissection. Photos are taken before, during, and after dissection to document findings. Once the vagus nerve, its branches, and the branches' targets are identified, the levels of key anatomic landmarks are painted onto the vagus nerve and each branch is painted and documented. After painting, nerve diameters and distances between landmarks are measured.

Attachments



Protocol05_Dissectio...

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Image Attribution

[Surgery icons created by monkik](https://www.flaticon.com/free-icons/surgery "surgery icons")
- Flaticon

Image: Flaticon.com

Materials

Personal Protective Equipment (PPE):

1. Nitrile gloves (at least 6 mil thickness, extended cuff, chemotherapy rated) ([link](#))
2. Optional, but recommended:
 - a. Lab coat or Disposable Tyvek sleeves ([link](#))
 - b. Lab coat or Tyvek apron ([link](#))
 - c. Eye protection (while using bone saw or performing splash-prone procedures)

Dissection Tools:

1. Scalpel handle ([link](#)) with blades (#10, #12, #15 recommended)
 - a. #10 blade ([link](#))
 - b. #12 blade ([link](#))
 - c. #15 blade ([link](#))
2. Variety of scissors
 - a. Fisherbrand Standard dissecting scissors ([link](#))
 - b. Fisherbrand Sharp point scissors ([link](#))
 - c. Mayo curved scissors ([link](#))
 - d. Mayo straight scissors ([link](#))
 - e. Fisherbrand Blunt-end scissors ([link](#))
 - f. Fisherbrand Blunt-tipped scissors ([link](#))
 - g. Integra Miltex Metzenbaum scissors ([link](#))
 - h. Fisherbrand Operating scissors ([link](#))
3. Mopec 5000 Swordfish Pathology Saw ([link](#))
4. Fisherbrand Bone-cutting shears ([link](#))
5. StoeltingTM Friedman Bone Rongeurs ([link](#))
6. 4000-6/50 High Performance Rotary Tool Kit ([link](#))
7. Fisherbrand Dissection probes
 - a. Seeker probe ([link](#))
 - b. Precision probe ([link](#))
 - c. Fine tip probes ([link](#))
 - d. Periodontal probe ([link](#))
 - e. Rung probe ([link](#))

8. Forceps

- a. World Precision Adson tissue forceps ([link](#))
- b. VWR Round-tip forceps ([link](#))
- c. VWR Fine tip forceps ([link](#))
- d. VWR Serrated forceps ([link](#))
- e. VWR Blunt-tip Adson forceps ([link](#))

9. Garland Wooden Mallet ([link](#))

10. DASCO Pro Chisels (variety of sizes – example [link](#))

11. Binocular Loupes, 2.5x magnification ([link](#))

- a. Suspected company: Oculus

12. Leica M525 F50 / M520 F40 Surgical microscope ([link](#))

Labeling Media:

1. Nerve-marking paint (made by mixing white latex paint with colored tissue dye)

a. The type of labeling paint can be varied so long as it does not damage the tissue, it does not interfere with other imaging or sample processing methods, and it stays adhered to the tissue.

- i. Davidson marking system dyes ([link](#))
- ii. White gloss latex paint ([link](#))
- iii. Black matte latex paint ([link](#))

2. Paint brushes ([link](#))

- a. Dissection probes can also be used to apply small dots of paint

Measurement Supplies:

1. Digital calipers ([link](#))
2. Castroviejo calipers ([link](#))
3. Fabric tape measure ([link](#))
4. Scale grid (custom-made via 3D laser cutter and acrylic ([link](#)))
 - a. ½ inch thick acrylic cut to 2 cm x 2 cm and etched with a 5 mm x 5 mm grid to be used as a scale in photos.
 - b. Company: McMaster-Carr. Color: white. Item number: 8505K745.

