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Simple methods of dissection protocols for the rapid isolation of rat dorsal root ganglia under the non-microscopic condition

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ABSTRACT: The dorsal root ganglia (DRG) neurons have long been studied to advance the understanding of sensory nerve function and growth. DRG neurons are also involved in determining the fundamental mechanisms of neurodegenerative and painful disorders of the peripheral nervous system. To explore the simple methods of rapid and accurate removal of DRG in anesthetized rats with the naked eye without destroying the spinal column or blood circulation. Male Wistar rats were fixed on the operating table, lumbar DRG was removed, and sufficient DRG neurons were harvested without microscopic equipment. At the same time, the procedure was accomplished without disruption to breathing and circulation. These approaches reduce the time required for DRG collection, thereby improving efficiency, and increasing the chances of generating healthy primary DRG cultures and reproducible experiments using DRG tissue.

Keywords: Rat; *In vivo*; Dorsal root ganglion; Sensory neuron; Peripheral neuropathy; Primary culture

1.0 INTRODUCTION

The cell bodies of sensory neurons can be found at all levels of the spinal column in paired structures called dorsal root ganglia (DRG). The segments of the DRG are slightly different in size and shape. In method two, the DRG of the first cervical nerve is about 1mm in diameter, and it is located in the atlas pterygoid foramen. The second cervical ganglion is the clamp between the atlas and the axis. The 3rd to 8th cervical ganglia are all enclosed in the spinal canal, located in the epidural close to the front of the intervertebral foramen, with a diameter of about 1.2mm. The DRG is situated on the

front of the intervertebral foramen in the thoracolumbar segment. It is a long fusiform, 1.4-1.6mm long, and the sacral ganglia are smaller, 1.2mm long, and 0.8mm thick (Xiong et al., 2006). Research on DRG in China has gained more attention over the last decades (Liyuan et al., 2018; Wang et al., 2010). The rapid and straightforward acquisition of highly active DRGs is a prerequisite for research. To meet the viability of *in vitro* culture neuronal cells, it is necessary to complete the collection in the shortest time with the most superficial possible equipment conditions, simultaneously ensuring a sufficient number of DRGs. The currently reported DRG collection methods in the literature are time-consuming, complicated, and require sophisticated microsurgical instruments (Tandrup, 2004). Therefore, the collection rate is not ideal. Thus, the two most important methods that reduce the dissection time for obtaining DRG without microscopic equipment were explored in these experiments, laying the foundation for further cell culture and research.

2.0 MATERIALS AND METHODS

Male Wistar rats (250-300g) were purchased from the College of Veterinary Medicine Yangzhou University (License # SCXK (Su) 2020-2005, Yangzhou, China. The animal's ethics committee approved the protocol for this study of Nanjing Medical University under the guidelines published by the research council of the School of Pharmacy, China Pharmaceutical University, and it conforms to the guidelines for the care and use of laboratory animals published by the US national institutes of Health (NIH, 1996). All surgeries were performed under 10% chloral hydrate (0.3 ml/100 g), and efforts were made to minimize suffering.

