Video Article

Laminotomy for Lumbar Dorsal Root Ganglion Access and Injection in Swine

Mark D. Unger[1], Timothy P. Maus[2], Ross C. Puffer[3], Laura K. Newman[1], Bradford L. Currier[4], Andreas S. Beutler[1]

[1]Departments of Anesthesiology and Oncology, Mayo Clinic, Translational Science Track, Mayo Graduate School

[2]Department of Radiology (Section of Interventional Pain Management), Mayo Clinic

[3]Department of Neurologic Surgery, Mayo Clinic

[4]Department of Orthopedic Surgery, Mayo Clinic

Correspondence to: Timothy P. Maus at maus.timothy@mayo.edu, Andreas S. Beutler at beutler.andreas@mayo.edu

URL: https://www.jove.com/video/56434
DOI: doi:10.3791/56434

Keywords: Neuroscience, Issue 128, Swine, large animal, laminotomy, dorsal root ganglion, convection enhanced delivery, intraganglionic injection

Date Published: 10/10/2017

Citation: Unger, M.D., Maus, T.P., Puffer, R.C., Newman, L.K., Currier, B.L., Beutler, A.S. Laminotomy for Lumbar Dorsal Root Ganglion Access and Injection in Swine. *J. Vis. Exp.* (128), e56434, doi:10.3791/56434 (2017).

Abstract

Dorsal root ganglia (DRG) are anatomically well defined structures that contain all primary sensory neurons below the head. This fact makes DRG attractive targets for injection of novel therapeutics aimed at treating chronic pain. In small animal models, laminectomy has been used to facilitate DRG injection because it involves surgical removal of the vertebral bone surrounding each DRG. We demonstrate a technique for intraganglionic injection of lumbar DRG in a large animal species, namely, swine. Laminotomy is performed to allow direct access to DRG using standard neurosurgical techniques, instruments, and materials. Compared with more extensive bone removal via laminectomy, we implement laminotomy to conserve spinal anatomy while achieving sufficient DRG access. Intraoperative progress of DRG injection is monitored using a non-toxic dye. Following euthanasia on post-operative day 21, the success of injection is determined by histology for intraganglionic distribution of 4',6-diamidino-2-phenylindole (DAPI). We inject a biologically inactive solution to demonstrate the protocol. This method could be applied in future preclinical studies to target therapeutic solutions to DRG. Our methodology should facilitate testing the translatability of intraganglionic small animal paradigms in a large animal species. Additionally, this protocol may serve as a key resource for those planning preclinical studies of DRG injection in swine.

Video Link

The video component of this article can be found at https://www.jove.com/video/56434/

Introduction

Dorsal root ganglia (DRG) are anatomically discrete, neuronal collections located along the vertebral column. Each DRG contains the primary sensory neurons that encode and relay peripheral stimuli to the central nervous system (CNS) from specific body regions. For instance, the pain of osteoarthritis begins when pain receptors located about a joint perceive noxious stimuli. This process is termed nociception. Long-term awareness of noxious stimuli leads to chronic pain [1].

Chronic pain is a frequent subject of preclinical study [2] where one goal is to develop useful methods for targeted delivery of analgesics to DRG, such as intraganglionic injection [3]. However, DRG are difficult to access because they reside within the boney confines of intervertebral foramina [4]. Several groups have successfully overcome this obstacle through the use of spine surgery in rodents [5,6,7,8,9,10].

In the clinic, laminectomy is a common spine operation and refers to surgical removal of the vertebral lamina, thereby unroofing the vertebral canal [11]. Incorporation of surgical techniques to afford direct DRG access has been successful in rodents [5,12], however, translation may be unrealistic considering differences in size of relevant structures and how that influences pharmacokinetics or technical feasibility [13,14]. For example, one study determined the transverse spinal cord diameter at T10 to be 3.0, 7.0, and 8.2 mm for rat, pig, and human, respectively [15]. Thus, large animal models better approximate human dimensions of nervous structures.