Additional Supplies:

1. Body bag ([link](#))
2. Camera with lens if applicable (camera [link](#); lens [link](#)) and appropriate memory card ([link](#))
3. Tripod ([link](#)) or c-stand ([link](#))
4. Cadaver wetting solution (0.01% liquid phenol (≥89% phenol; [link](#)) and 40% professional-grade concentrated bleach (8.25% sodium hypochlorite; [link](#)) (v/v) in water)



5. Spray bottles for hydrating nerves with continuous mist ([link](#))
6. Cheesecloth ([link](#))
7. String ([link](#)) or suture ([link](#) or [link](#))
8. Magnifying lamp ([link](#))
9. Overhead lights ([link](#)) with c-stands ([link](#))
10. Standard hand-held fan ([link](#)) or blow dryer ([link](#))
11. ColorBond Tissue Marking Dye Mordant spray ([link](#))
12. Phosphate buffered saline (PBS) 1x or normal saline
 - a. PBS can be diluted from stock 10x PBS using distilled water
13. Chemical absorbent pads ([link](#))
14. Pins
 - a. T-pins ([link](#))
 - b. Ball-top pins ([link](#))
15. Plastic wrap

Safety warnings

! Safety Warnings: This protocol might include items and/or substances that may pose hazards (e.g., chemical, physical, biological, or otherwise) to your health upon use or exposure. Before engaging in the processes described in this protocol, familiarize yourself with and follow the safety data sheets, manufacturer safety recommendations, and local regulations.

Ethics statement

Be sure to seek approval for or an exemption from human subjects research from your local regulatory body(ies) as required by local and/or institutional regulations before initiating studies. This study was determined to be exempt from IRB oversight by the Case Western Reserve University Institutional Review Board (IRB) because it involved de-identified cadaveric tissue and no protected health information was collected from the donors.

Before start

See the protocol for embalming a human cadaver (dx.doi.org/10.17504/protocols.io.kxygx4wm4l8j/v1).



Section 1: Pre-dissection Workup

- 1 Identify the gross anatomical landmarks on the body (Appendix 1, Table 1) pre-dissection or during dissection, as appropriate. As soon as an anatomical landmark is revealed and the vagus is accessible, paint the anatomical level on the vagus nerve (Appendix 1, Table 2; Appendix 4).
- 2 Take photos of the cadaver according to the photo checklist (Appendix 2).
- 3 Measure cranial circumference using a fabric ruler.
- 4 Measure head circumference at the nose using a fabric ruler.
- 5 Record general cadaver composition and notable superficial characteristics in the dissection notes document (template in Appendix 3).
- 6 Cut scalp hair using hair clippers as needed.
- 7

Note

Note: The order of dissection can vary from body to body as noted below. Photographs are obtained before, during, and after dissection according to the photo checklist (Appendix 2). Unique and/or notable findings related to the general composition of the cadaver and the vagus nerve (e.g., anatomic variants, pathologies, etc.) encountered during the dissection should be recorded in the dissection notes (Appendix 3) throughout the dissection process.

8

Note

Note: To reduce tissue drying during dissection, we spray the bodies with a wetting solution of 59.99% water, 40% bleach, and 0.01% phenol at 15-minute intervals while dissecting. When not in active dissection, we spray the body with the same wetting solution, cover the exposed tissue with wetted cheesecloth, and seal the cadaver in a body bag.

Section 2: Superficial Cervical Dissection

9

Note

Note: The superficial cervical dissection can be performed in two ways: (1) to gain access to the deep cervical region where the vagus nerve is located (described in Section 4) or (2) to characterize superficial nerves in the neck if the researcher is interested in these structures in addition to the vagus nerve (described in Sections 3). If performing the superficial cervical nerve dissection (Section 3), the transverse cervical nerve, cervical branch of the facial nerve, marginal mandibular nerve, supraclavicular nerves, ansa cervicalis, great auricular nerve, greater occipital nerve, lesser occipital nerve, third occipital nerve, and buccal branch of the facial nerve can be identified. Steps for the superficial cervical nerve dissection are **described in Section 3** after the superficial cervical dissection protocol described here in Section 2.

