Video Article

Dissection of Pelvic Autonomic Ganglia and Associated Nerves in Male and Female Rats

Martin M. Bertrand[1,2], Janet R. Keast[1]

[1]Department of Anatomy and Neuroscience, University of Melbourne

[2]Department of Visceral Surgery, CHU de Nîmes

Correspondence to: Janet R. Keast at janet.keast@unimelb.edu.au

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Abstract

The bilateral major pelvic ganglia (MPG; synonym, pelvic ganglia) are the primary source of postganglionic sympathetic and parasympathetic neurons innervating pelvic organs of rodents; the functionally equivalent structure in humans is the inferior hypogastric plexus. The major pelvic ganglia also provide the route by which lumbar and sacral sensory axons reach the pelvic organs. These complex, mixed ganglia can prove challenging to identify and dissect for further experimental study of normal autonomic mechanisms or to establish preclinical models of disease, injury or visceral pain. Here we describe a protocol to access and visualize these ganglia and their associated nerve tracts. We provide this protocol with schematics for both male and female rats, as the ganglion size and landmarks for identification differ between sexes. The protocol describes removal of the ganglion for in vitro studies, but this method can be integrated into a surgical recovery protocol for experimental interventions (e.g., nerve crush, nerve resection) or for mapping neuronal circuits (e.g., by microinjection of neural tracers). We also demonstrate the primary structures of the ganglion and its associated nerves immediately following dissection and following immunohistochemical staining.

Video Link

The video component of this article can be found at https://www.jove.com/video/60904/

Introduction

The rat is the one of the best characterized species used in the study of the pelvic organ physiology and anatomy. While excellent resources exist for descriptions of these organs[1,2], they generally do not provide information on the related neural structures or do so at insufficient resolution to guide an experimental study. As detailed further below, the organization of the autonomic ganglia that regulate pelvic organ function is quite different to the rest of the autonomic nervous system, making it difficult to accurately infer pelvic innervation features from neuroanatomical information available for other autonomic ganglia. This deficiency in resources to guide researchers entering this area may have slowed research on neural regulation of pelvic organs. Here we describe protocols for accessing this region of the nervous system for further in vitro studies or experimental intervention.

The bilateral major pelvic ganglia (MPG; synonyms: pelvic ganglia; paracervical ganglia [female]; Frankenhäuser's ganglion [female]) are the primary source of postganglionic sympathetic and parasympathetic neurons innervating pelvic organs of rodents; the inferior hypogastric plexus comprises the equivalent neuronal structure in humans[3,4,5,6]. Sensory projections from lumbar and sacral dorsal root ganglia also travel via the MPG to reach the pelvic organs. Therefore, understanding the neural circuitry and biology of the MPG is critical for preclinical studies on a myriad of clinical conditions relating to the development and adult function of pelvic organs. Several excellent descriptions of rodent MPG have been published[7,8], but our experience is that in general these descriptions do not always provide sufficient guidance to practically inform an experimental dissection or manipulation of these structures when recovery of the animal is required. Moreover, the majority of MPG studies focus on male rats. In female rats, the MPG are smaller[9] and have distinct anatomical landmarks, and therefore require a distinctly tailored guide to visualization and dissection.

Sympathetic and parasympathetic pathways are distinguished by their anatomy, specifically the location of their preganglionic neurons, with sympathetic pathways having preganglionic neurons in the thoraco-lumbar spinal cord and the parasympathetic preganglionic neurons located in the brainstem (cranial nerve projections) and sacral spinal cord. In most other regions of the autonomic system, their target ganglion neurons are located in distinct sympathetic or parasympathetic ganglia. However, the MPG are unusual in being mixed sympathetic-parasympathetic ganglia, and therefore at a macroscopic scale are sites of convergence from preganglionic axons of both thoraco-lumbar and sacral spinal regions. We have therefore included in our protocols the location and description of these primary nerve tracts that connect each spinal region with the MPG, facilitating experimental analysis or separate manipulation of these neural components. We also note for readers specifically comparing these ganglia across species, that in rodents the spinal preganglionic neurons that are 'functionally sacral', e.g., are active and required during micturition, defecation and penile erection, are located at spinal levels L6-S1 rather than exclusively in sacral segments[10]; likewise L6 and S1

dorsal root ganglia provide the major 'sacral' sensory input to pelvic organs. In rodents, sensory and preganglionic input from more rostral neural circuits is concentrated in spinal levels L1 and L2[10].

