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Laboratory Investigation

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Electron Microscopic Study in the Rat Model of Electrically Injured Myelopathy: Preliminary Report

Je Hoon Jeong [1], **Cheong Hoon Seo** [2], **and Dae Hoon Lee** [3]

[1]Department of Neurosurgery, Soonchunhyang University Bucheon Hospital, Bucheon, Korea
[2]Department of Rehabilitation Medicine, Hangang Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, Korea
[3]Korea Institute of Machinery and Materials, Daejeon, Korea

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Address for correspondence:
Je Hoon Jeong
Department of Neurosurgery, Soonchunhyang University Bucheon Hospital, 170 Jomaru-ro, Wonmi-gu, Bucheon 14584, Korea.
Email: neuri71@gmail.com
neuri71@schmc.ac.kr

ORCID iDs
Je Hoon Jeong
https://orcid.org/0000-0002-4656-0113
Cheong Hoon Seo
https://orcid.org/0000-0003-4091-6382
Dae Hoon Lee
https://orcid.org/0000-0001-5627-2054

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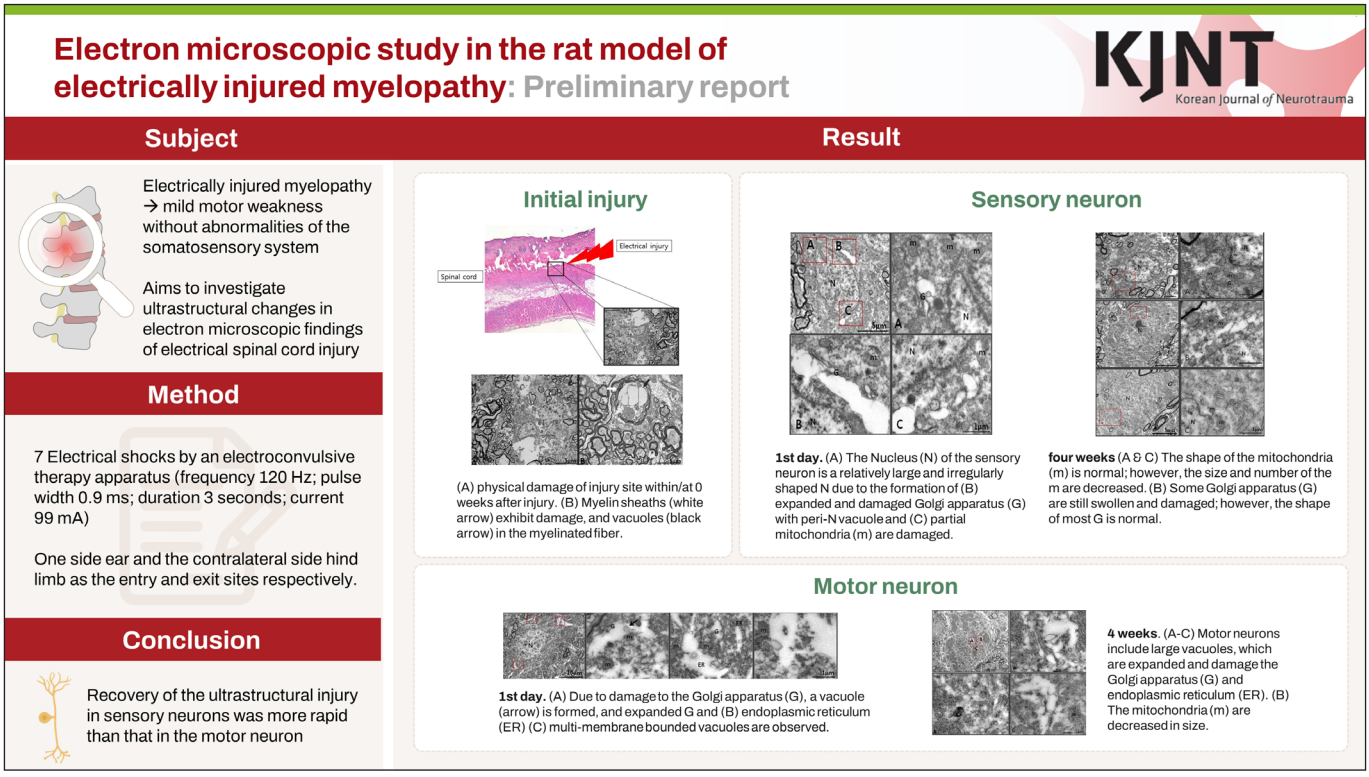
ABSTRACT