10 Take photos during the dissection according to the photo checklist (Appendix 2).

11 Create superficial skin incisions.

11.1 Begin the dissection with a superficial midline incision from the mental protuberance to the jugular notch.

11.2 Continue the incision diagonally from the mental protuberance to the angle of the mouth. From the angle of the mouth, continue this incision to the lateral canthus.

11.3 From the lateral canthus, continue the incision transversely across the temple to the superior edge of the tragus.

11.4 Make a lateral incision from the jugular notch to the acromioclavicular (AC) joint along the anterior surface of the clavicle.

12 Reflect the skin flaps laterally. The platysma is the first muscle that appears below the skin and should be removed.

13 The facial region, defined as the region from the inferior border of the mandible to the level of the incision between the eye and ear that was created in Step 11.3, can be cleaned down to the muscle.



- 14 Remove superficial veins of the neck, including the external jugular vein (EJV) superiorly until it branches into the retromandibular vein medially and the posterior auricular vein laterally. Continue following the retromandibular vein as it travels towards the parotid gland and remove it.
- 15 Identify the superior root of the ansa cervicalis.
- 15.1 Cut the sternal/clavicular attachments of the sternocleidomastoid (SCM) and reflect the SCM in a superolateral direction.
- 16 Clean any remaining muscles, fascia, and fat so the carotid sheath can easily be visualized.

Section 3: Superficial Cervical Dissection with Superficial Cervical Nerve Preservation

- 17 See Note at the top of Section 2.
- 18 Create superficial skin incisions.
- 18.1 Begin the dissection with a superficial midline incision from the mental protuberance to the jugular notch.
- 18.2 Continue the incision diagonally from the mental protuberance to the angle of the mouth. From the angle of the mouth, continue this incision to the lateral canthus.
- 18.3 From the lateral canthus, continue the incision transversely across the temple to the superior edge of the tragus.
- 18.4 Make a lateral incision from the jugular notch to the AC joint along the anterior surface of the clavicle.
- 18.5 Reflect the skin flaps laterally. The platysma is the first muscle that appears below the skin. The platysma should be left intact since protecting deeper structures is of utmost importance during this step.
- 19 The facial region, defined as the region from the inferior border of the mandible to the level of the incision between the eye and ear that was created in Step 11.3, can be

cleaned down to the muscle while preserving branches of the facial nerve that travel to the cervical region.

20 Find the transverse cervical nerve.

20.1 Extend the midline incision through the platysma from the mental protuberance to the jugular notch. Mobilize the platysma from the underlying structures from the midline to the SCM.

20.2 Starting from the sternal head of the SCM, remove the platysma muscle and associated fascia from the SCM in a superolateral direction (up and away from the midline). Continue until about $\frac{1}{3}$ - $\frac{1}{2}$ of the way up the SCM.

20.3 The transverse cervical nerve will either be sitting on the anterior surface of the SCM or on the posterior surface of the platysma, in both cases aligned along the lateral to medial direction.

21 Locate the nerve point of the neck (i.e., Erb's point), which is located on the lateral margin of the SCM at the mid-point between the jugular notch and mastoid process.

21.1 At this point, the transverse cervical, great auricular, lesser occipital, and supraclavicular nerves can be visualized emerging from the neck medial to the EJV.

22 Find the cervical branch of the facial nerve.

22.1 Follow the EJV superiorly until it branches into the retromandibular vein medially and the posterior auricular vein laterally. Follow the retromandibular vein as it travels towards the parotid gland.

22.2 Once the parotid gland has been reached, carefully dissect the anterior surface of the retromandibular vein. The cervical branch of the facial nerve will be found deep to the parotid on the lateral side of the retromandibular vein. Note that the cervical branch of the facial nerve crosses the anterior surface of the retromandibular vein, so caution is needed to prevent damaging it.