Here we describe a protocol to access the MPG and their associated nerve tracts in male and female rats, and support this with schematics to illustrate specific landmarks. This protocol guides surgical access to these structures in an experimental context of removing the tissue for in vitro studies, e.g., isolating MPG neurons for molecular characterization or primary culture. It can also be adapted to MPG removal after intracardiac perfusion with fixative, although this is a more difficult dissection because the neural tissue becomes more difficult to visualize when the adjacent tissues are devoid of blood. This protocol can also be integrated into a surgical setting for experimental intervention of these nerve pathways (e.g., nerve resection, microinjection of neural tracers). These types of dissections are increasingly important for the growing field of bioelectronic medicine, where new targets and approaches for neuromodulation to treat clinical conditions of the pelvic viscera are being developed[11]. We present the complete protocol first for male rats, then a replicate of the protocol tailored specifically for female rats.

Protocol

All procedures are to be conducted according the institutional and funding body requirements for animal experimentation. The use of animals for this dissection and the protocol for euthanasia have been approved by the Animal Ethics Committee at the University of Melbourne (Protocol number 1814639).

NOTE: The dissections illustrated here were performed on adult (~10 weeks) male and female Sprague-Dawley rats (Biomedical Sciences Animal Facility, University of Melbourne), weighing 280 g (female) and 350 g (male). Prior to these dissections, the rats were euthanized in a CO_2 chamber for 4−5 min. Immediately following death, MPG were dissected. If dissecting tissue from an animal that has undergone transcardial perfusion with fixative, take precautions to protect the operator from exposure to fixative, i.e., perform dissection in fume cupboard or downdraft cabinet and wear suitable personal protective equipment. A protocol for transcardial perfusion has been published in detail[12].

1. Major pelvic ganglion and adjacent nerves: access and resection in a male rat

NOTE: **Figure 1** shows anatomical landmarks for MPG visualization in a male rat.

1. Access to the abdominal cavity and pelvis
    1. Place the rat in a supine position and access the abdomen and pelvis through a ventral midline incision, taking care to avoid contamination of the surgical field with fur.
    2. Gently move the abdominal organs to one side using forceps or cotton-tipped applicators. Note the location of the ventral lobes of the prostate gland and the urinary bladder.
    3. Move the seminal vesicle to the contralateral side.
    4. Cut the vas deferens to provide better access to the area overlying the ganglion.
    NOTE: From this point of the dissection, the tissue must not dry out; keep the tissue moist with physiological saline (for fresh tissue dissection) or fixative (for perfusion-fixed animal). Keeping the tissue moist with saline not only benefits tissue structure but also makes the dissection easier as dry nerves are more fragile and tear more easily during handling.
    5. Identify the dorsolateral lobe of the prostate gland, on the dorsal surface of which is the location of the ganglion; this will not yet be visible.
    6. To visualize the ganglion, carefully clear away tissues near and overlying the ganglion. If necessary, use a retractor to keep the dissection field clear.
    7. Remove a nearby aggregate of adipose tissue and open the lateral fascia of the pelvis.