Objective: The patient with electrically injured myelopathy showed mild motor weakness without somatosensory pathway abnormalities. Few reports have been reported on the pathophysiological mechanisms of electrically injured myelopathy, and there is controversy about the exact pathological causes. This study aimed to investigate the ultrastructural changes in the electron microscopic findings of electrical spinal cord injury.
Methods: Nine rats were used in this study. We performed 7 electrical shocks (frequency, 120 Hz; pulse width, 0.9 ms; duration, 3 seconds; current, 99 mA) using an electroconvulsive therapy (ECT) apparatus (57800 ECT unit; UGO BASILE). We used one ear and one contralateral hind limb as entry and exit sites, respectively. We only enrolled rats with hind limb weakness and performed electron microscopy evaluations of the spinal cord on the first day and 4 weeks after injury.
Results: On the first day after injury, an electron microscopic examination showed a directly damaged area that appeared to be torn as physical damage, damaged myelin sheath, vacuolated axons in the myelin sheath, swollen Golgi apparatus, and injured mitochondria. Looking at changes in motor and sensory nerves, the sensory neurons showed recovered mitochondria and Golgi apparatus 4 weeks after injury; however, motor neurons still showed injured mitochondria, swollen Golgi apparatus, and endoplasmic reticulum.
Conclusion: This study showed that recovery from ultrastructural injury was more rapid in sensory neurons than in motor neurons.

Keywords: Spinal cord injuries; Burns, electric; Animals, laboratory; Electron microscopy

GRAPHICAL ABSTRACT



Conflict of Interest
Je Hoon Jeong serves as an Editor-in-Chief of the *Korean Journal of Neurotrauma*, but has no role in the decision to publish this article. Except for that, no potential conflict of interest relevant to this article was reported.

INTRODUCTION

The incidence of high-voltage electrical shock-induced spinal cord injury ranges showed 2% to and 5%.[3,5,9] Many electrical spinal cord-injured myelopathy patients usually do not show sensory pathway abnormalities.[1] Additionally, usual magnetic resonance imaging (MRI) of the spinal cord does not demonstrate the change or lesion related to electrically injured myelopathy.[1] We previously reported a novel animal model of electrically injured myelopathy.[5] In that report, we presumed that electrically injured myelopathy is too subtle to be detected by a routine MRI sequence or structural restoration of electrically injured myelopathy is very rapid; therefore, the available time for detecting a structural lesion is too short.[5]

The motor, sensory, and interneurons are found in specific spinal cord parts and adjacent anatomical structures. Most sensory neurons are pseudounipolar, which only have one axon and 2 split branches. Motor neurons have the most common type of nerve cell. They are multipolar and have one axon and several dendrites. The spinal cord's motor neurons are divided into visceral and somatic. Visceral motor neurons control the smooth muscle, and glands and somatic motor neurons control skeletal muscle. Somatic motor neurons are placed in the gray matter of the ventral horn in the spinal cord. Visceral motor neurons are in the intermediate horn between the dorsal and ventral horns.[4] The sensory neuron cell bodies are placed in the dorsal root ganglion, and their axons route the dorsal root into the gray matter of the dorsal horn. The interneurons located in the gray matter may connect-the sensory neurons.[8] We also thought that the different shapes and uneven distribution of the

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motor and sensory neurons in the spinal cord producing spatial non-uniformity, caused the difference in electrical impedance.[5]

Therefore, we tried to investigate ultrastructural changes in the spinal cord's electron microscopic findings and evaluate the relevance of unique myelopathy and ultrastructural changes in the electrical burn injury rat model.

MATERIALS AND METHODS

This study was performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the local ethical committee (HMC2009-2-0930).

Electrical injuries

We used the same animal model which previously reported.[5] Nine female Sprague-Dawley rats (250–300 g, 8 weeks) rat were anesthetized by inhalation of 5% isoflurane. We set the one side ear as the input point and the contralateral side hind limb as the output point and prepared with a depilatory cream. For generating a positive pulse with high voltage and constant current, we used the electroconvulsive therapy (ECT) apparatus (57800 ECT unit; UGO BASILE, Gemonio, Italy). This machine has a feedback network with a maximum voltage of 2.5 kV. We performed the electric shock (frequency 120 Hz; pulse width 0.9 ms; duration 3 seconds; current 99 mA). We applied 7 shocks (pulse width magnitude 99 mA; intervals 3 minutes) to each, and we used only rats showing hind limb weakness.[5] We injected 5 mL of 5% glucose balance solution into the rat abdominal cavity to avoid shock after the operation. To prevent infection, Penicillin (400,000 U) and gentamicin (80,000 U) were injected every day, and the input and output points were sterilized with an iodine solution.