In swine, Raore *et al.* used multi-level laminectomy to gain access to the cervical spinal cord for multiple intraspinal injections [16]. The procedure was well tolerated and led to a phase I clinical trial where comparable surgical outcomes were documented [17]. These results encourage continued use of preclinical large animal models as predictors of technical feasibility and safety in humans.

To date, no detailed methodology exists for surgical access and injection of DRG in a large animal species. To narrow this translational gap, we report a protocol for DRG exposure and injection via laminotomy in swine. Standard neurosurgical techniques, instruments, and materials were used and the method was designed to mimic modern surgical practice. We demonstrate intraganglionic injection using an aqueous solution for lumbar DRG and confirm successful delivery via histology after post-operative day 21.

Protocol

All methods described here have been approved by the Institutional Animal Care and Use Committee (IACUC) of Mayo Clinic.

1. Prerequisites of Rigor and Reproducibility

1. To ensure rigor of design, follow national standards of good laboratory practices are at all times and obtain internal approval by IACUC (or similar committee) prior to any animal involvement in experiments.
   NOTE: This protocol was designed to maintain a clinically faithful approach. Thus, the materials, instruments, and techniques involved are described in a fashion identical to the highest clinical standards in humans. For instance, strict sterile technique is followed and expired materials should never be used.
2. To support reproducibility of this methodology in an experimental setting, develop internal standard operating procedures and control for variability in swine breed, weight, gender, and age between separate cohorts.
   NOTE: The design of this protocol was based on the use of swine weighing 38 - 53 kg.

2. Pre-operative Animal Care

1. Administer prophylactic intramuscular (IM) ceftiofur, given at 5 mg/kg, 1 day prior to the procedure.
2. Fast animals of solid food and restrict animals from cosmetic treatments, *i.e.* oil baths, 12 h prior to the procedure.
3. Induce general anesthesia within 1 h of the procedure, using IM tiletamine and zolazepam, given as Telazol at 5 mg/kg, and IM xylazine, given at 2 mg/kg.
4. Once induced, administer subcutaneous (SC) buprenorphine sustained release (SR), given at 0.18 mg/kg.
5. Place an ear-vein catheter and perform rapid sequence intubation to place an endotracheal tube.
6. Attach a pulse oximeter with heart rate monitoring function to the tongue to monitor oxygenation and heart rate.
7. Place the animal in the prone position and clip the skin over the dorsum using an electric clipper. Clip hair over a large, bilateral area extending from the midline sagittal plane to the midline coronal plane and longitudinally from the sacral apex to the scapular spines. Use adhesive tape to remove hair and free-floating skin.
8. Scrub the clipped area up to 3 times with warm water and soap and dry the skin using a lint-free towel.
9. Mark bilateral anatomical landmarks using a surgical marking pen. Mark the last ribs, iliac crests, spinous processes, and transverse processes.
   NOTE: By marking the last ribs, iliac crests, and transverse processes, the lumbar spine is demarcated along its superior, inferior, and lateral boundaries, respectively. This protocol has been designed to guide access and injection of any lumbar DRG of interest. For reference, the superior iliac crests align with the L3 or L4 vertebral level.
10. Cover the animal with a warm blanket for transport to the operative suite.

3. Positioning in the Operative Suite

1. Gently lift and position the animal prone into a modified large humane animal sling with padded abdominal aperture.
   NOTE: The abdominal aperture allows for reduction in abdominal pressure similar to the Wilson frame used during human surgery of the spine. In turn, this decreases intraoperative bleeding from the spinal blood vessels. The sling is advantageous because the legs are allowed to hang freely through padded openings which protect the animal from peripheral nerve impingement. However, because the sling frame is made of metal, it should be padded with insulation to avoid electrical short-circuiting and inadvertent animal burn. A blanket roll can be placed to position the head and neck in a comfortable position, depending on the size of the animal.
2. Maintain general anesthesia using 1 - 3% inhaled (IH) isoflurane, titrated to effect. Moisten eyes with ophthalmic ointment and gently tape them shut.
3. Place lines for vital sign monitoring to document temperature, blood pressure, heart rate, and oxygenation. Monitor ventilation by capnography.
4. Place an adhesive, disposable electrosurgical dispersive electrode over the left or right scapula.
5. Administer warmed Lactated Ringer's as maintenance fluids through the ear-vein catheter. Give fluids at a rate of 5 - 10 mL/kg/h.
6. Place a forced-air warming device over the thoracic and cervical region and avoid covering the last ribs.