2. Dissection of the MPG and its associated nerves
    1. Identify the following locations that provide landmarks for the next steps of the dissection: the dorsolateral lobe of the prostate gland (the ganglion is located on the surface of this lobe, slightly more caudal than the junction between seminal vesicle and prostate) and the seminal vesicles (where they converge at the midline indicates the ganglion location on the animal's rostrocaudal axis).
    2. As required from this point, carefully remove any tissue that impedes complete view of the neural structures, avoiding damage to the thin capsule of the prostate gland or major vessels.
    3. Identify the pelvic nerve by visualizing the following landmarks and features.
        1. Find the internal iliac vein and its fine branch projecting towards the MPG and the bladder. This vascular branch runs parallel to and is sometimes embedded within the pelvic nerve, then traverses the ganglion.
        2. Gently place fine-tipped angled forceps under the pelvic nerve and slide the forceps along to free it from surrounding tissue.
        NOTE: It may also be possible to isolate the pelvic nerve from the small vessel running parallel to it, but for most types of experiments this is not essential. Confirm that the structure is the pelvic nerve by viewing under high magnification to determine that the nerve contains several loosely aggregated fascicles, which are easily distinguished under the dissecting microscope and are characteristic of the pelvic nerve, as none of the other major nerves associated with the ganglion show this clear fasciculation.
    4. Identify the cavernous nerve by visualizing the following landmarks and features.
        1. After following the pelvic nerve to its junction with the ganglion, follow the cavernous nerve as it travels across the prostate and then caudally towards the cavernous bodies of the penis.
        2. If microscope magnification permits, note that there is a small group of delicate nerves emerging from the ganglion between the pelvic and cavernous nerves; these are the rectal nerves that travel to the lower bowel.
    5. Identify the hypogastric nerve by visualizing the following landmarks and features.
        1. Identify where the hypogastric nerve joins the ganglion at its cranial edge, after travelling alongside the ureter.

2. Confirm that the hypogastric nerve is much thinner than either the pelvic or cavernous nerves and is not accompanied by large vessels.

6. Identify the MPG by visualizing the following features.
    1. Visualize the ventral, dorsal and cranial edges of the ganglion, forming a triangular shape.
    2. Confirm the location of each major nerve: the pelvic nerves emerging from the ganglion's dorsal edge, the cavernous nerve at the most caudal corner of the ganglion, the hypogastric nerve from its cranial edge, and the accessory nerves emerging from the ganglion's ventral edge.

7. Identify the accessory nerves by visualizing the following landmarks and features.
    1. After clearing tissue to enable visualization of the ventral edge of the ganglion, identify a cluster of nerves that project towards the urinary and reproductive tracts.
    2. If microscope magnification permits, identify one caudal group of nerves that enter between the prostate lobes and one rostral group between the seminal vesicle and the bladder.

3. Removal of the MPG with its associated nerves
    1. Gently slide forceps between the ganglion and the underlying prostate gland, being careful not to puncture the thin capsule of the prostate. Disrupt any connections between the ganglion and the prostate.
    2. Clear any final connections with surrounding tissues for the lengths of nerves required for the experiment, then cut each nerve.
    3. Using fine forceps, move the ganglion with its nerves to the appropriate solution for the experiment and confirm that each of the main nerves are intact.

2. Major pelvic ganglion and adjacent nerves: access and resection in a female rat

NOTE: **Figure 2** shows anatomical landmarks for MPG visualization in a female rat.

1. Access to the abdominal cavity and pelvis
    1. Place the rat in a supine position and access the abdomen and pelvis through a ventral midline incision, taking care to avoid contamination of the surgical field with fur.
    NOTE: From this point of the dissection, the tissue must not dry out; keep the tissue moist with physiological saline (for fresh tissue dissection) or fixative (for perfusion-fixed animal).
    2. Gently move the abdominal organs to one side using forceps or cotton-tipped applicators. Note the location of the uterine horn, urinary bladder and rectum.
    3. Cut the ovarian and uterine vessels and retract the uterine horn.
    4. Enter the peritoneal space and gently clear away an aggregate of adipose tissue located near the uterine cervix.

