Simple and reproducible approaches for the collection of select porcine ganglia

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Abstract

Background—The anatomy and physiology of the pig nervous system is more similar to humans compared to traditional rodent models. This makes the pig an attractive model to answer questions relating to human health and disease. Yet the technical and molecular tools available to pig researchers are limited compared to rodent researchers

New Method—We developed simple and rapid methods to isolate the trigeminal, nodose (distal vagal), and dorsal root ganglia from neonatal pigs. We selected these ganglia due to their broad applicability to basic science researchers and clinicians

Results—Use of these methods resulted in reproducible isolation of all three types of ganglia as validated by histological examination.

Comparison with Existing Method(s)—There are currently no methods that describe a step-by-step protocol to isolate these porcine ganglia.

Conclusions—In conclusion, these methods for ganglia collection will facilitate and accelerate future neuroscience investigations in pig models of human disease.

Keywords

porcine; trigeminal; vagal; dorsal root ganglia; nodose

INTRODUCTION

Large animal models are increasingly utilized in biomedical research as a useful and alternative model for the study of human diseases[1–3]. Large animals models offer an

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advantage in that the anatomy and physiology of many organ systems more closely parallel human organs compared to traditional rodent models[4–8]. For example, the growth and development of the porcine brain is more similar to humans when compared to rodents, carnivores, and other ungulates[8]. Yet rodent animals dominate many areas of research, including neuroscience[9]. This is due in part to the greater number of resources and tools available to rodent researchers. Therefore, developing and making available tools and resources for large animal models[10] might expand research programs utilizing large animal models and accelerate new research discoveries. This might be particularly relevant given the recent surge in neuroscience funding initiatives[11].

Here, we describe user-friendly methods for dissection and collection of the trigeminal, nodose (distal vagal), and dorsal root ganglia. These major ganglia provide sensory feedback from multiple organ systems and are involved in autonomic control. Their association with nociception and pain[12], which is a common manifestation of various neurological diseases[13], is also of broad interest. Following the methods herein allows for rapid and reproducible isolation of ganglia that may be used for numerous downstream processes, such as histology, RNA isolation, and cell culturing.

METHODS

Animals: Newborn pigs (~1 week of age, n=2, male, n = 2 female) were used for this study. Pigs were sedated and anesthetized by masked inhalation of 8% Sevothesia (Henry Schein Animal Health) and euthanized by intravenous Euthasol (Virbac). Tissues (n=8 ganglia, right and left ganglia from each pig) were collected following protocols that were developed from combined experiences (LR and DM) to result in simple and repeatable approach for each ganglia (trigeminal, nodose, dorsal root) in pigs of both sexes. The University of Florida Animal Care and Use Committee approved all procedures.

In young pigs, basic dissecting tools that are required include a scalpel, thumb forceps, mayo scissors (or chicken shears) and occasionally a bone rongeur. In older animals with additional calcification of bones, additional tools (e.g. oscillating saw, hand saws, etc.) may be necessary.

Histology: After collection, tissues were fixed in 10% neutral buffered formalin (~7–10 days) then routinely processed, paraffin-embedded, sectioned (~4 μm) and stained with Masson's trichrome stain. Tissues were examined by a veterinary pathologist (DKM) for confirmation of ganglia and digital images were collected with specialized equipment (BX51 microscope and DP73 digital camera, Olympus) and software (CellSens Pathology Edition, Olympus).

RESULTS

Nodose Ganglion

The pig is placed in dorsal recumbency. A midline incision is made at the neck and the skin is reflected back to form a window viewing the larynx and soft tissues surrounding the trachea (Figure 1A, B right side). The soft tissues (muscles, adipose tissue) ventral and

lateral to the trachea are removed so as to see the neurovascular tracts containing nerve, artery and vein (Figure 1B). From this point, the nerve is carefully dissected and followed cranially (1–3+ cm) to find the bulge associated with the nerve – which is the nodose ganglion (Figure 1C). This approach helps to avoid accidental collection of other ganglia that are in the region such as the cranial cervical ganglion[14]. Moreover, this approach provides a very simple strategy to identify the nodose ganglion (via tracing of the nerve towards the skull); such descriptions are lacking in other resources[15,16].