22.3 The marginal mandibular branch of the facial nerve should be observed exiting from a similar point and running medially along the mandible towards the mental protuberance.

23 Find the connection between the transverse cervical nerve and the cervical branch of the facial nerve.

23.1 Follow the transverse cervical nerve superiorly until a connection is found with the cervical branch of the facial nerve. Alternatively, the cervical branch of the facial nerve



can be followed inferiorly until a connection with the transverse cervical nerve is found.

24 Identify the superior root of the ansa cervicalis.

24.1 Cut the sternal/clavicular attachments of the SCM and reflect the SCM in a superolateral direction.

24.2 The ansa cervicalis should be visible superficial to the carotid sheath. If needed, follow branches from the strap muscles back to the ansa cervicalis or locate the hypoglossal nerve and locate the superior root of the ansa cervicalis branching from it.

25 Once all the nerves have been located and identified, clean any remaining muscles, fascia, and fat so the nerves can easily be visualized, measured, and traced.

Section 4: Deep Cervical Dissection

26 This dissection will expose the vagus nerve in the cervical region. Photos are taken according to the photo checklist (Appendix 2) as dissection progresses.

27 Identify the spinal accessory nerve (CN XI) on the deep surface of the SCM.

28 Identify the hypoglossal nerve and the full ansa cervicalis.

28.1 Follow the superior root of the ansa cervicalis superiorly to its origin on the hypoglossal nerve, if not completed in Section 3.

28.2 Confirm the hypoglossal nerve is crossing anterior to the carotid sheath and coursing medially toward the floor of the mouth.

28.3 Follow the superior root of the ansa cervicalis to the inferior root of the ansa cervicalis, as well as any branches emerging from the ansa cervicalis.

29 Remove the zygomatic arch.

29.1 Using a bone saw, bone shears, or rongeurs, cut the zygomatic arch at its midpoint and at the origin of the zygomatic process of the maxilla. Take care to preserve the jugale.



- 30 Remove the mandible – **be sure to retain it after removal.**
- 30.1 Remove the parotid gland in its entirety down to the mandible.
- 30.2 Remove any connective tissue from the temporomandibular joint to the midpoint of the body of the mandible.
- 30.3 Using a bone saw, cut the body of the mandible just lateral to the mental foramen.
- 30.4 Incise the temporomandibular joint using sharp dissection.
- 30.5 Remove muscular attachments to the ramus, coronoid process, and angle of the mandible using sharp dissection.
- 30.6 Be sure to retain the removed segment of the mandible for marking landmarks later in the dissection process.
- 31 Clean the carotid sheath.
- 31.1 Remove fascia, fat, and connective tissue located between the posterior surface of the SCM and the anterolateral surface of the carotid sheath from the superior border of the clavicle to the superior-most cervical region that can be accessed to his point (up to the mandible).
- 31.2 The omohyoid muscle crosses over the anterior surface of the carotid sheath. This muscle can be reflected at the intermediate tendon or at the superior attachment.
- 32 Identify and remove the posterior belly of the digastric muscle and the stylohyoid muscle.
- 32.1 Cut the digastric muscle at its intermediate tendon and reflect superolaterally, or remove it by cutting at its origin.
- 32.2 Cut the stylohyoid muscle as it splits around the intermediate tendon of the digastric muscle, and cut at its origin to remove it.