2. Dissection of the MPG and its associated nerves
    1. Identify the lateral wall of the uterine cervix, just caudal to its junction with the uterine horns; this region is the primary landmark for defining the MPG location on the animal's rostrocaudal axis.
    2. As required from this point, carefully remove any tissue that impedes complete view of the neural structures, avoiding damage to major vessels.
    3. Identify the pelvic nerve by visualizing the following landmarks and features.
        1. Find the internal iliac vein and its fine branch projecting towards the MPG and the bladder. This branch runs parallel to and is sometimes embedded within the pelvic nerve, then traverses the ganglion.
        2. Confirm that the structure is the pelvic nerve by viewing under high magnification to determine that the nerve contains several loosely aggregated fascicles, which are easily distinguished under the dissecting microscope and are characteristic of the pelvic nerve, as none of the other major nerves associated with the ganglion show this clear fasciculation.

    4. Identify the hypogastric nerve by visualizing the following landmarks and features.
        1. Identify where the hypogastric nerve joins the ganglion at its cranial edge, after travelling alongside the ureter.
        2. Confirm that the hypogastric nerve is much thinner than either the pelvic or cavernous nerves and is not accompanied by large vessels.

    5. Identify the cavernous nerve by visualizing the following landmarks and features.
        1. After following the pelvic nerve to its junction with the ganglion, follow the cavernous nerve as it travels caudally along the lateral wall of the cervix towards the vagina.
        2. If microscope magnification permits, note that there is a small group of delicate nerves emerging from the ganglion between the pelvic and cavernous nerves; these are the rectal nerves that travel to the lower bowel.

    6. Identify the accessory nerves by visualizing the following landmarks and features.
    NOTE: The accessory nerves are difficult to see but project from the medial aspect of the MPG. After clearing tissue to enable visualization of the ventral edge of the ganglion, identify a cluster of very delicate nerves that project towards the urinary and reproductive tracts.
    7. Identify the MPG by visualizing the following features.
        1. Visualize the ventral, dorsal, and cranial edges of the ganglion, which form a triangular shape.

2. Confirm the location of each major nerve: the pelvic nerves emerging from the ganglion's dorsal edge, the cavernous nerve at the most caudal corner of the ganglion, the hypogastric nerve from its cranial edge, and the accessory nerves emerging from the ganglion's ventral edge.

3. Removal of the MPG with its associated nerves
    1. Gently place fine-tipped angled forceps under the pelvic nerve and slide the forceps along to free it from the underlying uterine cervix and surrounding tissue.
    NOTE: It may also be possible to isolate the pelvic nerve from the small vessel running parallel to it, but for most types of experiments this is not essential. If demonstrating the dissection, place a suture under the pelvic nerve, to facilitate its visualization.
    2. Repeat the process for the cavernous nerve, then the hypogastric nerve, and finally the accessory nerves.
    3. Gently slide forceps between the ganglion and the underlying uterine cervix. Disrupt any connections between the ganglion and the cervix.
    4. Clear any final connections with surrounding tissues for the lengths of nerves required for the experiment, then cut each nerve.
    5. Using fine forceps, move the ganglion with its nerves to the appropriate solution for the experiment and confirm that each of the main nerves are intact.

3. Confirmation of ganglion components (optional)

1. After removal of the ganglion, immerse ganglion in a conventional histological fixative (e.g., 4% buffered formalin) for a minimum of 1 h, wash out fixative with 0.1 M phosphate buffer and process tissue for cryosectioning and fluorescence immunohistochemistry, as previously described[13].
    NOTE: Many high-quality antibodies that specifically recognize these three neural markers are commercially available. See **Table of Materials** for the reagents used for the labeling shown in **Figure 3**.
2. Alternatively, process ganglia intact (wholemounts) for immunohistochemistry using a similar method as above but increasing the incubation times for the antibodies to 4 days (primary antibody) and 2 days (secondary antibody).
3. To demonstrate a major population of sensory axons, use antibodies against calcitonin gene-related peptide (CGRP).
    NOTE: The recommended dilution of the antibody used in this study is 1:5,000.
4. To demonstrate noradrenergic sympathetic neurons, use antibodies against tyrosine hydroxylase (TH).
    NOTE: The recommended dilution of the antibody used in this study is 1:5,000.
5. To demonstrate a major population of cholinergic neurons, use antibodies against neuronal nitric oxide synthase (NOS).
    NOTE: The recommended dilution of the antibody used in this study is 1:500.

