Synapse involvement of the dorsal horn in experimental lumbar nerve root compression.

A light and electron microscopic study.

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A running head: Changes of dorsal horn induced by root compression

Key Words: Nerve root, Compression, Spinal cord, Synapse, Pathology, Radiculopathy, Lumbar lesion
**Study Design:** This study was aimed at investigating changes in the dorsal horn of the lumbar cord induced by mechanical compression using an *in vivo* model.

**Objectives:** To determine the effect of axonal flow disturbance in the dorsal horns induced by nerve root compression.

**Summary of Background Data:** Few studies have looked at changes of synapses within the dorsal horn caused by disturbance of axonal flow and the axon reaction as a result of mechanical compression of the dorsal root.

**Methods:** In mongrel dogs, the 7th lumbar nerve root was compressed for 1 week, or 3 weeks using a clip. After intravenous injection of Evans blue albumin, they were observed under a fluorescence microscope (FM) for the purpose of clarifying the function of the blood-spinal cord barrier. Morphologic changes of the synapses in the dorsal horn secondary to the nerve fiber degeneration were examined by light (LM) and electron microscope (EM). Changes on immuno-staining for substance P (SP), calcitonin gene-related peptide (CGRP), and somatostatin (SOM) in the dorsal horn were also examined.

**Results:** LM observation conducted 1 week after compression of the nerve roots revealed Wallerian degeneration of the myelinated nerve in the dorsal horn, and FM observation of these areas demonstrated edema formation resulting from damage of the blood-spinal cord barrier. Three weeks after the compression, EM observation revealed shrinkage of the axon terminals, ubiquitous presence of high electron density degeneration and presence of synapses whose contact with synapses was disrupted. Immuno-histochemical studies showed a marked decrease of SP, CGRP, and SOM.
staining in substance gelatinosa with Wallerian degeneration after compression of nerve root.

**Conclusion:** It is important to recognize that compressive disturbance of the nerve roots caused Wallerian degeneration not only at the site of compression of nerve roots but also at the synapses of spinal cord dorsal horns.
Key Points

• Morphologic changes in the dorsal horn of the lumbar cord induced by nerve root compression were examined by light and electron microscopy.

• It should be remembered that compressive nerve root damage is not restricted to the site of compression, but also extends to the synapses in the dorsal horn.

• Prolonged compression of the nerve root appears to result in irreversible damage to the synapses in the dorsal horn.
Introduction

Many patients suffer from radicular symptoms due to degenerative disease of the lumber spine. Nerve root compression associated with lumbar canal stenosis and disc herniation is a frequently encountered clinical problem. It now is commonly acknowledged that the nerve root is compressed, deformed, or stretched by the disc, facet, pedicle, or ligaments. However, acute compression of a normal nerve root does not always cause pain; instead, it can cause numbness, paresthesia, and motor weakness. So far, many papers about the reaction pattern of nerve roots to experimentally applied compression have been published.\textsuperscript{1-13} Consequently, it has been considered that development of radiculopathy associated with degenerative diseases of the spine is related to the circulation and metabolism in the nerve root. Compressed nerve roots can exist without causing any pain. These facts suggest that some secondary changes in and around the nerve root may be a critical factor in radicular pain.\textsuperscript{14-17} Understanding the anatomical substrate of the transmission of pain and temperature information to the central nervous system is an essential step in developing clinical approaches for pain relief. The primary afferent nerve fibers that convey pain and temperature information terminate in the spinal cord dorsal horn on interneurons and on spinoreticular and spinothalamic neurons.\textsuperscript{18,19}

Dorsal horn cells of the lumbar spinal cord project many terminals to Rexed laminae I to VI. In the zona spongiosa (Rexed I) and the substantia gelatinosa (Rexed II and III), synapses are formed by axon terminals of thin myelinated and unmyelinated fibers originating from the dorsal root that are involved in the transmission of pain and temperature sensation. In Rexed laminae I and II, 4 types of synapses can be identified, including axo-dendritic, dendro-axonic, axo-axonic, and dendro-dendritic
synapses. Investigation of the cat lumbar cord showed that 96.5% of the synapses in the substantia gelatinosa are axo-dendritic synapses. Large cells have been discovered in Rexed lamina I by Clarke and these correspond to the special cell layer that was termed marginal cells by Waldeyer. These cells are considered to be secondary sensory neurons in the pain pathway because retrograde degeneration occurs transection of the anterolateral funiculus of the spinal cord in humans to achieve analgesia.