Trigeminal Ganglion

The cervical spine (near C1) is sectioned so as to disarticulate and decapitate the head from the spine (Figure 2A). The skin over the head is removed. The skull is cut in the horizontal plane so as to remove the calvarium and the brain. The bone tissue overlying each globe and optic nerve is cut as shown in Figure 2A (dashed lines) and detached to expose the eyes and orbital sockets (Figure 2B). The globe, optic nerve and adjacent soft tissues are removed to reveal the trigeminal nerve (Figure 2C, arrows). A larger window is cut through the ventral temporal bone to further uncover the trigeminal nerve (Figure 2D, blue arrows). The trigeminal nerve is traced caudally towards its exit point from the skull, where the trigeminal ganglia are located (Figure 2D, white arrow). Cutting the nerve at the exit point frees it from the base of the skull and allows for easy manipulation and removal, while keeping the ganglia intact (Figure 2E, white dashed circle). The intact ganglia appear as bulbous tissue (Figure 2F, white arrows) adjacent to the trigeminal nerve near its origin. Collection in this manner allows for functional and morphological characterization of the trigeminal ganglia[17].

Dorsal Root Ganglia (DRG)

For the purposes of this methods paper, we will focus on the cervical DRG, but DRG collection in the thoracic or lumbar regions can be similarly made using slight modifications in approach. For cervical DRG, the cervical spine (near C1) is sectioned as done for the trigeminal collection (see above) and the spine is also sectioned in the thoracic spine. The epaxial muscles and ribs are removed for easier handling (Figure 3A). After initial sectioning, the cervical spine can be examined at the sectioned interface for the spinal nerves (Figure 3A, arrow) that can be often seen exiting the vertebral canal. Dissection in this region can further expose the spinal nerves (Figure 3B, small arrows) traversing the neuroforamina into the tissues and DRGs (Figure 3B–C, large arrows) are seen as prominent bulges along the spinal nerves. This technique is useful for all applications, but is optimal for collection of intact DRG to be used for ganglia morphology and morphometric applications.

DRGs are also collected for molecular studies (e.g. RNA transcription) and another modification to the technique can provide larger quantity of DRG for multiple molecular analyses. In this approach, the spine is prepared as in Figure 3A. Then using rongeurs or scissors, the vertebral lamina are bilaterally sectioned over a defined region (e.g. cervical spine) to remove the posterior covering of the spinal canal. Here, the spinal cord and spinal nerve roots can be clearly seen (Figure 3D). The spinal nerves are cut with scissors near the spinal cord and the spinal cord is removed leaving visible the remnant spinal nerves in their respective neuroforamina (Figure 3D, arrows). Forceps can be used to reach into the neuroforamina and remove the spinal nerve and DRG (Figure 3E). The tissues can

sometimes acquire crush artifacts during this process that compromise the DRG morphology, so this technique may be best suited for molecular studies. Histological examination is useful to confirm proper collection of the ganglia, especially when the techniques are first being learned. Also, histological images of ganglia can be useful for data publication to further corroborate the proper collection of ganglia for molecular studies. The respective ganglia in this study were readily identified histologically by aggregates of neurons adjacent to the respective nerves (Figure 4 A–D) and histological evidence of ganglia were found in all samples (100%).

DISCUSSION

Pigs are studied as models of human disease because of their similar anatomy, physiology, metabolism, and size[5,18]. In recent years, their use in translational research has significantly expanded due to the development of genetically modified and experimentally-induced pig models of human disease. These include cystic fibrosis[19–21], muscular dystrophy[22], diabetes[23], cardiovascular disease[24,2526], diabetes[27], cancer[28,29], environmental toxicology[30], cutaneous wound healing[31], metabolic syndrome[32], among others. Porcine models have also been used to investigate traumatic brain injury[3], neurodegenerative diseases[33,34], brain development[35], seizures[36], cognition[37], and neurogenesis[38].

In the current study, we demonstrated practical methods for the collection of three key ganglia (the trigeminal, nodose, and dorsal root) from juvenile pigs. The trigeminal ganglia provide sensory innervation to the face, oral and nasal cavities and dura mater of the CNS[39]. These neurons relay mechanosensitive, thermosensitive, and nociceptive information to the brainstem and upper spinal cord. Thus, the trigeminal ganglia are often implicated in pain associated with toothaches, headaches, migraines, rhinosinusitis, temporomandibular joint (TMJ) disorder and trigeminal neuralgia. The vagal ganglia, consisting of the jugular and nodose ganglion, send projections to multiple visceral organs, including the respiratory[40], gastrointestinal[41], and cardiovascular[42] systems. The vagal ganglia have been implicated in several diseases, including airway hyperreactivity[43,44], chronic pulmonary disease[45], inflammatory bowel disease[46], esophageal motility disorders[47], and obesity[48]. The DRG are a collection of sensory neurons that convey information from the periphery to the CNS[49]. They have been associated with neuropathic pain following nerve injury[50]. Thus, the trigeminal, nodose, and dorsal root ganglia are of broad interest to basic scientists and clinicians.