Histological examination

We performed electron microscopic evaluations of the spinal cord at 1st day and 4 weeks after injury.

Electron microscopic analysis

We used the same methods in the previously reported.[6] We immediately cut the spinal cords into small pieces after surgical removal, and reserved them in the fixation solution (phosphate-buffered saline [PBS]-buffered 2.5% glutaraldehyde and 4% paraformaldehyde) for 2 hours on ice (**FIGURE 1**). To perform the post-fixation, we used in 1% PBS-buffered osmium tetroxide. We performed the dehydration process through a graded ethanol series and embedding process in epoxy resin (Epon 812). We completed ultra-thin sections (75 nm) by an RMC MTXL ultramicrotome (RMC Boeckeler, Tucson, AZ, USA). We used A JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan) to exam the sections. We completed staining with uranyl acetate, and lead citrate were done.

RESULTS

On 1st day after the injury, an electron microscopic examination showed the direct damaged area, which appeared to be torn as physical damage, the damaged myelin sheath, and a vacuolated axon in the myelin sheath (**FIGURE 2**).

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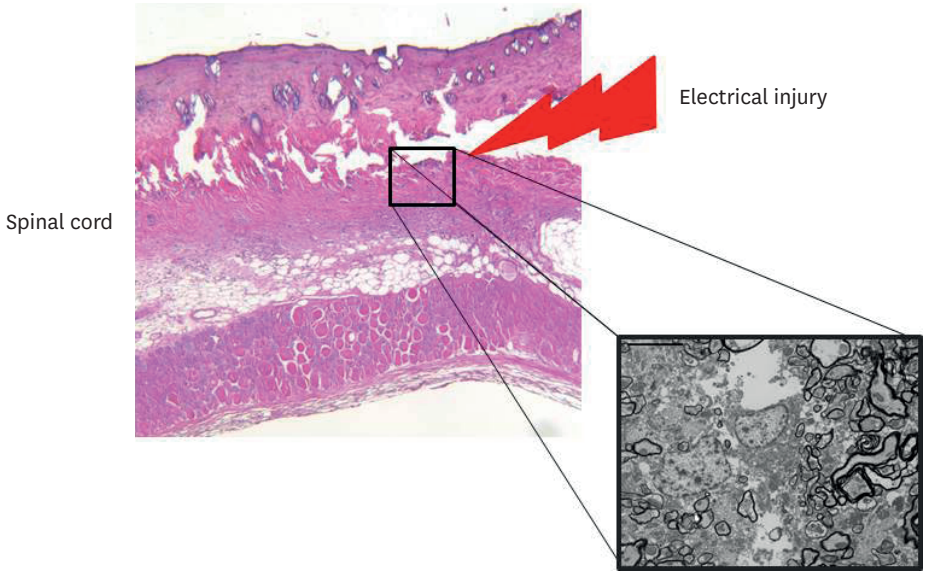

FIGURE 1. Photography with the hematoxylin and eosin staining and electron microscopic pictures. this figure shows the detailed information about the location in the spinal cord where the electron microscopic figures were taken.