Numerous immunohistochemical studies of neurotransmitters in the spinal dorsal horn and dorsal root ganglia have been conducted in a range of animals. However, there have been few studies into the effect of mechanical lumbar nerve root compression on neurotransmitter dynamics in the primary sensory neurons and the primary afferent nerve fibers. This was followed by a radioisotope study of Weinstein et al. showing a decrease of SP and an increase of VIP in the dorsal root ganglia of rabbits after exposure of the ganglia to 5-Hz vibration. Bandalamente et al. confirmed that mechanical compression of the dorsal root ganglia in rats resulted in an increase of the SP concentration in ipsilateral dorsal root ganglia cells and in the spinal dorsal horn, as measured using immunofluorescence and isotopes. Rothman et al. showed the changes of substance P and CGRP after cervical nerve root injury (compression or transection) using rats. They described spinal substance P and CGRP were increased over normal at day 1 for both injuries and decreased with time for CGRP after transection, which paralleled behaviors and substance P was significantly correlated with CGRP expression for both injuries. In these studies, the changes were linked to the appearance of pain, although different noxious stimuli were used. These studies also suggest that disruption of
axonal flow in the central branches of the primary sensory fibers caused by nerve root compression affects neurotransmitter metabolism in the synapses of the spinal dorsal horn and in the sensory neurons of the dorsal root ganglia, and may be closely implicated in the onset of pain and sensory disturbance. Thus, it appears that various neurotransmitters produced by the primary sensory neurons in the dorsal root ganglion (DRG) and transported to the spinal dorsal horn by axonal flow in the central branches of the dorsal root neurons are implicated in the development of radicular pain and sensory disturbance associated with nerve root compression. The synapses of lumbar dorsal horn should not be overlooked when considering the mechanism of low back pain, sciatica and sensory disturbance in the legs so it is important to understand the morphologic and functional changes that occur in synapses of the lumbar dorsal horn as a result of nerve root compression.

The aim of the present study was to investigate the mechanism underlying radicular symptoms by examining changes of lumbar dorsal horns following nerve root compression. In this study, we employed morphological and immunohistochemical methods to examine the changes in lumbar dorsal horns using the nerve root compression model. In this immunohistochemical study, we investigated substance P (SP) and calcitonin gene related peptide (CGRP), which is thought to play a role in pain sensation, and somatostatin (SOM), which is believed to be involved in temperature sensation.
Materials and methods

The experiment was carried out under the control of the local animal ethics committee in accordance with the guidelines on animal experiments in our university, Japanese government animal protection and management law, and Japanese government notification on feeding and safekeeping of animals. Twelve adult dogs, weighing 7 to 15 kg, were anesthetized with intramuscular injection of 3 ml of Ketalar (Ketamine 50 mg/ml; Warner-Lumbert, Morris Plains, NJ) and ventilated on a respirator under general anesthesia (O₂: 3 ml/min, N₂O: 3 ml/min, Halothane: 1.5 ml/min). Animals were maintained at constant physiologic levels during experiment. Each animal was placed in the prone position on a frame. The sixth and seventh lumbar laminae were removed, and the seventh lumbar nerve root was exposed widely on one side. The nerve root was clamped with a clip for microvascular suturing (Kouno Co, Chiba, Japan) at the midpoint between the dural sac and dorsal root ganglion. The 7th nerve root was exposed to compression at 7.5 gram force (gf) clipping power as our previous studies. In the previous study, the axonal flow was disturbed by compression at 7.5 gf (about 105.7 mmHg) or higher after 1 week of compression. That is, when compared with the pressure applied to the nerve root measured by Take et al. in patients with disc herniation, intensification of axonal flow disturbance observed in the present study was assumed to correspond to the pressure (20-235 mmHg [mean 111 mmHg]) applied to the nerve root by SLR of 30 degrees or more.