4. Sterile Preparation of Operative Field for a Left-sided Injection

NOTE: From this point forward, proceed in strict sterile fashion.

1. Prepare the skin overlying the lumbar spine beginning with wide application of 0.7% iodine povacrylex and 74% isopropyl alcohol according to the manufacturer's instructions. To ensure that the guide needle can later be placed in a sterile fashion, lateralize application to the side of planned injection by extending antisepsis towards the midline coronal plane past the marked transverse processes.
2. Place disposable surgical towels in rectangular fashion to outline the planned incision site, which is over the midline along the marked lumbar spinous processes.
3. Apply an adhesive antimicrobial incise drape over the operative towels and exposed skin. Clamp drapes in place and extend the drapery edge off the operative field.
4. Secure a vertical drape to poles at the head of the sling between the operative field and the monitoring technician.
5. Secure lines for suction and electrosurgery within the operative field by clamping to the sterile drapes. Pass the free ends of tubes and wires out of the sterile field.

5. Skin Incision and Subperiosteal Dissection

1. Palpate the lumbar spinous processes along the midline and identify 3 consecutive vertebral levels.
2. Use a #15 scalpel to open an 8 - 12 cm midline sagittal incision through the incise drape directly posterior to the spinous processes. Maintain hemostasis using gauze tamponade and monopolar electrosurgery.
   NOTE: Care should be taken not to deviate from the midline as the incision is advanced in the anterior direction because this limits bleeding from the paraspinal muscles. Periodic palpation for the spinous processes facilitates advancement. Self-retaining Weitlaner, Meyerding, or Gelpi retractors may be placed and repositioned as needed to facilitate the dissection. Suction is used to maintain visibility.
3. Dissect the subcutaneous tissue and fat using monopolar electrosurgery until the thoracolumbar fascia is reached. Palpate the spinous processes deep to the thoracolumbar fascia and cut the fascia along the midline to expose the supraspinous ligament spanning between spinous processes.
   NOTE: The thoracolumbar fascia is identified as an organized, aponeurotic sheath with a connective tissue grain that interweaves in an oblique, lateral to medial direction. At this point, the incision may be lengthened in either the superior or inferior direction to ensure that 3 spinous processes are fully visible with the centermost spinous process aligned within the center of the dissection field.
4. Use a #15 blade to place a 2-mm deep parasagittal incision through the supraspinous ligament posterior to each spinous process. Place each incision along the left third of the posterior surface of the spinous process.
5. Gently release the supraspinous ligament at each level along each incision using a 5-mm Freer elevator.
6. Identify the subperiosteal plane and dissect within that plane along the lateral surface of each spinous process.
7. Perform subperiosteal dissection at each spinous process in a parallel fashion to ensure that a gentle, even dissection is achieved.
8. Incise the paraspinal muscle attachment along the interspinous spaces using monopolar electrosurgery in concert with subperiosteal dissection.
9. Identify the lamina at each level and continue subperiosteal dissection laterally to reach the lateral border of the 2 zygapophyseal joints that connect the 3 exposed vertebrae and to reach the lateral edge of the lamina between the joints, called the pars interarticularis.
   NOTE: The pars interarticularis is the posterior border of the intervertebral foramen wherein the DRG resides. Occasionally, a small vein arises from a foramen located on the posterior surface of the lamina. These veins have a tendency to rupture during the subperiosteal dissection. Hemostasis is easily achieved by using a combination of bipolar electrosurgery and bone wax applied to the foramen.