2.1 Method one

All the rats were shaved, weighed, disinfected, and scrubbed with 75% ethanol and anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g). Anesthetized rats were placed in a prone position and fixed on the operating table. The surgery was performed strictly aseptic to avoid DRG contamination. The skin was pinched with thumb force, and a caudal to cranial incision was given at the dorsal midline scissors. The skin was undermined and separated from the underlying muscles (**Fig. 1A**). Tendons attached to the spinous process were cut with a tissue clip; muscles around the spine were separated bluntly with mosquito-type hemostatic forceps until the peritoneum. The spinal spinous and transverse processes were exposed as much as possible. The hemostatic forceps should be used carefully during separation to avoid piercing the pleural cavity or the inferior vena cava and veins (**Fig. 1B**). The abdominal oblique muscles on both sides of the spine were cut to prevent obstructing the sightline during subsequent operations, and the peritoneal cavity was opened. The operation was carefully performed with correct shear to avoid the death of rats due to vascular rupture (**Fig. 1C and D**). The lumbar spine was gently lifted to see the inferior vena cava and veins; the hemostatic forceps were used to separate it from the spine with blunt dissection to prevent the rat's death caused by a ruptured blood vessel (**Fig. 1E and F**). Using forceps, the sciatic nerve was identified from inside the hindlimb and cut from the distal spinal cord. To find the position of the intervertebral foramen, along the sciatic nerve near the spinal cord, connective tissue was removed from the intervertebral foramen, and the intervertebral foramina were exposed (**Fig. 1G and H**). Pull the nerve fibers with forceps to pull the DRG and nerve fibers out of the intervertebral foramen. The enlarged DRG attached to the heel can be seen about 1.5 mm in size, oval in shape, with a smooth and shiny yellowish surface. The nerve fibers at both ends of the ganglion were cut, frozen, and used for cell culture or placed in a 10% neutral formaldehyde (**Fig. 1I and J**).

2.2 Method two

In this method, skin incision and muscles on both sides of the spinal column were separated and similar to method one. After exposing the spinous and transverse processes, the vertebral arch plates were exposed to facilitate the later opening of the spine (**Fig. 2A, B, and C**). Ribs were cut on both sides longitudinally along the spine (**Fig. 2D**), and the spinal column was separated (**Fig. 2E**). The spine from the vertebral arch plate was cut apart, spinous processes were cut apart, and transverse processes were isolated from both sides to expose the spinal cord fully (**Fig. 3F**). It was gently pulled from its place (**Fig. 3G and H**). The nerve attached can be seen in the tapered hole below the spinal cord. The enlarged ganglion on the posterior root, about 1.0-1.5mm in size, is light yellow, round, and smooth on the surface; it is pulled gently along the posterior root with forceps (**Fig. 3I**). The spinal nerves at the two ends of the ganglion were subtracted from the ganglion, and the bulk of the spinal nerve was preserved. The DRG was cryo-preserved immediately to avoid affecting the activity of the neurons.

2.3 Hematoxylin and eosin staining

Formalin-fixed DRGs were embedded with paraffin, and the sections were adjusted to a thickness of 15µm for sectioning. DRG slices were washed with sterile water