The incision was closed and the animal was allowed to recover. The animals were evaluated at 1 week (n=6) and 3 weeks (n=6) after clipping. After the appropriate period of nerve root
compression, Evans blue albumin (EBA, 10ml/kg, molecular weight approximately 59,000, Sigma Chemical Co., St.Louis, MO) was injected intravenously and allowed to circulate for 30 minutes. EBA was prepared by mixing 5% bovine albumin (Wako Chemical Co., Osaka, Japan) with 1% Evans blue (Sigma). The animals were fixed by intraaortal perfusion with 4% paraformaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.2 at 20°C.

The lumbar cord section was divided into 2 groups. At first, EBA tracer technique was used to investigate the status of the blood-spinal cord barrier function in the lumbar cord. EBA was prepared by mixing 5% bovine albumin (Wako Chemical Co., Osaka, Japan) with 1% Evans blue (Sigma). After the clamp was released, the tracers were administered intravenously. The EBA was allowed to circulate for 1 hour before the lumbar cord was removed. After the lumbar cord section was fixed with 4% paraformaldehyde for 24 hours, twenty-micron thick sections were mounted with 50% aqueous glycerin to be examined under the fluorescence microscope at 380 μW. The other sections were rinsed in 0.05M tris-HCl buffer, postfixed at room temperature for 3 hours in 2% OsO₄ in 0.1 M sodium cacodylate buffer, impregnated with 2% uranyl acetate, dehydrated in graded ethanol, and embedded in epoxy resin. For light microscopy, 1-3μm thick toluidin blue stained sections were used. For electron microscopy, ultrathin sections contrasted with uranyl acetate and lead citrate were examined under JSM2000 electron microscope.

Another 20 animals were used for immunohistochemical studies. The animals were evaluated at 1 week (n=5) and 3 weeks (n=5) after nerve root compression by clip. As the control group, 10 animals were evaluated at 1 week (n=5) and 3 weeks (n=5) after hemi-laminectomy. These animals
only had the seventh nerve root exposed. After the appropriate period of nerve root compression, potassium chloride was injected intravenously under general anesthesia to kill the animals and cardiac arrest was confirmed. The animals were perfused intracardially with saline followed by 0.1 mol/L phosphate buffer containing 4% paraformaldehyde (pH 7.4).

The spinal cord within the 7'th lumbar nerve root was harvested, and was immersed in the same fixative at 4°C for 24 hours. The specimens were rinsed with 10-30% sucrose in PB for 3 days. Then, the lumbar cords were cut transversely into 20-μm sections with a cryostat. The sections were immersed in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 for approximately 1 week, and then in hydrogen peroxide for 15 minutes to inactivate the peroxidase in red blood cells. The peroxidase-anti-peroxidase (PAP) method was used for immunohistochemical analysis. Sections were immersed in PBS containing 20% normal swine serum (NSS) for 1 hour, and reacted with antibodies for SP (1:500 dilution; Incstar, Stillwater, MN), CGRP (1:2000 dilution; Incstar, Stillwater, MN) or SOM (1:500 dilution; Incstar Stillwater, MN) at 4°C for 3 days. Then the sections were incubated in goat antirabbit IgG serum (1:200 dilution; Miles Labs, USA) for 2 hours at room temperature, followed by incubation in PAP complex (1:200 dilution; Dako, Denmark) for 30 minutes at room temperature. Next, the sections were reacted with 3',3'-diaminobenzidine (DAB, 0.4 mg/ml, Sigma) and 0.01% H2O2 for 10 minutes in 50 mM Tris-HCl buffer (pH 7.4) at room temperature. Finally, the sections were washed in PBS, mounted in DPX mountant for microscopy (BDH, England) without counterstaining, and examined under a standard light microscope.
The spatial extent of SP, CGRP and SOM immunoreactivity in Rexed I and II was quantitated using a color image analyzer (Mac SCOPE, Mitani Corp., Fukui, Japan).\textsuperscript{40} The image of an optical field of a stained specimen was collected using the objective lens. Each area in which the density significantly exceeded the determined threshold in each regular-setting region of interest (ROI, 144 X 144 pixels) was coded by colors indicating the intensity of staining. For each section, 3 ROI sampling was also performed to quantify intensity in the general background of the slide. Likewise, ROI sampling was also performed to quantify intensity in the general background of the slide. Average intensity of Rexed I and II was determined and subtracted from average background intensity, normalizing each section. Immunoreactivity was expressed as a ratio of the density in the spinal cord dorsal horn ipsilateral and contralateral to the lesion. The compressed and control groups were compared using a non-paired t test. Data were entered into a database and analyzed using SPSS statistical software, version 15.0.J (SPSS Inc, Chicago, IL).
Results