6. Single-level Laminotomy

1. Identify the target of laminotomy as the centermost lamina located between and medial to the 2 zygapophyseal joints.
2. Trace the lamina to its inferior edge, to a point just medial to the contiguous inferior articular process of the caudal zygapophyseal joint.
3. Use a 5-mm Freer elevator or curette to palpate the transition between the caudalmost edge of the lamina and central canal.
   NOTE: Care is taken to not force the palpating instrument anterior as this will contact the dural sac and spinal cord. Note that the spinal cord in swine extends past the lumbar spine [18]. An intervertebral disc rongeur can be used to remove additional soft tissue overlying this area to facilitate palpation.
4. Use a 2-mm up-biting, 45-degree Kerrison rongeur to extract bone in a piece-wise fashion. Remove bone along the base of the spinous process superiorly to a level just caudal to the caudal surface of the pedicle and out laterally to its full extent.
5. Use angled bone rongeurs to assist with bone removal. Leave the inferior articular process that was connected to the lamina in place until the laminotomy is largely completed.
6. Confirm that the inferior articular process is freely mobile and attached only by the zygapophyseal joint capsule. Incise the capsule using a #15 or #11 blade.
7. Remove the inferior articular process in a piece-wise fashion but leave the adjoining superior articular process intact.
   NOTE: As the laminotomy is completed, hemostasis is performed with bipolar electrosurgery. Monopolar electrosurgery is not used because of the proximity of neural structures. Bone wax can be placed along sites of bleeding from exposed bone and absorbable gelatin sponges can be used to obtain hemostasis near soft tissue. Cottonoid is a helpful tool to wick serous fluid and blood away from the dissection.

7. Dissection of DRG

1. Evacuate the epidural fat in a piece-wise fashion from superficial to deep beginning medially and proceeding laterally. Remove fat by gentle dissection using bipolar forceps and suction with 6 - 10 French Frazier suction tips.
   NOTE: Loupe magnification or the use of a dissecting microscope is helpful in providing the level of detail needed to safely evacuate the epidural fat and achieve meticulous hemostasis of the epidural venous plexus using bipolar electrosurgery.
2. Identify the dural sac along the midline running in a superoinferior direction, parallel to the axis of the skin incision. Remove epidural fat along the dural sac until the dural sac can be seen to give rise to the dural nerve root sleeve.
3. Trace the dural sleeve laterally and inferiorly by epidural fat evacuation until it is seen to enlarge around the DRG.
   NOTE: Identify the DRG for its oval shape and yellow to orange color. At the mid-lumbar spine, the DRG is typically 4 - 6 mm in size, longest in the medial to lateral direction, and located directly inferior or 2 - 3 mm medial to its respective pedicle. A blunt, right-angled nerve hook can be used to gently palpate for the pedicle.
4. Evacuate epidural fat laterally, past the DRG, until the adjoining spinal nerve is seen.
   NOTE: If durotomy occurs, repair it by watertight closure using 6-0 nylon suture and smooth micro needle driver in a simple running stich.

8. Injection of DRG

1. Use a 22-gauge spinal needle to guide the trajectory of a 32-gauge convection enhanced delivery (CED) needle. Puncture the 22-gauge guide needle through the skin and paraspinal muscles.
   NOTE: The CED needle is designed to achieve fluid convection in tissue, also known as bulk flow, due to pressure gradients [19,20].