- 33 If dissecting the carotid sinus nerve, follow the [carotid sinus nerve dissection protocol](#).
 - 34 Open the carotid sheath.
 - 34.1 Open the carotid sheath between the superior border of the clavicle and the laryngeal prominence, taking care not to disturb or cut the vagus nerve. Ideally, the carotid sheath should be opened inferior to the carotid bifurcation.
 - 35 Identify the vagus nerve within the carotid sheath.
 - 35.1 Document the location and orientation of the nerve relative to the internal jugular vein (IJV) and common carotid artery (CCA).
 - 36 Identify the glossopharyngeal nerve (CN IX) and its branches by:
 - 36.1 Identifying the stylopharyngeus muscle that originates from the styloid process and preserving the branch of the glossopharyngeal nerve to the stylopharyngeus.
 - 36.2 Follow the branch to the stylopharyngeus to the glossopharyngeal nerve.
 - 37 Continue dissecting the cervical vagus nerve. Follow the vagus nerve both superiorly and inferiorly, clear the surrounding tissues, and identify vagus nerve branches.
 - 37.1 Follow each vagus nerve branch distally to the point where there is a high level of confidence in naming it.
- Note**
- Note: Some branches will not be identified until further dissection is completed.
- 37.2 While following vagus nerve branches, any tissue that is not innervated by the vagus can be removed. This is necessary to facilitate visualization of the entirety of the cervical vagus nerve.



- 37.3 Select branches are labeled with suture to preserve or facilitate easy identification throughout the remainder of the dissection.
- 38 Sympathetic trunk dissection.
 - 38.1 The sympathetic trunk (ST) can be found medial/posteromedial to the ICA.
 - 38.2 The vagus nerve is likely to have connections to the sympathetic trunk.
- 39 Spinal nerve dissection (optional if researcher is interested in spinal nerve connections).
 - 39.1 Follow the inferior root of the ansa cervicalis proximally until it bifurcates into the C2 and C3 spinal nerves.
 - 39.2 Follow the superior root proximally towards the C1 spinal nerve.
 - 39.3 Follow any other branches of the vagus nerve that travel to spinal nerves to their origin from the spinal nerve(s).

Section 5: Cranial Dissection (can occur before or after cervical dissection)

- 40 Create scalp flaps.
 - 40.1 Make a midline incision from the superior portion of the forehead to the mid-occipital region and a coronal incision from ear to ear.
 - 40.2 Pull these flaps as laterally as possible to expose as much of the cranium as possible.
 - 40.3 Use a chisel or the back end of a scalpel to scrape off the periosteum that covers the cranium.
- 41 Remove the calvarium.

41.1 Use a bone saw to cut the circumference of the skull just superior to the margin of the skin flaps.

41.2 Remove the calvarium using a chisel. Be cautious: do not damage brain tissue by inserting the chisel too deeply. Wedge the chisel between the inferior and superior pieces of the cranium made by the cut and lift.



42 Remove the cerebrum.

42.1 Remove the falx cerebri and dura mater and lift the cerebrum posteriorly from the anterior cranial fossa. Insert a large knife and cut at the level of the midbrain.

42.2 Remove the cerebrum, tentorium cerebelli, and falx cerebelli.

Section 6: Jugular Foramen Dissection

43 Remove the cerebellum.

43.1 Remove the cerebellum in a piece-by-piece manner by incising the cerebellar peduncles. Take care not to damage CNs IX and X as they enter the jugular foramen.



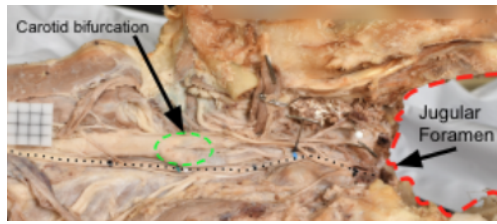
43.2 Trim the brainstem to visualize the cranial nerve roots of the glossopharyngeal and vagus nerves. All other cranial nerve roots can be removed by cutting them off the brainstem as they enter their respective foramina.

44 For increased visibility of the nerves transitioning through the jugular foramen, the distal sigmoid sinus (including the jugular bulb) can be removed.

45 Open the lateral cranium.

45.1 Remove any overlying temporalis muscle and use a chisel to scrape off pericranium as well as any remaining zygomatic arch.

45.2 Using a bone saw or Dremel, make a U-shaped cut in the cranium that extends 2-3 cm anterior and 2-3 cm posterior to the external auditory meatus. This cut can extend all the way through the cranium posteriorly, if desired.