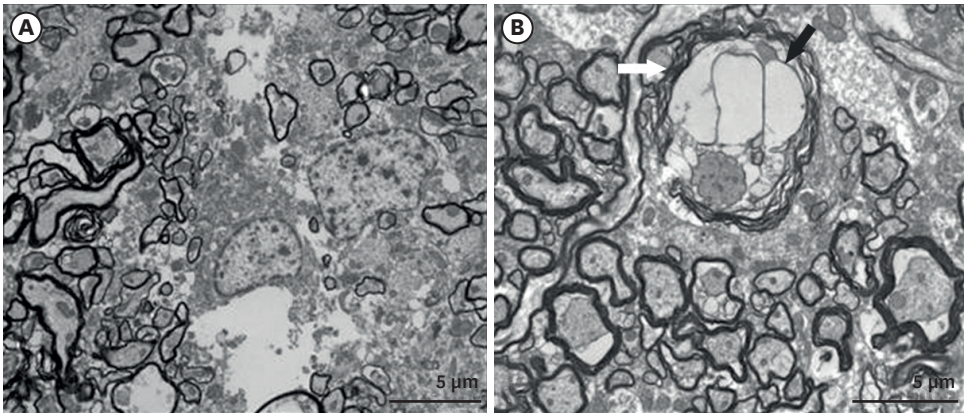

FIGURE 2. Electron microscopic findings of the injured point of the spinal cord. (A) At the injury point of spinal cord, cells and neurites seem to exhibit physical damage within/at 0 weeks after injury. (B) Myelin sheaths (white arrow) exhibit damage, and vacuoles (black arrow) are observed in the myelinated fiber.

On 1st day after the injury, the nucleus of the sensory neuron was a relatively large and irregularly shaped nucleus due to the formation of expanded and damaged Golgi apparatus with peri-nucleus vacuole (**FIGURE 3**). In motor neurons, similar to the sensory nerve, damaged. Expanded Golgi apparatus and multi-membrane bounded vacuoles in the endoplasmic reticulum (ER) were also observed at 1st day after injury (**FIGURE 4**).

At 4 weeks after injury, most of the sensory neurons were found to be normalized, although the size and the number of mitochondria were slightly decreased Golgi apparatus was still swollen and damaged (**FIGURE 5**). The motor neurons including large vacuoles were in a little improved state, these vacuoles were expanded and damaged the Golgi apparatus (**FIGURE 6**).

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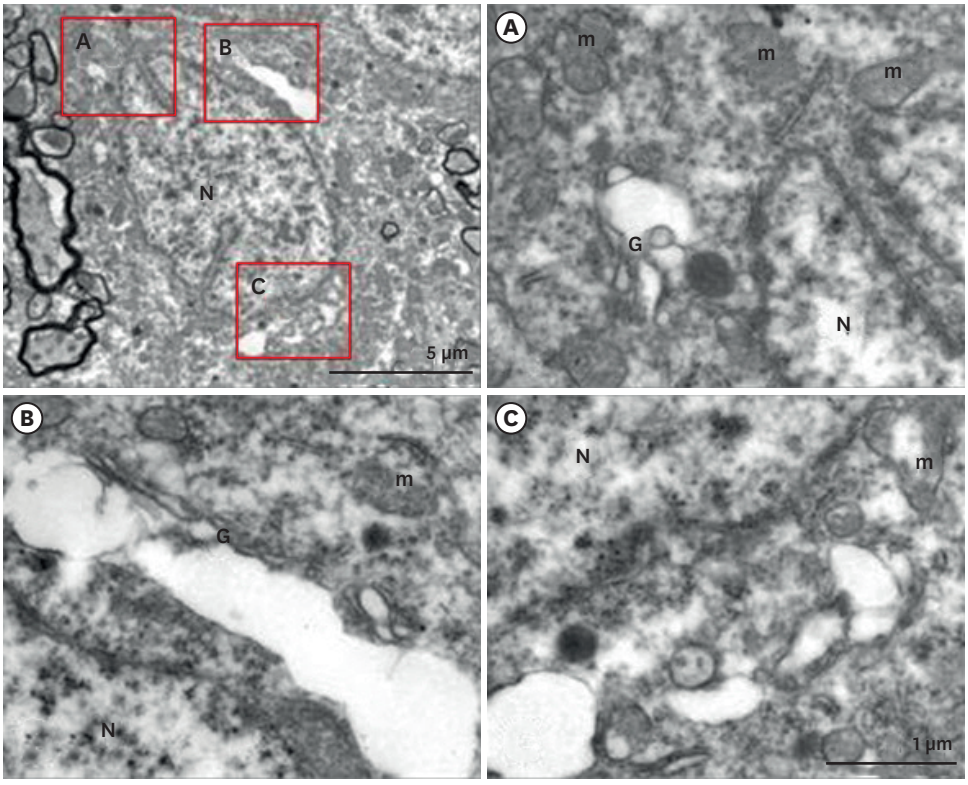

FIGURE 3. Ultrastructural observations of sensory neurons within/at 1st day after electrical injury. The second to the fourth photographs show higher magnification of red solid line boxes on the first photographs (A & C). The N of the sensory neuron is a relatively large and irregularly shaped N due to the formation of (B) expanded and damaged G with peri-N vacuole and (C) partial m are damaged.
N: nucleus, G: Golgi apparatus, m: mitochondria.