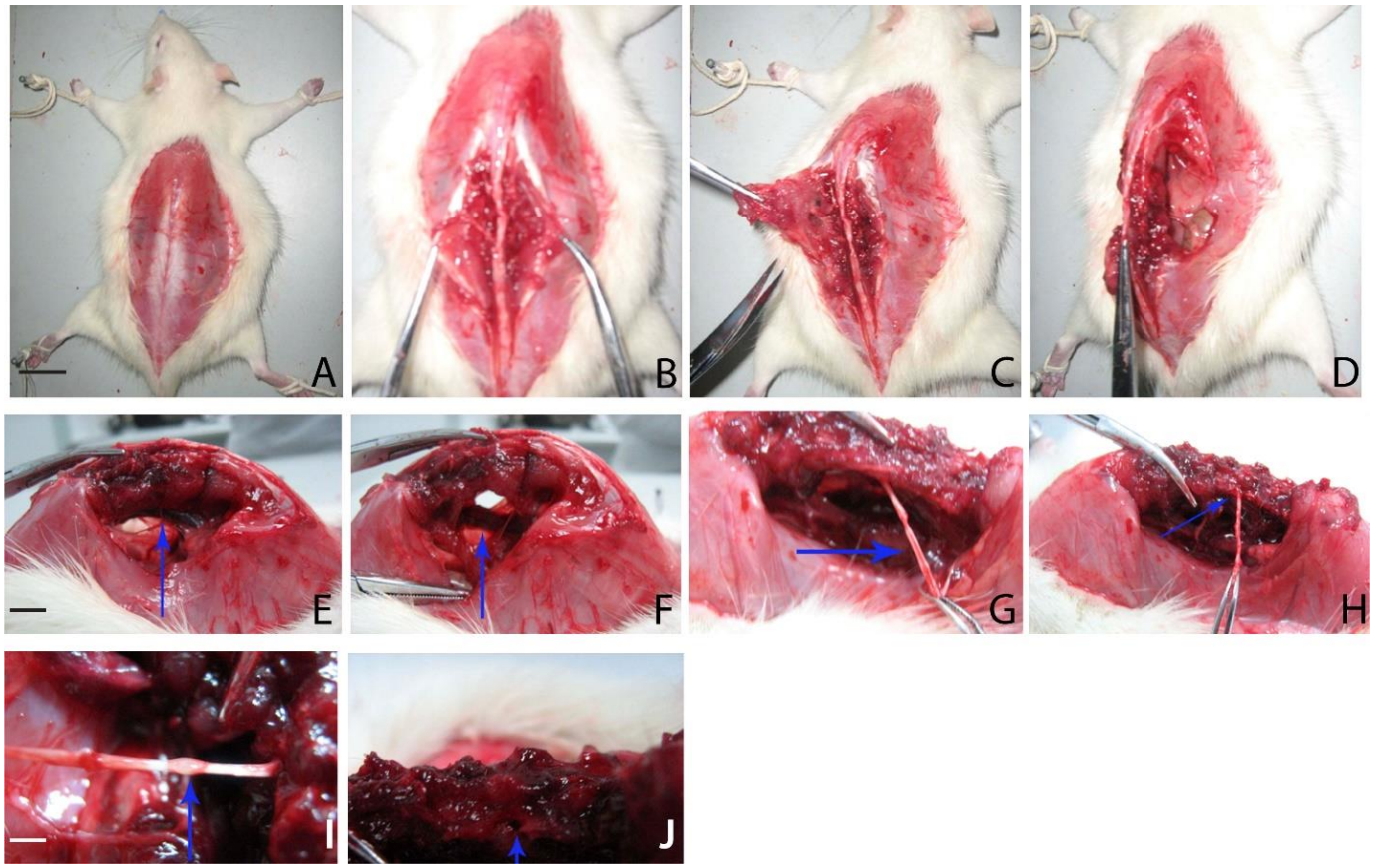

Figure 1: Dissection of DRGs (blue arrows) from the rat and identification of specific ganglia. The first incision is made to expose the muscle **(A)**, and blunt separation of the muscles around the skin **(B)**. Before **(C)** and after **(D)** cutting the abdominal oblique muscles obstructing the view. Before **(E)** and after **(F)** the separation of inferior vena cava and artery. Identifying and locating the sciatic nerve **(G)** and intervertebral foramen **(H)**. Removal of DRG (blue arrows), **(I)** and the intervertebral foramen **(J)** behind the dorsal root ganglion. Scale bar = 2 cm.

for several seconds, stained with hematoxylin and eosin (H&E) stain for 3-5 minutes, differentiated with an ethanolic solution of hydrochloric acid for several seconds, reversed blue in ammonia water for 20 seconds, stained with 0.5% eosin solution for 1 minute, followed by 75-95%, anhydrous ethanol was dehydrated for 1 minute, and made transparent with xylene twice for 1 minute. Finally, it was sealed with neutral gum.

3.0 RESULTS

3.1 Method one

The surgical device did not touch the dorsal root ganglion during the experiment. The blood circulation in the spinal cord was not damaged, so the DRG may be highly active. Each operation lasts 30-40 minutes, and each case can take out 5-7 dorsal root ganglia. Through slicing observation, it can be determined that the obtained DRG is correct. Rat lumbar segments DRG were successfully harvested.

3.2 Method two

The DRG was successfully removed without surgical instruments. The experimental procedure was rapid and accurate. The DRGs were complete and sufficient in quantity. The total time of the operation was 35-45 minutes, and the time for DRG removal was 15-20 minutes. In other cases, 20 to 30 DRGs could be taken, with an average of 1-2 minutes.

3.3 Hematoxylin and eosin staining

The prepared slices were placed under an inverted microscope, and 3-5 fields were randomly selected under low magnification and high-power magnification for morphological observation (Fig. 3C and D).