2. Aim the guide needle along a trajectory that approximates the longitudinal axis of the DRG and results in the needle tip emerging from the lateral paraspinal wall of the dissection field.
3. Fine tune the needle path until the needle lumen aligns with the center of the DRG.
   NOTE: The guide needle should never be allowed to contact the DRG.
4. Draw up sterile injectate into a sterile syringe and connect the syringe to the sterile infusion tubing.
5. Secure the tubing to the CED needle and hand the syringe out of the sterile field. Connect the syringe to a programmable syringe pump.
   NOTE: Tubing is prepared to a length of 5 feet to ensure that sterility and mobility are maintained. Also, it is of paramount importance that no air bubbles be introduced into solution.
6. Advance the injectate until expression is seen from the CED needle tip.
7. Place the CED needle within the guide needle lumen and slowly advance the CED needle until it emerges from the guide needle tip. Ensure that the DRG is not punctured during needle alignment.
8. Fine tune the guide needle position along the long axis of its trajectory to determine the final location of the CED tip.
9. Secure the guide needle and CED needle together using interlocking needle hubs once depth and alignment of the guide and CED needles is achieved.
10. Confirm that all injection apparatus connections are fully secured, including the guide needle, CED needle, and connected tubing loaded with injectate.
11. Advance the guide needle along its long axis to approximate the CED needle tip and DRG.
12. Puncture the DRG with the CED needle tip.
13. Submerse the CED needle tip into the three-dimensional center of the DRG.
    NOTE: Because the DRG is a three-dimensional structure of variable size and shape, DRG exposure must be complete in order to accurately place the CED needle tip at the true center of the DRG. The true DRG center is located at the intersection of its three anatomical axes, namely, the anterior to posterior, lateral to medial, and superior to inferior axes.
14. Deliver 100 µL of injectate by CED using a graduated rate and volume of 3 steps.
15. Deliver 4 µl at 2 µL/min for the first step. Deliver 8 µL at 4 µL/min for the second step. Deliver 88 µL at 8 µL/min for the third and final step.
    NOTE: Allow a 3-min pause between steps and after the final step to allow for pressure equilibration.
16. Withdraw the injection apparatus after the final injection step and 3-min pause along its long axis in a smooth, gentle motion.
    NOTE: For injected solutions that are colorless, colored dye is included in the solution at a concentration of 0.1% weight/volume to assist in visual assessment of injectate distribution [12]. Also, the vital dye 4',6-diamidino-2-phenylindole (DAPI) is included in solution at a concentration of 0.25 µg/µL when the study design requires histologic assessment of injectate distribution [5].

9. Closure

1. Apply 3 rounds of warm saline irrigation to the surgical site prior to closure to mobilize and flush the site of debris, *i.e.* bone fragments. Use suction to recover the saline and debris.
   NOTE: Meticulous hemostasis is ensured when irrigation remains clear. Hemostatic agents (gelatin sponge) and cottonoid are removed at this time. Ensure that all materials and instruments have been cleared from the incision site prior to closure.
2. Use a 3-layered technique for closure.
3. Suture the thoracolumbar fascia using 0 suture in a simple, interrupted, noninverted fashion. Place a stich every 5 - 8 mm to achieve watertight closure.
4. Suture the subcutaneous tissue using 2-0 suture in a simple, interrupted, inverted fashion with a stitch placed every 5- to 8-mm to achieve adequate strength.
5. Close the skin using 0 suture in a simple, running or interrupted fashion.
6. Use a needle counter to ensure that no sharps are unaccounted for.
7. Irrigate the skin with saline, dry the skin, and place adhesive bandage strips perpendicular to the incision.
8. Place gauze on top of the bandage strips and attach a final adhesive antimicrobial incise drape.

10. Post-operative Animal Care

1. Extubate, cover with warm blankets, and transport the animal to recovery.
2. Follow standard institutional operating procedures for post-operative monitoring and recovery from survival surgery. At a minimum, observe the animal every 15 min until return of consciousness, hourly until full anesthetic recovery is achieved, and twice-daily thereafter.
3. Provide post-operative pain management by administering IM or oral carprofen, given at 4 mg/kg, once daily for 5 days beginning on post-operative day 0. Administer SC buprenorphine SR, given at 0.18 mg/kg, once on post-operative day 2.
4. Administer post-operative antisepsis by giving IM ceftiofur, given at 5 mg/kg, once on post-operative day 4.
5. Remove the bandage on post-operative day 5 - 7. Remove the sutures when wound healing is complete, typically on post-operative day 10 - 14.
6. Humanely euthanize the animal according to standard institutional operating procedures once the study endpoint is reached.