Representative Results

A successful dissection will not only remove the complete body of the MPG intact, but also retain the first segment of each of the major nerves still attached. These nerves are valuable indicators of ganglion orientation in vivo and therefore provide essential information for many types of anatomical studies (e.g., mapping expression patterns or cellular changes after an experimental perturbation). Although preserving the associated nerves can be of less importance for some experiment types (e.g., ganglion dissociation for culture of isolated neurons), the presence of nerves also provides a way of handling the ganglion without touching (and potentially damaging) the neuronal cell bodies.

An unsuccessful dissection will have an incomplete or damaged ganglion, or where the primary nerves are no longer attached. It is also possible that ganglia or nerves are unknowingly damaged during dissection, either because the physical damage is too subtle to detect under the dissecting microscope or because the damage only becomes apparent during certain types of assays. For example, if the ganglion tissue becomes dry during dissection, the tissue may appear normal during later handling, but will show high levels of non-specific fluorescence on the surface.

Examples of dissected MPG are shown in **Figure 3**, which provides examples of the entire MPG visualized as a whole thickness complete ganglion (**Figure 3A**) and an MPG that has been cryosectioned for performing immunofluorescence to demonstrate noradrenergic and cholinergic neurons (**Figure 3B,C**).

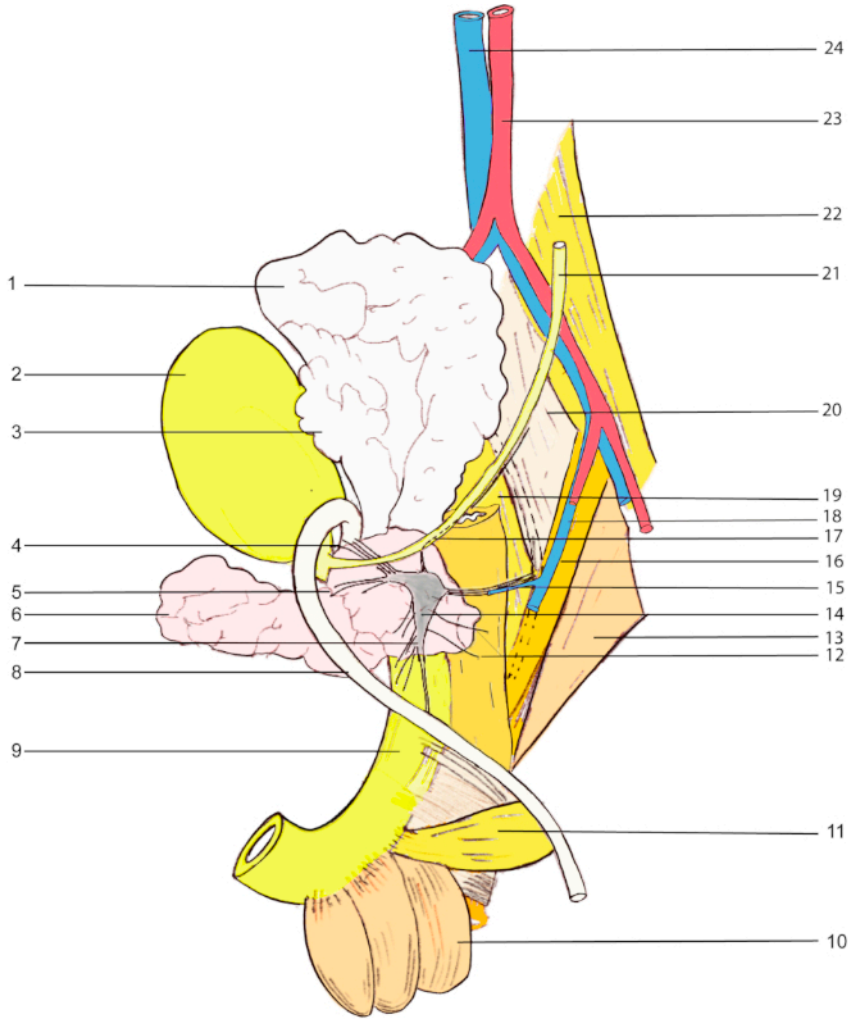

Figure 1: Anatomical landmarks for MPG visualization in a male rat. 1, seminal vesicle; 2, urinary bladder; 3, coagulating gland; 4 & 5, accessory nerves; 6, prostate (ventral lobe); 7, cavernous nerve; 8, vas deferens; 9, urethra; 10, bulbocavernosus muscle; 11, ischiocavernosus muscle; 12, rectal nerves; 13, abductor caudae externus; 14, major pelvic ganglion; 15, pelvic nerve; 16, abductor caudae internus; 17, hypogastric nerve; 18, internal iliac vein; 19, flexor caudae brevis; 20, flexor caudae longus; 21, ureter; 22, psoas major; 23, abdominal aorta; 24, inferior vena cava. Please click here to view a larger version of this figure.