- 45.3 Chisel around the jugular foramen as necessary to fully open the jugular foramen.
- 46 Follow the spinal accessory nerve (CN XI) superiorly from the SCM to the inferior margin of the jugular foramen.
- 47 Carefully remove the IJV if it is immobile and obstructs the view of the nerves emerging from the jugular foramen. Otherwise, reflect the IJV to enable visualization of the nerves exiting the jugular foramen.
- 48 Remove the styloid process and all muscles attached to it using bone shears.
- 49 Remove the internal carotid artery (ICA).
- 49.1 Separate the glossopharyngeal nerve from the adventitia of the ICA.
- 49.2 Cut the ICA approximately 1 cm superior to the carotid bifurcation, taking care not to cut any branches of the vagus nerve.
- 49.3 Cut the ICA superiorly as it enters the carotid canal.

Section 7: Thoracic Wall Dissection

- 50 Create incisions and skin flaps.

- 50.1 Make a midline incision from the inferior aspect of the xiphoid process to the jugular notch of the manubrium.
- 50.2 Make another incision from the jugular notch to the acromion bilaterally while following the curvature of the clavicle on its anterior surface.
- 50.3 Make an incision from the xiphoid process along the anterior surface of the costal cartilage and bone of the 10th rib and extend the incision to the midaxillary line, taking care not to incise the peritoneum or underlying viscera.
- 50.4 Grab the skin near the sternum and use a scalpel or scissors to remove as much muscle and tissue as possible.
- 50.5 The pectoral muscles can be reflected or removed.
- 51 Open the thoracic cavity.
 - 51.1 Use a chisel to scrape off any periosteum or muscle (serratus anterior) that is adhered to the ribs along the midaxillary line.
 - 51.2 Using bone cutters or a bone saw, cut the ribs at or posterior to the midaxillary line on both sides, starting inferiorly and progressing superiorly. A more posterior cut will allow for better access to the thoracic vagus dissection.
 - 51.3 Mark the level of the superior margin of the clavicle on the vagus nerve by placing a colored pin in soft tissue adjacent to the vagus nerve at that level. The pin must be securely placed so it does not get dislodged during the remainder of the dissection.
 - 51.4 Mark the level of the superior margin of the midpoint of the jugular notch by placing a colored pin at that level in the soft tissue adjacent to the vagus nerve. The pin must be securely placed so it does not get dislodged during the remainder of the dissection.
 - 51.5 Mobilize the clavicles. Cut the clavicle as laterally as possible, ideally where it articulates with the acromion process. Grasp the lateral cut end of the clavicle and lift in a medial direction. Detach the subclavius muscle. Clean the inferior and posterior surfaces of the clavicle while protecting the vagus nerve. Transect the subclavian vein distally to visualize the vagus nerve entering the thoracic cavity.
 - 51.6 Free the chest wall by using a scalpel or scissors to cut the attachments between the posterior surface of the ribs and the anterior surface of the lungs/heart.



- 51.7 Remove the anterior chest wall. Optional: Wrap the chest wall in plastic wrap to avoid drying and shrinking.
- 51.8 Estimate the location of the sternal angle by placing a colored pin in the heart at that location. A notch can be cut in the anterior chest wall to facilitate accurate placement of the pin in the heart.

Section 8: Mediastinum Dissection

- 52 Identify the thoracic left and right vagus nerves.
 - 52.1 Start from the superior border of the clavicle and follow the vagus nerve as it travels into the thorax.
 - 52.2 Remove fascia and identify branches progressing inferiorly from the level of the clavicle.
 - 52.3 Identify the recurrent laryngeal nerves and other proximal branches of the thoracic vagus nerves.
- 53 Remove the lungs.
 - 53.1 Mobilize the lungs by lifting them away from the wall of the thorax before proceeding.
 - 53.2 Use sharp dissection to remove the lungs, taking care to avoid damaging the vagus nerves. The cuts should remain at least 2-3 cm lateral to the lung hilum to protect the vagus nerves.
- 54 Mobilize the heart.
 - 54.1 Cut along the inferior margin of the pericardium, separating the heart from the diaphragm. Lift the free edge of the pericardium to identify the heart and inferior vena cava.
 - 54.2 Cut the inferior vena cava and reflect the heart superiorly. With the heart reflected, continue careful, blunt dissection of the posterior pericardial wall and remove excess tissue to visualize the esophagus and esophageal plexus.
- 55 Identify the esophageal plexus of the vagus nerve.