Representative Results

Histologic assessment of injectate spread

Successful delivery of injectate to DRG is determined by histologic assessment of DAPI spread. The technique involves positioning the needle tip in the three-dimensional center of the DRG. Therefore, successful delivery is determined by evaluating the extent of DAPI staining from histologic sections both near (central DRG sections) and distant (peripheral DRG sections) to the needle tip. **Figure 1A** and **Figure 1B** represent a successful injection of one DRG. DAPI staining was evenly dispersed through both the central and peripheral DRG parenchyma. Thus, a successful DRG injection is illustrated by the diffuse spread of DAPI staining throughout the three-dimensional DRG architecture. Sub-optimal injection is illustrated by inconsistent staining. For instance, minimal staining (**Figure 1C**) or focal staining along the outer rim but not inner

aspect of DRG parenchyma (**Figure 1D**) indicates unsuccessful injection. Also, considered together, **Figure 1C** (central DRG section) and **Figure 1D** (peripheral DRG section) illustrate a lack of consistent staining within three dimensions for this single lumbar DRG.

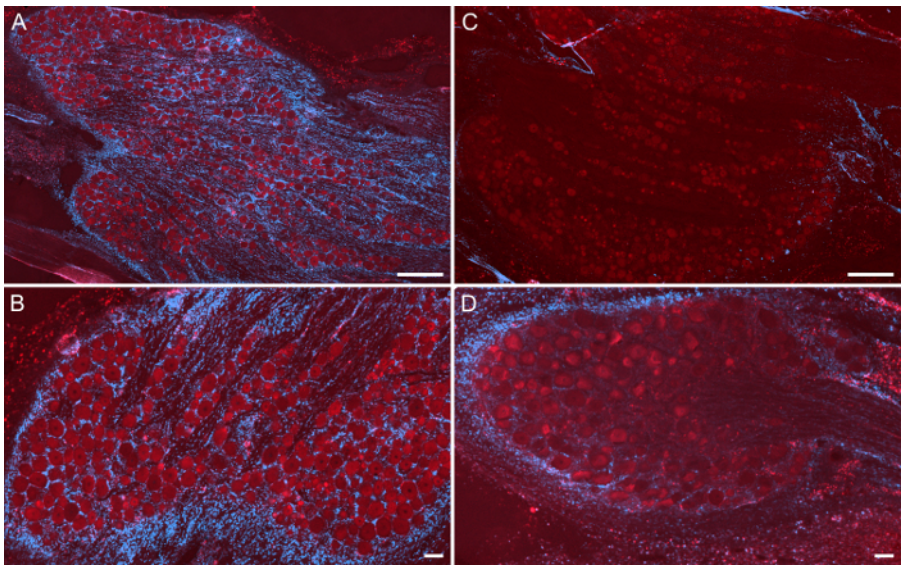

Figure 1: Three-dimensional Assessment of DAPI Distribution in Injected DRG. (A) A central section from an injected lumbar DRG representative of a successful outcome. The staining of the marker dye DAPI is evenly dispersed throughout the entire DRG in two dimensions. **(B)** A parallel, peripheral section of the same DRG in (A), illustrating the consistency of DAPI spread within a second plane of section, confirming a successful injection in three dimensions. **(C)** A central section from an injected lumbar DRG representative of a sub-optimal outcome. Minimal to no staining of DAPI is seen except for occasional foci. **(D)** A parallel, peripheral section of the same DRG in (C), illustrating partial distribution of DAPI along the DRG periphery. Blue: DAPI. Red: autofluorescence. Scale bars = 500 µm (A and C),100 µm (B and D). Please click here to view a larger version of this figure.