4.0 DISCUSSION & CONCLUSION

These experiments aim to extract DRGs quickly and accurately without microscopic involvement. These methods may result in highly active dorsal root ganglion. Since microscopy equipment has been used in previous studies (Liu et al., 2013) to avoid damage to the

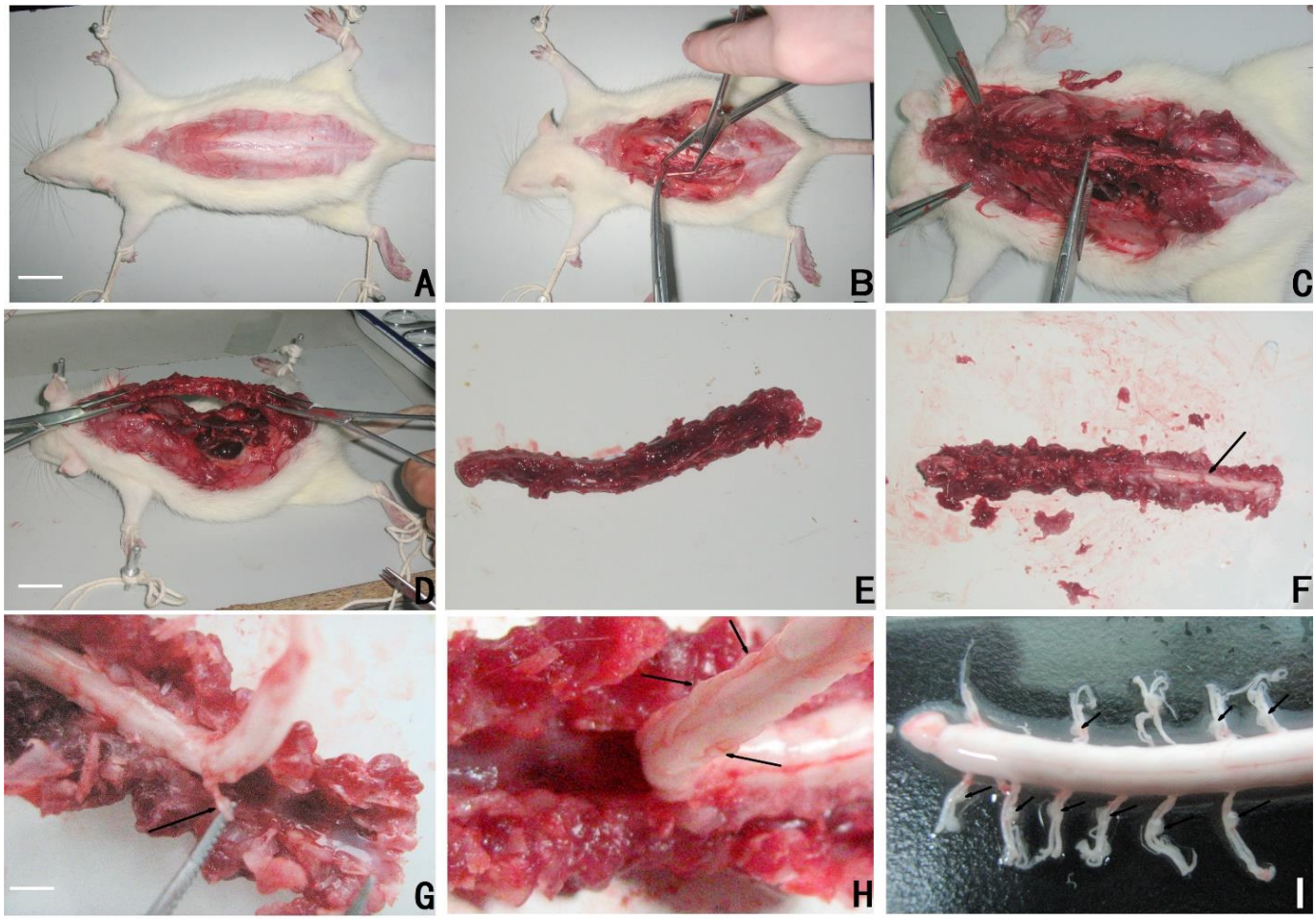

Figure 2: Dissection of DRGs (black arrows) from the rat and identification of specific ganglia. The first incision is made across the spine **(A)**. Next, parallel cuts are made on both sides of the spine and overlying muscle [before **(B)** and after **(C)**] are removed. After that, cut the ribs on both sides of the spine **(D)** and cut the desired spine **(E)**, to extract dorsal root ganglion from the removed spine. Exposed spinal cord **(F)**, and pull up the spinal cord and pull out the ganglion from the taper hole **(G)**. The ganglion attached to the nerve heel **(H)** and dorsal root ganglion **(I)** were removed from the spinal cord. Scale bar = 2 cm.

spine, the main focus of this study was the gentle manipulation of the spine to locate the position of the DRG without damage to the spine. Depending on the genetic makeup, rats possess 30 or 31 pairs of DRG, 8 cervical, 13 thoracic, 5 or 6 lumbar, and 4 sacral (Malin et al., 2007) (**Figure 3A**). These DRG contains pseudo-unipolar sensory neurons (Krames, 2015) and is the main afferent neuron of the peripheral nervous system. Peripheral nerves send many types of sensory information to the central nervous system (CNS) and transmit signals from the CNS to other parts of the body. This peripheral sensory information starts at its outer weekend shoots and is transmitted from the periphery to the spinal dorsal horn (**Figure 3B**). The neurotransmitters released from the central nervous terminals act on postsynaptic target cells, perform preliminary processing on sensory information, and complete primary sensory input transmission (Harrison et al., 2018; Kent et al., 2018).

In method one, a relatively large sciatic nerve can be used as a landmark to quickly find the position of the foramen without separating the muscles around the spine. It can effectively shorten the operation time. In addition, the following points should be noted in this method: (1) Separation of the muscles around the spine should separate the inferior vena cava and veins from the spine. Rupture of the vena cava and veins may result in blood loss and the death of the rat. (2) The connective tissue at the intervertebral foramen should be separated as cleanly as possible to prevent the nerve fibers from breaking when pulled, causing the ganglia to be removed. (3) The manipulation of the spine while pulling during the operation should be meticulous and gentle; too vigorous manipulation may cause the rupture of the pleural cavity in the rat, resulting in the death of the rat.