DISCUSSION

There are 2 types of electrically injured myelopathy early or late after electrical trauma. One of the representative examples of the early type is struck by lightning.[5] The early type of electrically injured myelopathy occurs a few hours after trauma: however, the aspect of recovery shows rapid and nearly complete within hours to days.[3,5] In this study, we used the same electrically injured myelopathy model of the previous report, and it is comparable to an early type injury model.

The neuron's cell body contains the nucleus, the neuron's control center. The cell body and the nucleus control the functions of the nerve cell. The cell body contains organelles in the nucleus. These tiny organelles are the ER, Golgi apparatus, and mitochondria, which produce energy, neurotransmitters, and protein. The mitochondria produce the energy for the proper function of the cell. The Golgi apparatus and ER produce and transport protein together. These proteins are the crucial elements for the new dendrites formation. New dendrites formation can make new links with other neurons.[2] According to the results of this study, in both the sensory and motor nerves, electron microscopic examination on 1st day after injury showed the direct damaged area, which appeared to be torn as physical damage, the damaged myelin sheath, and a vacuolated axon in the myelin sheath. But 4 weeks after injury, both sensory nerves had healed to a greater extent better than the motor neurons.

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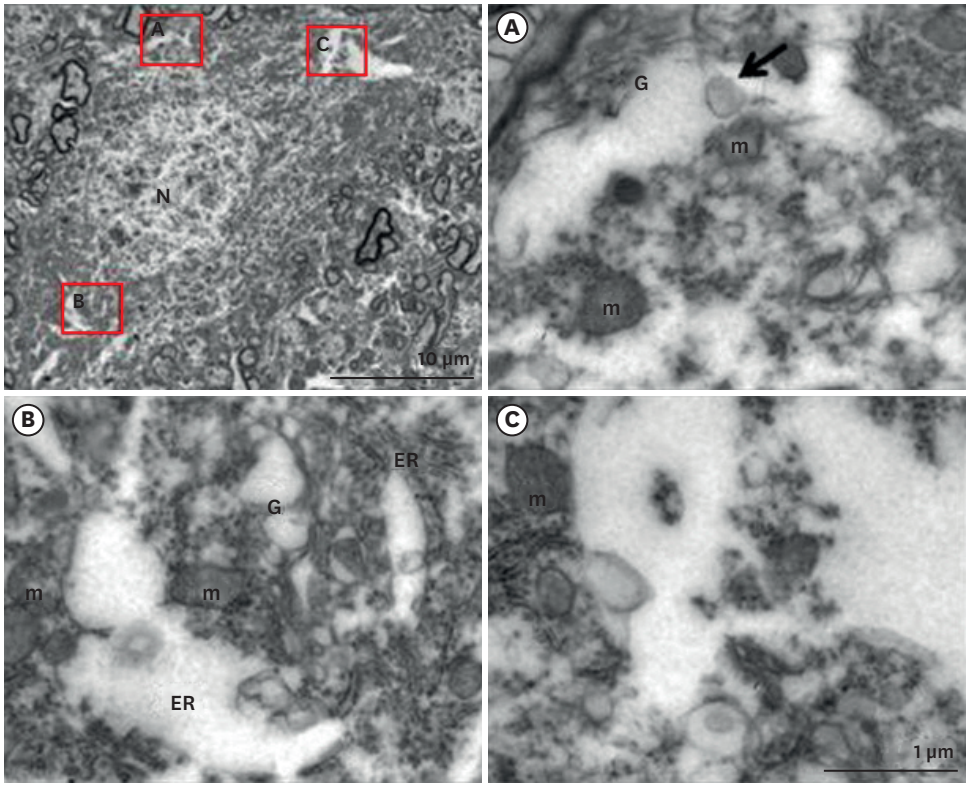

FIGURE 4. Ultrastructural observations of motor neurons within/at 1st day after electrical injury. The second to the fourth photographs show higher magnification of red solid line boxes on the first photograph. (A) Due to damage to the G, a vacuole (black arrow) is formed, and expanded G and (B) ER, (C) multi-membrane bounded vacuoles are observed.
N: nucleus, m: mitochondria, G: Golgi apparatus, ER: endoplasmic reticulum.