Discussion

We sought to describe a method for surgical exposure of DRG *via* laminotomy and intraganglionic injection in a healthy large animal species, specifically, swine. In rodents, a similar method has been detailed [12] and used to deliver conventional pharmacologic agents [8,10] and viral vectors [6,7,9,12] to DRG. The results from the above small animal studies are promising and we hope our protocol will pave the way for others to translate these prior findings to swine. Healthy and diseased animals were used in the above studies, supporting the utility of small animals in preclinical research. Inevitably, large animal models will be required to make the best available comparison to human DRG in terms of size and injectate distribution. For instance, the discrepancy in DRG size is clearly evident between rats and humans. L5 DRG in adult, male rats measure approximately 2.6 mm x 1.5 mm [5] compared to 11.6 mm x 6.6 mm in adult, male humans [21]. Based on live radiographic measurements in swine, L5 DRG were found to be approximately 8.0 mm x 6.0 mm [22]. Therefore, swine are situated as a particularly useful species for preclinical study due to structural similarities with the human nervous system and nearby musculoskeletal anatomy. This is evidenced by the reverse translation used to design this protocol according to that performed in the clinic. Moreover, swine are use animals and are of growing importance in biomedical research. This protocol will support preclinical studies of intraganglionic delivery of injectable solutions to advance prior findings from work in rodents to large animals. Thereby, this protocol may foster novel strategies for treating chronic pain that integrate anatomically selective delivery techniques with novel molecularly selective agents, which, we predict, may have the potential to transform pain medicine [3].

Additional notes for successful DRG injection

The prelaminar periosteum at lumbar vertebral levels in swine continues cephalad in place of the ligamentum flavum. During laminotomy, the periosteum can be easily separated by placement of the Kerrison rongeur and can mimic the appearance of dura mater. It is a critical step to differentiate the ligamentum flavum, periosteum, epidural fat, and dura sac as the dissection is carried out. Furthermore, exposure of the epidural space is more efficient and incurs less bleeding if the periosteum is removed simultaneously with the lamina. If the periosteum is not removed along with bone, it can be incised with a #11 blade and removed to expose the underlying epidural fat.

To do no harm is a top priority and this must be balanced with the goal of DRG access and injection. Thus, care is taken not to advance the evacuation of epidural fat farther anteriorly or anteromedially than is needed to allow positive identification of the dural sac, dural nerve root sleeve, and DRG. Dissection in the anteromedial direction where the dural sac gives rise to the dural nerve root sleeve is particularly dangerous as a longitudinal epidural vein will be encountered. Furthermore, dissection in this direction increases the risk for unintended durotomy, signaled by the outflow of cerebrospinal fluid from the surface of the dural sac.

A final critical point is that of identifying the dural sac, dorsal roots, DRG in its entirety, and spinal nerve. This helps to establish 4 pieces of converging anatomical evidence that ensure complete DRG definition. Defining the DRG in its entirety is required in order to position the needle tip at its three-dimensional center, which permits the CED needle to establish a consistent pressure gradient while maximizing the distance to the surrounding anatomical boundaries. Both factors greatly increase the volumes delivered and range of intraparenchymal spread [19,20]. Delivery of injectate at a location that is not the true anatomic center results in sub-optimal injection because inconsistent pressures result when injectate leaks from the nearby site of DRG puncture [20].

One difficulty with using a CED needle for DRG injection is that of compliance. Once the injection has started, the needle tip must be kept as still as possible or else pressure gradients will dissipate due to abrupt changes in compliance [20]. Respiratory motion is a source of continuous movement during the injection. However, the risk of needle movement secondary to respiratory excursion is largely removed by anchoring the CED and guide needle within the paraspinal musculature prior to puncture of the DRG as both the needle and DRG move in synchrony with respiration. The injection duration for a volume of 100 µL totals 24 min at the stepped rate described here. Care should be taken to limit external disruption of the entire injection apparatus during this time. Arrangement of the surgical field, personnel, and surrounding obstacles should be modified as needed before the injection is started to ensure an undisturbed interface between the CED needle tip and DRG.

Disclosures

None; the authors have no conflicts of interest related to this study.

Acknowledgements

The study was performed with support by the Schulze Family Foundation (to A.S.B.).

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