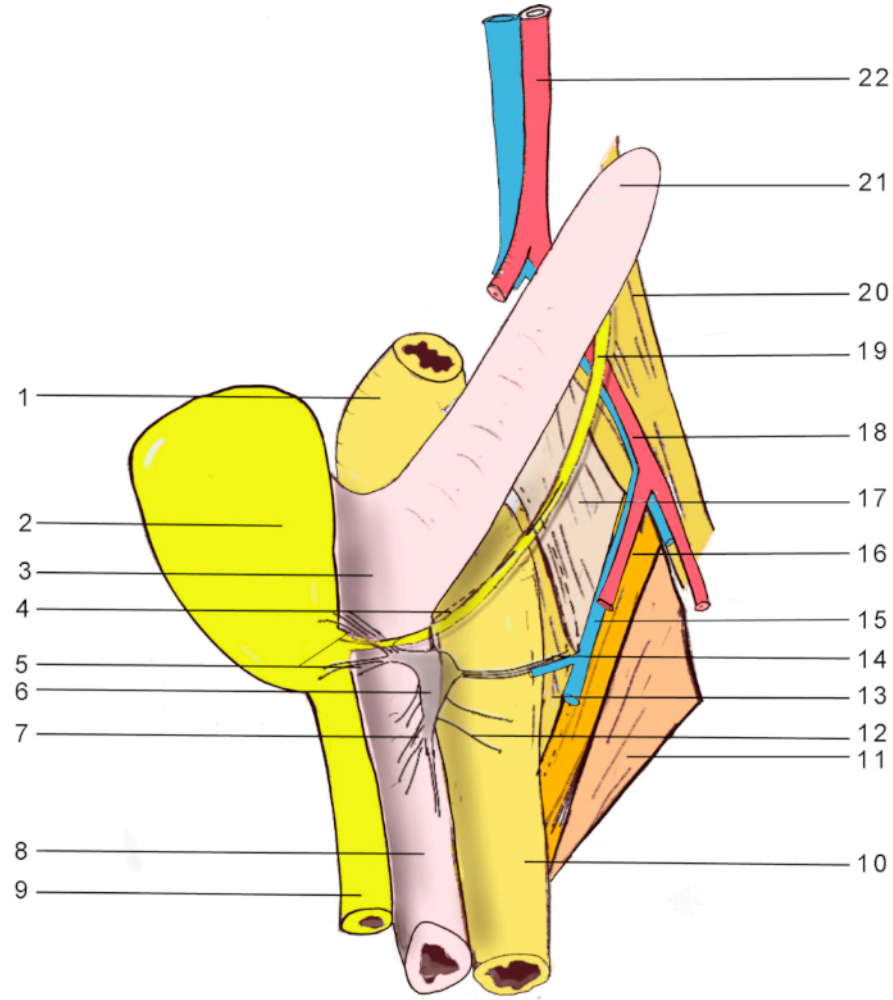

Figure 2: Anatomical landmarks for MPG visualization in a female rat. 1, distal colon; 2, urinary bladder; 3, uterine body; 4, hypogastric nerve; 5, accessory nerves; 6, major pelvic ganglion; 7, cavernous nerve; 8, vagina; 9, urethra; 10, rectum; 11, abductor caudae externus; 12, rectal nerves; 13, flexor caudae brevis; 14, pelvic nerve; 15, internal iliac vein; 16, abductor caudae internus; 17, flexor caudae longus; 18, external iliac artery; 19, ureter; 20, psoas major; 21, uterine horn; 22, abdominal aorta. Please click here to view a larger version of this figure.

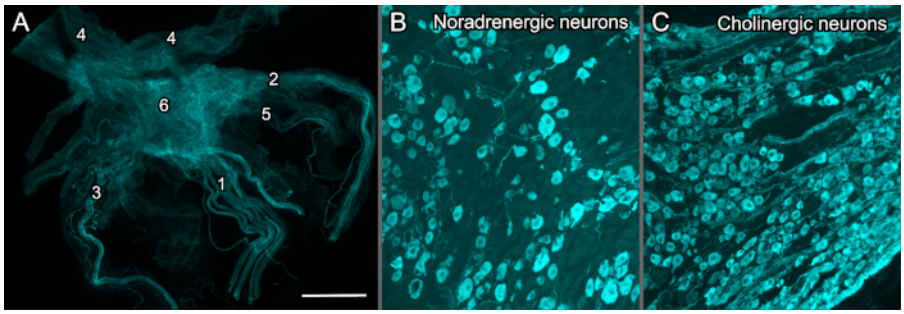

Figure 3: Immunohistochemically labelled MPG from adult male rats. All preparations have been visualized with a conventional widefield fluorescence microscope equipped with a monochrome camera, then digitally colorized. (**A**) Wholemount (complete thickness), fixed MPG with associated nerves, immunohistochemically labelled for the sensory nerves that express calcitonin gene-related peptide (CGRP); 1, pelvic nerve (showing the multiple fascicles); 2, cavernous nerve; 3, hypogastric nerve; 4, accessory nerves; 5, rectal nerves; 6, major pelvic ganglion (MPG). (**B,C**) Cryosections (14 µm) of fixed MPG, immunohistochemically labelled to demonstrate the mixed noradrenergic-cholinergic nature of the ganglion; (**B**) noradrenergic neurons demonstrated by antibody for tyrosine hydroxylase and (**C**) a major population of cholinergic neurons by antibody for neuronal nitric oxide synthase. Calibration bar represents (**A**) 1,000 µm, (**B,C**) 200 µm. Please click here to view a larger version of this figure.

Discussion

Neural control of the pelvic organs is mediated by complex pathways including somatic, parasympathetic, sympathetic and visceral sensory components[14,15,16,17]. Most of these pathways originate in or pass through the MPG. The dissection protocols outlined here provide an introduction to MPG anatomy, the related associated nerves and nearby macroscopic anatomical landmarks; the latter are illustrated by anatomical schematics. Other approaches to MPG dissection may also be successful, but we find the one described here to be robust and suitable for a researcher new to this area of the nervous system.

The most critical aspects of the protocol are correct identification of each major nerve and the complete removal of MPG tissue. With careful viewing and handling of the tissues, MPG tissues can be removed for anatomical, molecular and electrophysiological in vitro studies[18,19,20,21,22]. The protocol can also be adapted for in vivo experimental manipulation[23,24,25], noting that in this case, great care must be taken to minimise contact with the primary nerves associated with the ganglion or to damage nearby vasculature. If the experiment requires selective denervation by interruption of one or more nerves, it is recommended to ligate the severed nerve to prevent reinnervation and confounding of analyses. This dissection protocol could also be utilized for the mouse, where there is also an MPG with comparable function[26,27,28].