Usual electrical injured myelopathy is very subtle, and even MRI cannot show meaningful change or lesions.[1] To detect and evaluate anatomical changes of electrically injured myelopathy, we used Manganese-enhanced (ME)-MRI in our previous study.[5,7,10] In that study, ME-MRI at one day after electrical injury illustrated disruption of enhancement which directed spinal cord injury. However, ME-MRI showed normalization of the MRI findings 1 week, 2 weeks, and 1 month after injury. Therefore, we presumed that electrically injured myelopathy is too subtle to be detected by a routine MRI sequence and that structural repair of electrically injured myelopathy is speedy.

Furthermore, in a previous study, findings of cresyl violet staining illustrated a more decrease of neural cells in the ventral horn than in the dorsal horn.[5] Actually, we have yet to find the reason for these findings. We only presumed the different shapes and uneven distribution of the spinal cord's motor and sensory neural cells, which produces spatial non-uniformity. In this study, we showed similar damage to sensory and motor nerves on 1st day after the injury. Still there was a difference in the degree of recovery rate at 4 weeks after injury. Therefore, a larger current might pass through the ventral horn, and it may cause a difference in the injury severity. Our study results might be one of the causes of predominant motor weakness produced by electrically injured myelopathy clinically. A further study to assess this finding is needed, and it may provide a clue to understanding and treating spinal cord injury.

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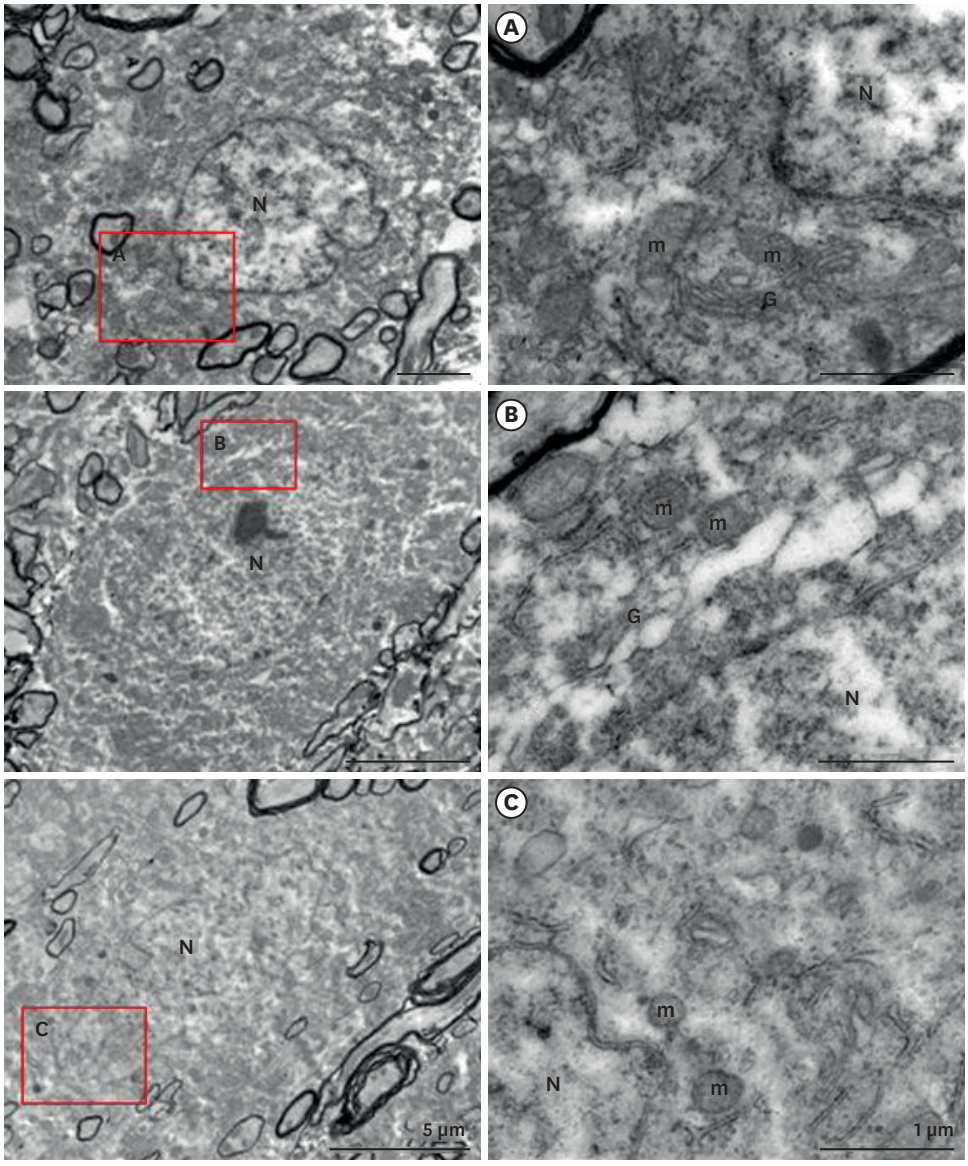