- 55.1 If accessible, remove the liver in segments as needed to facilitate visualization of the esophageal plexus, taking care to preserve hepatic and cystic branches of the vagal trunks. If needed (if the liver is obstructing access to the esophageal plexus), jump to Section 9, Step 57, then return here.
- 55.2 Identify any branches from the esophageal plexus to the heart, lungs, and esophagus.
- 56 Identify the anterior and posterior vagal trunks emerging from the esophageal plexus.

Section 9: Abdominal Dissection

- 57 Open the abdomen.
 - 57.1 Make a midline incision from the xiphoid process to the umbilicus and reflect skin flaps laterally. Take care not to cut the underlying viscera when making this incision.
 - 57.2 Incise the peritoneum using sharp dissection, taking care to avoid damaging abdominal viscera.
 - 57.3 Remove the greater omentum to facilitate visualization of abdominal viscera.
- 58 Place colored pins in the lateral walls of the esophagus at the location of the superior margin of the esophageal hiatus.

Note

Note: Pins might need to be adjusted as dissection progresses. Care should be taken when adjusting pins to ensure that the location of the esophageal hiatus is preserved.
- 59 Make an incision along the midline of the diaphragm and reflect laterally.
- 60 Dissect the anterior abdominal vagal trunk through the esophageal hiatus and identify its branches (e.g., celiac, cystic, gastric, hepatic, intestinal, pancreatic, pyloric).



- 60.1 To find cystic branches, trace hepatic branches distally toward the gallbladder.
- 61 Many abdominal branches form a plexus around the celiac trunk. Separate the nerves from the arteries and then remove the arterial branches of the celiac trunk as much as possible (i.e., cut the arterial branches and remove them from the body).
- 62 Dissect the posterior abdominal vagal trunk and identify its branches (e.g., celiac, cystic, gastric, hepatic, intestinal, pancreatic, pyloric).
- 63 Follow all branches as far distally into the abdominal organs as possible.
- 63.1 Remove small visceral branches as needed to facilitate clear identification of major vagal branches (i.e., cut the small visceral branches and remove them from the body).

Section 10: Measurements and Painting

- 64 Anatomical landmarks and branches of the vagus nerve are labeled using paint after dissection is complete.
- 64.1 Landmarks are painted and allowed to dry using the handheld fan, then sprayed with mordant before painting branches.
- 64.2 Paint colors are selected according to the branch pattern spreadsheet (Appendix 4).
- 65 Document the location around the circumference of the vagus nerve where each branch originates, its trajectory, and its target.
- 66 During the dissection, the diameter of the vagus nerve and its branches at specified anatomical landmarks (Appendix 4) are measured using calipers.
- 67 Record the diameter of the vagus nerve at anatomical landmarks (in millimeters) using calipers:
- 67.1 Reorder the anatomical landmark columns on the Nerve Measurements spreadsheet to reflect the landmarks in order from superior to inferior.



- 67.2 Record the width of the nerve in the ventral-dorsal (sagittal plane) axis, the right-left (coronal plane) axis.
- 67.3 Only record the diameter in the planes that are accessible without damaging the vagus nerve and its branches.
- 67.4 If the diameter cannot be measured in a specific plane, then any diameter can be measured and considered a "non-plane specific diameter".
- 68 Measure the distance between anatomical landmarks to the vagus nerve using calipers.

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