Changes of the nerve root after mechanical compression

On macroscopic examination of the 7’th lumbar cord following intravenous injection of EBA at 1 week after compression, there was dark blue staining of the dorsal root (Fig. 1A) and the dorsal horn (Fig. 1B) at the compressed side. After 1 and 3 weeks, intraradicular edema was observed not only at the site of compression but also in the central zone of the compressed dorsal root (Fig. 1C). The evidence of active Wallerian degeneration was also seen in the area of intraradicular edema (Fig. 1D). After 1 and 3 weeks, however, there was no extravasation of EBA in the dorsal root of the uncompressed site under fluorescence microscope (Fig. 1E). Histological examination revealed nerve fiber deformation but no appreciable Wallerian degeneration (Fig. 1F).

Changes of the dorsal horn after mechanical compression

After 1-week of compression, fluorescence microscopic observation of dorsal horn demonstrated edema formation resulting from damage of the blood-spinal cord barrier (Fig. 2B). However, the extravasation of EBA didn’t show in the normal side and the red fluorescence-emitting EBA was confined to the blood vessels, indicating that the blood-spinal cord barrier was intact. The nerve fibers emitted a green fluorescence (Fig. 2A). After 3 weeks compression, fluorescence microscopy showed marked extravasation of EBA. This fluorescence was stronger than that observed in the 1-week group, suggesting an increase of intramedullar edema (Fig. 2C).
Optical microscopic observation conducted 1 week after compression of the nerve roots revealed Wallerian degeneration at the myelinated nerve in the dorsal horn. Nodular changes of the myelin could be seen in the dorsal horn, and Wallerian degeneration was also evident in the area where edema was observed by fluorescence microscopy (Fig. 2E). After 3 weeks of compression, these changes became more marked, there were many deformed nerve fibers in the affected layers of Rexed I and II (Fig.2F). After 1 and 3 weeks, histological examination of the uncompressed side revealed nerve fiber deformation but no appreciable Wallerian degeneration (Fig. 2D) (Table 1).

On electron microscopy, somato-dendritic and axo-dendritic synapses were present in the unaffected layers of Rexed I and II. Synapse vesicles in the axon terminal constituting chemical synapses were abundantly found in which neurotransmitters were localized. In the normal side, spherical vesicles were concentrated at the presynaptic axon terminal in the somato-dendritic (Fig. 3A,B) and axo-dendritic synapses (Fig. 4A,B). Some dense core vesicles were seen in the somato-dendritic synapse. Spherical (S)-type axon terminals with stimulatory spherical vesicles and Flattened (F)-type axon terminals with inhibitory elliptical vesicles could be observed in the axo-dendritic synapses. In contrast, one week after compression, enlargement of the axon terminal was evident in Rexed I (Fig.3 C,D) and II (Fig. 4 C,D). Deformed vesicles, some of which were fused, were scattered about the axon terminal. Three weeks after compression, nerve filaments and degenerated mitochondria were increased in the postsynaptic terminals or dendrites, while ballooning of rough endoplasmic reticulum and fusion of ribosomes were observed in the neurons (Fig.3 E, F). There were many atrophied axon terminals showing high-electron-density degeneration, and
aggregation of degenerated vesicles at a site distant from the synapse could be seen (Fig. 4 E,F) (Table 1). These findings showed the disruption of synaptic contact.