The bilateral nature of ganglia can be a strategic advantage for investigators. First, both ganglia can be collected as samples of treatment effects on each animal, to help mitigate intragroup variances and outliers. Second, investigators can utilize each ganglia for different approaches such as one for those requiring intact morphology (histopathology, immunohistochemistry, immunofluorescence, *in situ* hybridization, etc.) and the other ganglia for molecular techniques where the morphology is not essential (e.g. proteomics, RNA, and DNA analyses). This approach of parallel usage of ganglia from the same animal/ experiment can increase the redundancy and rigor of data for a given project[51].

While pigs are increasingly studied as preclinical models of disease, compared to rodent species, there is a paucity of published resources, expertise, reagents and other tools available. The implementation of the National Swine Resource and Research Center[10] and the increased recognition of the importance of porcine models is helping to close this gap. As more investigators embrace large animal models, this technical resource, along with additional emerging resources, will continue to enhance the research landscape.

Acknowledgments

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Highlights

- Reproducible and rapid techniques for isolating three common porcine ganglia

- Image-guided methods to accommodate novice to experienced audiences

- Methods emphasize preservation of ganglia structure for morphometric examination

- Dual approaches for DRG collection depending on anticipated downstream uses
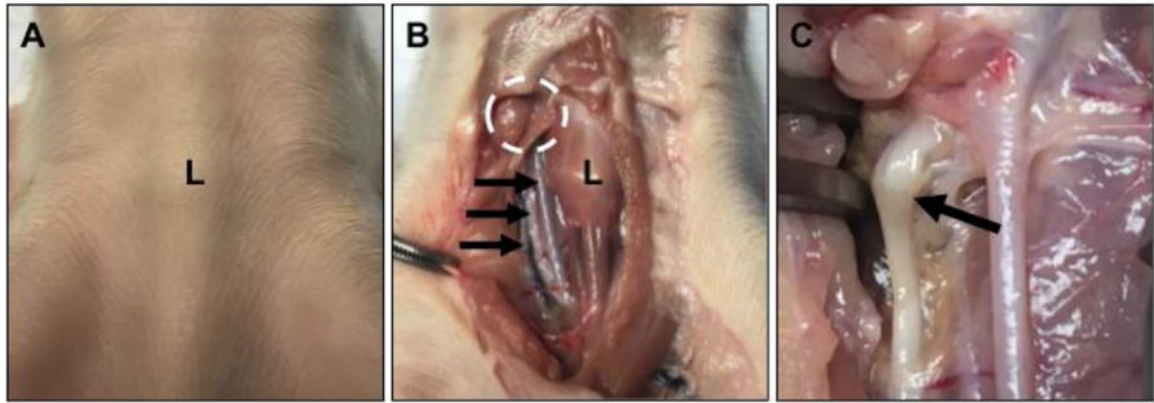
Figure 1.
Cervical neck, ventral midline view with the cranial aspect being in the upper portion of image. A) The larynx (L) can be seen as a landmark in the newborn pig. B) The overlying skin is removed to form a window over the larynx (L) and adjacent soft tissues (see example of soft tissues on right side of larynx in image). These soft tissues are carefully dissected away from the larynx/trachea (see dissected example on left side of larynx in image) to reveal the nearby tracts of artery (top arrow), vagus nerve (middle arrow) and vein (bottom arrow). Once the vagus nerve is identified, it is traced cranially toward the expected locale of the nodose ganglion (dashed circle). C) The nodose ganglion (arrow) is morphologically defined as a "bulge" along the vagus nerve.
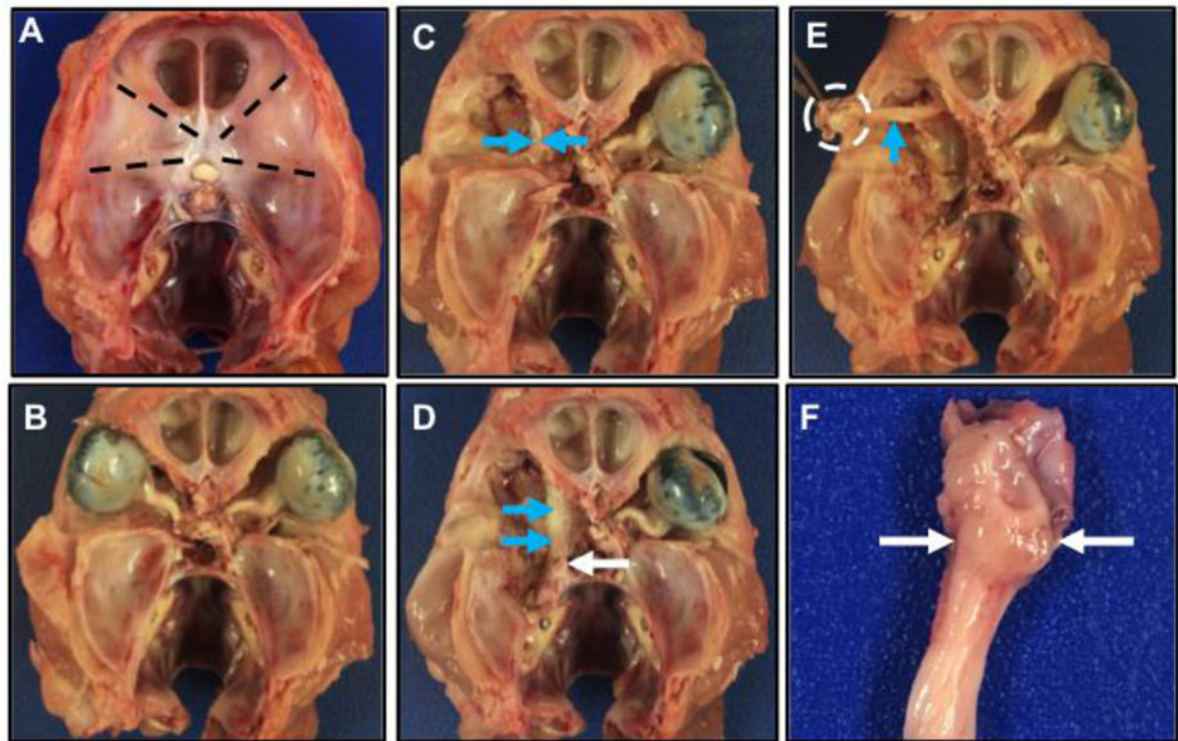
Figure 2.
Cranial view of the skull with the calvarium and brain removed. A, B) The bones overlying the eyes (bulbus oculi) are removed in a roughly triangular pattern (A, dashed lines) to expose the eyes and the optic nerve (B). C) The eye, optic nerve and soft tissues are carefully removed and the large trigeminal nerve (blue arrows) can start to be seen tracking along the medial aspects. D) The trigeminal nerve (blue arrows) is carefully dissected caudally towards its origin (white arrow) near its attachment to the brain. E) The trigeminal nerve (blue arrow) can be dissected free from the adjacent tissue and ganglia will generally be located near its origin (dashed circle). F) The trigeminal ganglia (arrows, slight bulge that is less white than adjacent nerve) and nerve near its origin (top of image).