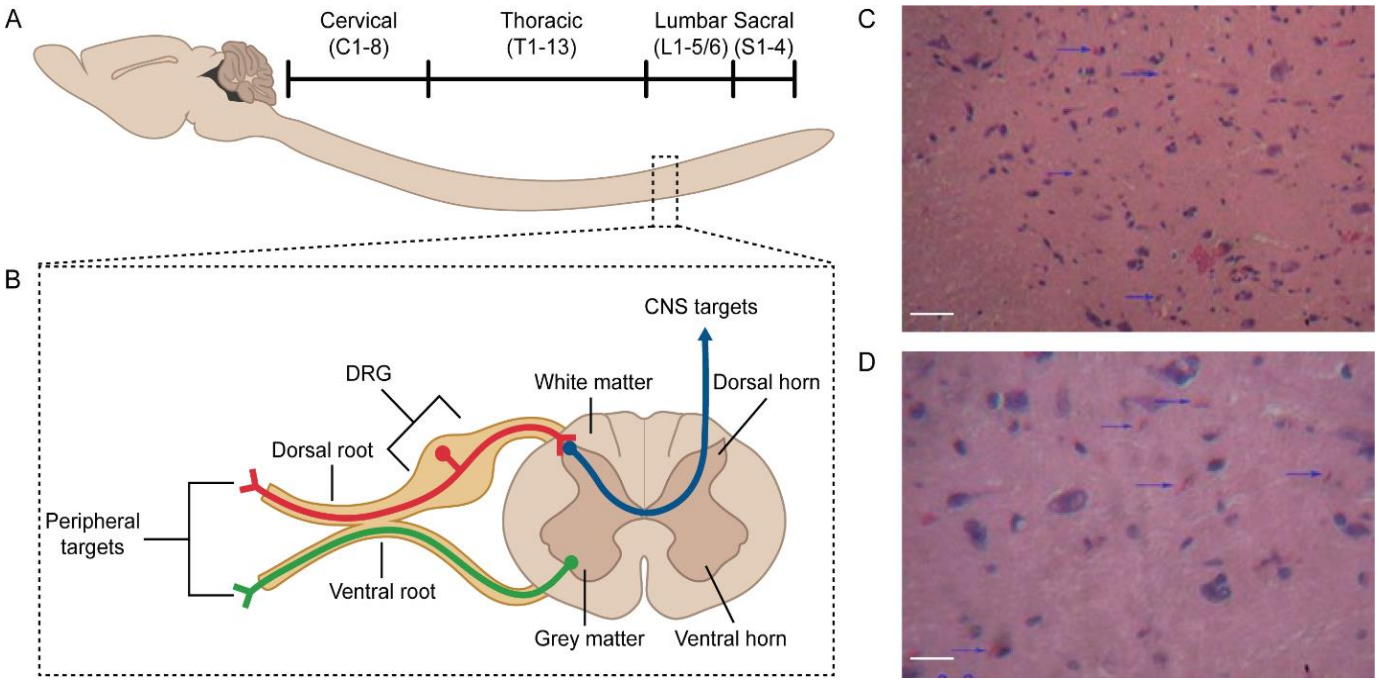

Figure 3: The schematic representation of the spinal column and isolated DRG neurons. Rats have 8 cervicals, 13 thoracics, 5 or 6 lumbar, and 4 sacral DRG pairs totaling 60 or 62 individual ganglia in terms of genetic makeup **(A)**. The diagram represents a transverse section of the spinal column **(B)**. Separated DRG can be enzymatically detached and grown in culture or sectioned for H&E analysis (method 1). Representative images of H&E stained primary DRG sensory neurons (blue arrows) by inverted microscope (**C** and **D**). **(C)** Seen under low magnification (10×10), **(D)** Seen under high magnification (10×40).

When the method is compared with Harrison *et al*. (2018) and Liem (2015), the disadvantages are: the number of DRG obtained from each rat is reduced; the advantages are: the operation process and the required instruments are more straightforward, without ruining the spine, preventing the destruction of the DRG, and obtaining higher ganglion activity. The anterior spinal artery and posterior spinal artery from the vertebral artery continue to receive supplementation of segmental arterial branches as they descend to protect the spinal cord from sufficient blood supply. Once the spinal cord is damaged, the blood supply is insufficient, resulting in damage to the spinal cord, ganglion, and inner neurons (Bai et al., 2006; Smith, 2001). Therefore, in the case of requiring the higher activity of DRG neurons, the surgical method used in this experiment may be more appropriate.

In method two of this study, care should be taken during surgery that: (1) the ganglion at the neck and lumbar region is large and easy to identify, if there is no requirement for the section where the ganglion is located, choose here; (2) after cutting the vertebral arch plate, the spinous process must be removed, and the transverse process separated as far as possible; otherwise, the nerve fibers can easily break during the process of removing the ganglion, obstructing the ganglion removal. (3) In the process of pulling the ganglion out of the cone hole, care should be taken to pull the ganglion intact to keep its activity at its highest. Compared with other methods of harvesting DRG, these methods have the advantages of not requiring microscopic equipment, simple experimental conditions, rapid and accurate operation, and provide a sufficient number of ganglia. The disadvantage of the method is mainly due to the limitations of experimental conditions. The experimental procedure, when performed too fast, could easily damage the ganglia to a certain degree, which may affect the activity of neurons. When the operation is performed too slowly, it will affect the extraction efficiency. This requires experimental operators to have a certain degree of proficiency and adjust the picking rate according to their needs.

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Conflicts of Interest: The authors have declared no conflict of interest.

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