**Immunohistochemical changes of the dorsal horn after mechanical compression**

There are many synapses in the dorsal horn where the central branches of the primary sensory fibres terminate. There was a rich network of SP (Fig.5A)- and CGRP (Fig.5B)-positive fibers in Rexed I and II, as well as SOM (Fig.5C)-positive fibers in Rexed II, in the control group and the contralateral side of the compression group, suggesting that these neurotransmitters are involved in transmission of information at the synapses. When the nerve roots were compressed for 3 weeks, the immunoreactivity of SP (Fig.5D)-, CGRP (Fig.5E)-, and SOM (Fig.5F)-positive fibres in the central branches was appreciably lower on the ipsilateral side than on the healthy (contralateral) side and the control group. Results of quantitative analysis of SP-, CGRP- and SOM immunoreactivity in dorsal horn are shown in Figure 5G and H. There was no reduction in the immunoreactivity of SP-, CGRP- and SOM-positive fibres in the dorsal horn after nerve root compression for one week (Fig.5G). After compression for 3 weeks, however, the immunoreactivity of these neurotransmitters was around 60-70% lower than in the control group. This appeared to be secondary to synaptic degeneration.
Discussion

A voluminous literature exists regarding the neuroanatomy and neurophysiology of nociceptive pathways in mammalian spinal cord.\textsuperscript{41-45} Synapses can be electrical or chemical, and the dorsal horn of the lumbar spinal cord principally contains chemical synapses. One of the major functions of chemical synapses is to mediate signalling between cells via neurotransmitters. According to the morphological classification of Gray, asymmetrical synapses classified as type I are involved in excitatory signalling and symmetrical synapses classified as type II are involved in inhibitory signaling.\textsuperscript{46} Uchizono\textsuperscript{47} reported that there are two types of synapses, one containing spherical vesicles in the axon terminal classified as spherical (S)-type synapses that is involved in excitatory signaling and the other containing elliptical vesicles classified as flat (F)-type synapses that is involved in inhibitory signalling, with the function of synapses being indicated by the morphology of the synaptic vesicles. Nalotzky et al.\textsuperscript{48} observed marginal cells in the cat by electron microscopy, and reported that 4 types of axon terminals (S-type, F-type, S-type containing cored vesicles, and a mixed S- and F-type) form synapses. They also reported that S-type terminals in the excitatory synapses of thin myelinated and unmyelinated fibers originating from the dorsal root ganglion undergo degeneration at the interfaces with marginal cells after transection of the dorsal root. On the other hand, after transection of fibers from neurons in the substantia gelatinosa that run in Lissauer’s tract, and provide inhibitory inputs to marginal cells, degeneration of F-type terminals (symmetric synapses) occurs in Rexed lamina I, while the cell bodies and proximal dendrites are inhibited by connecting to more than 3 times the number of F-type terminals compared with the distal dendrites.
The secondary degeneration of dorsal horn synapses secondary to Wallarian degeneration caused by crushing or cutting of the dorsal root and peripheral nerve have been investigated in detail by animals experiments.\textsuperscript{49} The present study revealed that almost all of the synapses in the dog lumbo-sacral dorsal root ganglion were axodendritic synapses on electron microscopy, while a large number of synaptic vesicles containing neurotransmitters were observed in the presynaptic terminals (chemical synapses).\textsuperscript{50} However, it is seldom realized that nerve root compression associated with intervertebral disc herniation and spinal canal stenosis influences the posterior horn of the spinal cord, so elucidation of the synaptic changes in the posterior horn is important for understanding the pathology of nerve root compression.

Axonal transport, a mechanism peculiar to the nervous system, conveys neurotransmitters and nerve growth factors and is closely involved in the transmission of information about environmental changes in the axon and target organ. Disruption of axonal flow therefore threatens nerve cell survival and is one cause of neural dysfunction. Recently, we reported on the changes occurring in the nerve root\textsuperscript{36} and dorsal root ganglion\textsuperscript{37} after mechanical compression of the dorsal root through which the central branches of primary sensory neurons travel. In the ventral root, we found that the Wallerian degeneration which occurs after nerve root compression, advances peripherally from the site of compression. In the dorsal root, however, Wallerian degeneration advances toward the spinal cord. Immunohistochemical examination of the site of nerve root compression, revealed the accumulation of SP, CGRP, and SOM in the axons peripheral to the site of compression in the dorsal root at one week after compression.\textsuperscript{30,33} We also showed that intraradicular edema not only occurs at the site of
nerve root compression, but also in areas affected by Wallerian degeneration. After nerve root compression, cells exhibiting central chromatolysis were seen in the dorsal root ganglion. Staining for SP, CGRP, and SOM was appreciably reduced in the small chromatolytic cells. In other words, nerve root compression disrupts axonal flow in the nerve fibers of the dorsal root. The resulting Wallerian degeneration leads to degeneration of the synapses in the dorsal horn where these nerve fibers terminate, and the axonal reaction causes central chromatolysis in the dorsal root ganglion where the cells bodies of these nerve fibers are located. One week after compression of the dorsal horn, Wallerian degeneration was seen in Rexed laminae I and II, while intramedullary edema appeared due to breakdown of the blood-spinal cord barrier. This change was considered to be caused by extravascular migration of monocyte-derived macrophages engaged in the removal of necrotic materials such as myelin.