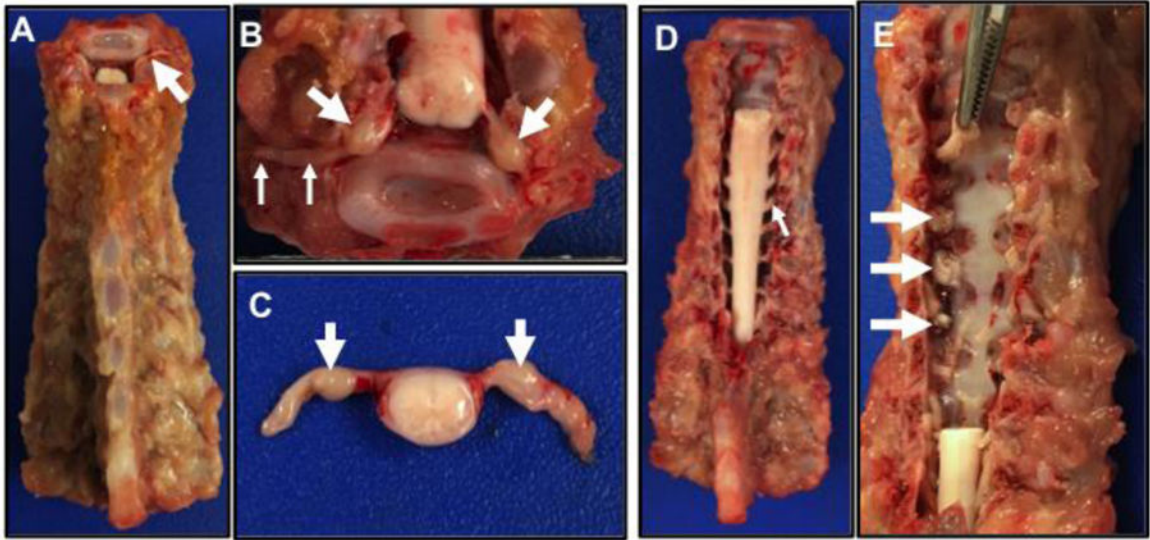
Figure 3.
Cervical to thoracic spine, dorsal view. A) The spine is sectioned from the head (at top of image) with the epaxial muscles and ribs removed. Note the small spinal nerve root (arrow) from the sectioning of the cervical spine. B) Cervical section through the spine (see top of A) that has been further dissected to reveal the dorsal root ganglia (large arrows) and spinal nerves (small arrows) extending from the spinal cord. C. Section of spinal cord and nerves with dorsal root ganglia (arrows). D) Dorsal view of cervical spine following laminectomy to reveal the spinal cord and nerve roots (arrow). E) The cervical spinal cord (see D) with spinal nerves was removed. Remnant spinal nerves can be seen exiting into the neuroforamina (arrows). Forceps (top of image) can be inserted into the neuroformina to grasp and remove the spinal nerve with dorsal root ganglia.
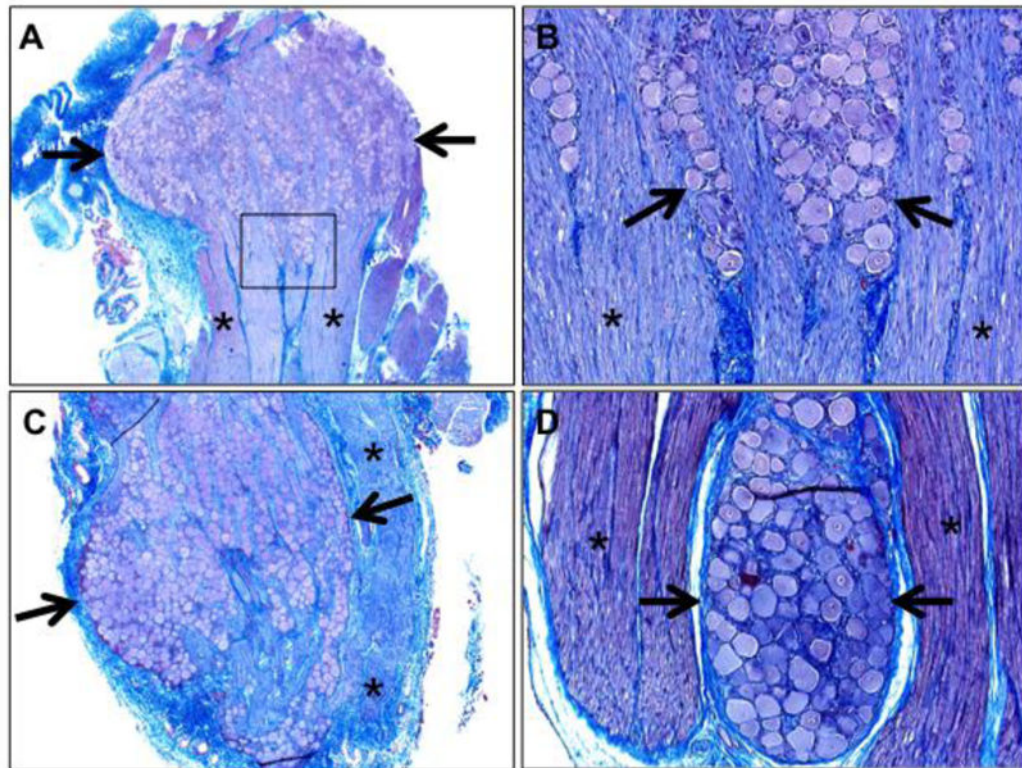
Figure 4.
Histological confirmation of ganglia collection. A) Trigeminal ganglia, 20×. B) Trigeminal ganglia (box in figure 4A), 100×. C) Nodose ganglia, 40×. D) Dorsal root ganglia, 100×. Note the ganglia that are observed by aggregates of neurons (A-D, arrows) and are adjacent to nerves (A-D, highlighted by asterisks), Masson's trichrome stains.