For neuroanatomical studies, the best preservation of antigens and tissue structure is obtained by dissecting the MPG from an anesthetized animal that has been perfused transcardially with histological fixative appropriate to the experiment[29]; however, identification of the ganglion and nerve structures are more difficult after this process, as the tissue coloration is lost. It is recommended to become proficient in identifying and dissecting the ganglion from nonperfused animals before attempting this dissection after perfusion. Likewise, it is recommended to first become proficient in the dissection in males because for animals of equivalent age and body size, the MPG and its associated nerves are much smaller in females.

To validate that the tissue removed is indeed the MPG, the researcher is first advised to check the location and features of each primary nerve. Many dissectors find the pelvic nerve and cavernous nerve the easiest to identify in situ; the hypogastric and accessory nerves are more delicate and more difficult to distinguish from the surrounding tissue. If these nerves are no longer available because of problems during dissection, or if there is uncertainty regarding their structure, it is recommended that initial MPG dissections are characterized with conventional histology (to confirm presence of neuronal cell bodies[8]) and secondly with immunohistochemistry (to identify that both cholinergic and noradrenergic neurons are present[30,31]) (**Figure 3**). To validate correct identification of the major nerves, the cavernous nerves are readily identified by their high density of neuronal cell bodies in their initial portion close to the MPG; most of these neurons express markers of cholinergic, nitrergic neurons[32,33]. The pelvic, hypogastric and accessory nerves have very few neuronal cell bodies[34].

There are several common pitfalls in performing this dissection. If novice dissectors have problems finding any of the major nerves or the MPG, they are encouraged to return to the steps that describe the key landmarks. It is very common to become so focused on finding the microstructures that one loses track of the macroscopic context. Most commonly, novice dissectors either move too far rostral in their dissection site or remain too 'superficial'-i.e., too close to the ventral opening of the abdomen, rather than examining deeper (i.e., more dorsal) structures. A common problem during dissection is damage to the vasculature during dissection. If bleeding starts, gently hold a cotton-tipped applicator over the source until bleeding stops, then flush the area liberally with saline before recommencing dissection. It is possible the MPG will not be usable for experiments if contaminated with too much blood or if the dissection is delayed too long while waiting for bleeding to stop. Another common dissection error is damage to the capsule of the prostate gland which significantly impairs the MPG visualization and removal. This capsule is a very delicate structure that is easily punctured while removing the fat from the lateral wall of the prostate, even if using only a cotton-tipped applicator. Finally, the main nerves associated with the MPG are easily damaged during the process of identifying each one and then during removal of the MPG. Dissectors are encouraged to develop a routine whereby each nerve is isolated in turn, in a particular order, so that there is less opportunity for confusion during the final steps of ganglion removal.

This dissection did not seek to trace each of the components of the accessory nerves to specific organs, or to identify each of the many microganglia that lie at various points between the pelvic ganglia and the pelvic organs[8]. These are quite difficult to visualize in vivo without using specific stains; however, they can be removed by following each of the nerve tracts towards the organs, and utilizing specific neural stains *post hoc* to determine ganglion location. These microganglia, even though comprising only small fraction of the neuronal population compared to the MPG, may provide specific types of input to the organs to which they are most closely located. We note here a limitation in the field that neither these microganglia nor many of the small nerve tracts exiting the MPG to travel to pelvic organs yet have broadly accepted names. Moreover, a similarly detailed study of microganglia has not yet been conducted in female rats.

In summary, the protocol and schematics provided here provide researchers with tools to study the primary structures providing the autonomic nerve supply to the pelvic organs, as well as the major peripheral conduits of sensory nerves from lumbosacral dorsal root ganglia that travel via the MPG to pelvic organs.

Disclosures

The authors have nothing to disclose.

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