FIGURE 5. Ultrastructural changes of sensory neurons within/at 4 weeks after electrical injury. The right-side photographs show a higher magnification of red solid line boxes on the left side photographs. (A & C) The shape of the m is normal; however, the size and number of the m are decreased. (B) Some G are still swollen and damaged; however, the shape of most G is normal.
N: nucleus, m: mitochondria, G: Golgi apparatus.

Our study has some limitations. First, we only evaluated the electron microscopic findings and did not evaluate any other detailed data such as time course, neurological findings etc. Second, we did not perform a comparative evaluation between traumatic and electrically injured model.

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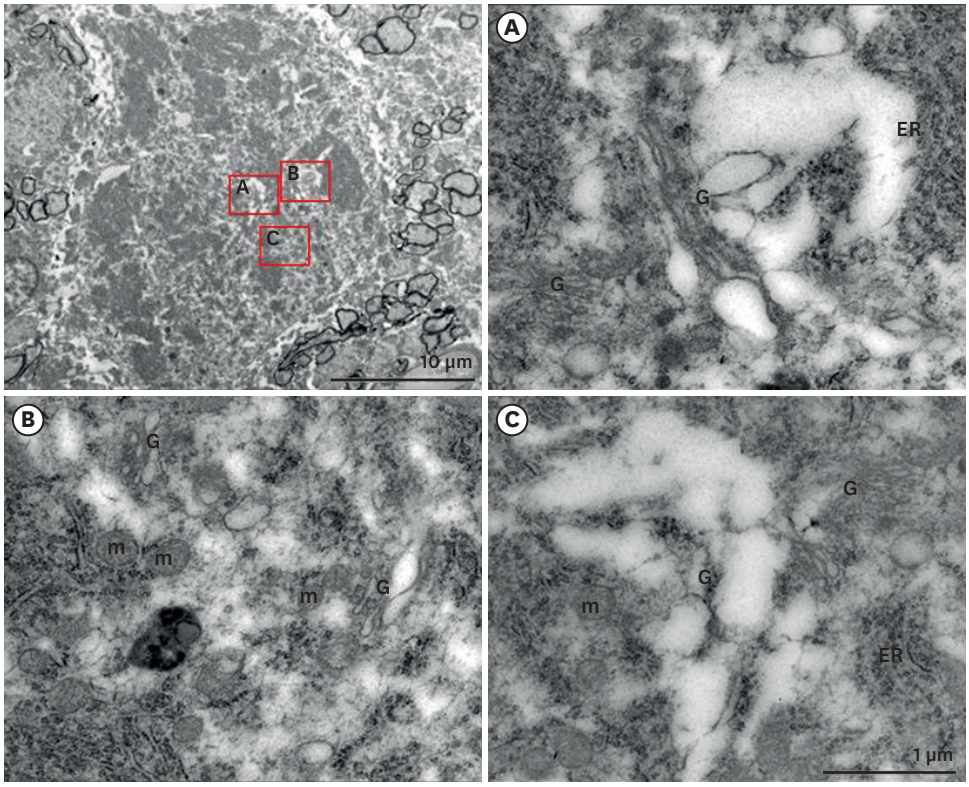

FIGURE 6. Ultrastructural changes of motor neurons within/at 4 weeks after electrical injury. The second to fourth photographs show higher magnification of red solid line boxes on the first photographs. (A-C) Motor neurons include large vacuoles, which are expanded and damage the G and ER. (B) The m are decreased in size.
N: nucleus, m: mitochondria, G: Golgi apparatus, ER: endoplasmic reticulum.

CONCLUSION

This study demonstrates the recovery of the ultrastructural injury in sensory neurons was more rapid than that in the motor neuron. Furthermore, the difference in the recovery of the neurons may be one of the causes of unique myelopathy in electrical injury.

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