Winkelstein et al. examined the relationship between injury severity in painful radiculopathy and spinal glial activation using rat lumbar radiculopathy model. In the result, more severe ligations of nerve root produced greater microglial activation, indication injury severity modulates spinal microglial activation. However, there is no mention about the changes of synapses in dorsal horn. In the our result, an immunohistochemical study indicated that there was a marked decrease in the staining of Lissauer’s tract, the SP and CGRP in Rexed I and II, and SOM-positive nerve terminals and these changes were considered to be attributable to degeneration of nerve terminals, i.e., the synapses of primary sensory fibers involved in the transmission of pain and temperature sensation. Accordingly, atrophy of presynaptic terminals and formation of high-electron-density degeneration
products in presynaptic terminals through the fusion of vesicles, as observed by electron microscopys, were considered to be suggestive of the loss of synaptic function. These morphological changes appear to explain the hypesthesia and analgesia of the lower limbs seen after nerve root compression caused by intervertebral disc herniation and spinal canal stenosis. However, this experimental model does not explain the mechanism responsible for pain, such as sciatica. If radiculopathy is mild, removal of the source of the compression will probably result in neuronal recovery and nerve fiber regeneration. However, prolonged compression of the nerve root appears to result in irreversible damage to the neurons in the dorsal root ganglion and the synapses in the dorsal horn.

As clinicians, we often come across patients with cauda equina and nerve root compression due to lumbar disc herniation or lumbar canal stenosis who continue to experience sensory disturbance and numbness long after surgical decompression of the nerve root, particularly patients with a long history of sensory disturbance in the lower extremities before surgery. It is therefore important to be aware that in patients with mechanical nerve root compression, dysfunction is not confined to degeneration at the site of compression, but also extends to the primary sensory neurons within the DRG as a result of the axon reaction. Patients with chronic sensory disturbance and numbness should therefore be fully informed of the fact that these symptoms will not resolve immediately after surgery.
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The submitted manuscript does not contain information about medical devices or drugs. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. Mr. Naruo Yamashita provided expert help with the photography. The authors would like to thank Mrs. Kyoko Shimokawa, Ms. Mika Osaki and Ms. Yukiko Horiuchi for their dedicated assistance in this study.
References


of intravenously injected protein tracers and gadolinium-enhanced magnetic resonance imaging.


Figure legends

Fig. 1. Changes of the L7-lumbar cord and nerve root after mechanical compression.

(A) Macroscopic micrograph of the 7th-lumbar root following intravenous injection of EBA. After 3-week compression, blue staining of the dorsal root was extending to the point of lumbar cord entry from the compression site. D: dura mater, DR: dorsal root, LC: lumbar cord, VR: ventral root, Arrow head: compression site.

(B) A transverse section of the L7-lumbar cord. There was dark-blue staining of the dorsal horn central to the compression site (A, arrow)

(C) A longitudinal section of the dorsal root under fluorescence microscope. Pooling of red fluorescent EBA was evident between the degenerative nerve fibers. The blood-nerve barrier was disturbed in the dorsal root central to the site of compression. Scale bars = 200 μm

(D) A transverse section of the dorsal root under light microscope. Marked intraradicular nerve fiber degeneration appeared. Toluidin blue stain, Scale bars = 100 μm.

(E) A longitudinal section of the uncompressed dorsal root under fluorescence microscope. EBA emits a bright red fluorescence in clear contrast to the green fluorescence of the nerve tissue. EBA was limited inside the blood vessels, and the blood-nerve barrier was maintained. Scale bars = 200 μm

(F) A transverse section of the uncompressed dorsal root under light microscope. No Wallerian degeneration was evident in the nerve root. Toluidin blue stain, Scale bars = 100 μm.
Fig. 2. Fluorescence (A-C) and light (D-F) micrographs of 7th-lumbar dorsal horn after nerve root compression.

A-C, There was no extravasation of the red fluorescence-emitting EBA and the blood-spinal cord barrier was preserved in the uncompressed side (A). After 1 week compression, however, intramedullary edema was evident (B). After 3 weeks compression, this fluorescence was stronger than that observed in the 1-week group, suggesting an increase of intramedullar edema (C). Scale bars = 100 μm, G: gray matter, W: white matter, I: layer of Rexed I, II: layer of Rexed II.

D-F, No Wallerian degeneration was evident in the dorsal horn of the uncompressed side (D). After 1 week compression, however, Wallerian degeneration was apparent in the dorsal horn at the compression side. Histological examination of these regions showed deformation of the myelin sheath (B). After 3 weeks compression, there was an increase of nerve fibers exhibiting obvious Wallerian degeneration in compared with the normal side (C). Toluidin blue stain. Scale bars = 100 μm, Lis: Lissauer tract, arrows: marginal cells.

Fig. 3. Electron micrographs of the somato-dendritic synapses obtained after dorsal root compression.

A, B, A bouton making contact with a marginal neuron dendrite in the unaffected layer of Rexed I. This type of bouton contained a mixture of clear spherical and dense-cored vesicles. (Original magnification, A: X3000, B: X30000).

C, D, After dorsal root compression for 1 weeks, there is clumping of synaptic vesicles and increased
electron-lucency of the bouton indicating early degeneration. (Original magnification, A: X5000, B: X30000).

E,F, After dorsal root compression for 3 weeks, a bouton contacting a marginal cell showing more advanced degenerative changes, with clumping and dissolution of vesicles and abnormal mitochondria. (Original magnification, A: X3000, B: X30000).


Fig. 4. Electron micrographs of the axo-dendritic synapses obtained after dorsal root compression

A,B, Many axo-dendritic synapse existed in the unaffected neuropil (Rexed II). The spherical and flattened vesicles were abundantly found in which neurotransmitters were localized and were concentrated at the synapse in the presynaptic axon terminal. (Original magnification, A: X10000, B: X 30000).

C,D, After dorsal root compression for 1 weeks, a degenerative bouton contacting a dendrite appeared in the affected layers of Rexed II. Clumping and dissolution of vesicles and increased electron-lucency of the bouton are shown. (Original magnification, A: X10000, B: X 30000).

E,F, After dorsal root compression for 3 weeks, shrinkage of the axon terminal in the course of osmiophilic (high-electron-density) degeneration and presence of synapses whose contact with synapses was disrupted. (Original magnification, A: X10000, B: X 30000).

At: axon terminal (At-F: with flat vesicles, At-S: with spherical vesicles), D: dendrite, d: dendritic
Fig. 5. Changes of SP (A,D), CGRP (B,E), and SOM (C,F) immunoreactivity after dorsal root compression. (A,B,C: control group, D,E,F: compression group) After 3 weeks of compression (D,E,F), there was an appreciable decrease in the number of SP-, CGRP-, and SOM-positive fibers in the Rexed I and II on compression side (arrows) when compared with the control group (A,B,C). Scale bars = 200 μm. G,H. Densitometric image analysis. G. After compression for 1 week (*: p = 0.084, #: p = 0.029, +: p = 0.132), G. After compression for 3 weeks (**: p<0.05).
Table 1. Changes of the dorsal horn after nerve root compression. Fluorescence microscope, 0: indicates no extravasation of Evans blue albumin (EBA), 1: extravasation of EBA in gray matter, 2: marked extravasation of EBA in dorsal horn, Optical microscopic, +: Wallerian degeneration in the dorsal horn, -: No Wallerian degeneration, Transmission electron microscope (TEM), +: Enlargement of the axon terminal and/or high-electron-density degeneration occurred in the dorsal horn. -: No degeneration of synapses.