Effects of Vibration Therapy on Immobilization-Induced Hypersensitivity in Rats
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Background. Cast immobilization induces mechanical hypersensitivity, which disturbs rehabilitation. Although vibration therapy can reduce various types of pain, whether vibration reduces immobilization-induced hypersensitivity remains unclear.

Objective. The purpose of this study was to investigate the preventive and therapeutic effects of vibration therapy on immobilization-induced hypersensitivity.

Design. The experimental design of the study involved conducting behavioral, histological, and immunohistochemical studies in model rats.

Methods. Thirty-five Wistar rats (8 weeks old, all male) were used. The right ankle joints of 30 rats were immobilized by plaster cast for 8 weeks, and 5 rats were used as controls. The immobilized rats were divided randomly into the following 3 groups: (1) immobilization-only group (Im, n = 10); (2) vibration therapy group 1, for which vibration therapy was initiated immediately after the onset of immobilization (Im+Vib1, n = 10); and (3) vibration therapy group 2, for which vibration therapy was initiated 4 weeks after the onset of immobilization (Im+Vib2, n = 10). Vibration was applied to the hind paw. The mechanical hypersensitivity and epidermal thickness of the hind paw skin were measured. To investigate central sensitization, calcitonin gene-related peptide (CGRP) expression in the spinal cord and dorsal root ganglion (DRG) was analyzed.

Results. Immobilization-induced hypersensitivity was inhibited in the Im+Vib1 group but not in the Im+Vib2 group. Central sensitization, which was indicated by increases in CGRP expression in the spinal cord and the size of the area of CGRP-positive neurons in the DRG, was inhibited in only the Im+Vib1 group. Epidermal thickness was not affected by vibration stimulation.

Limitations. A limitation of this study is that the results were limited to an animal model and cannot be generalized to humans.

Conclusions. The data suggest that initiation of vibration therapy in the early phase of immobilization may inhibit the development of immobilization-induced hypersensitivity.
Joint immobilization causes various degenerative and atrophic changes in intact organs and tissues, including muscular disuse atrophy and joint contractures in humans. In addition, it has recently been shown to cause thermal and mechanical hypersensitivities that are observed as symptoms of pain disorders in humans and animals. In humans and animals with these hypersensitivities, a withdrawal response occurs even when thermal and mechanical stimuli are below the normal threshold. In particular, Terkelsen et al conducted an experiment in humans who underwent cast immobilization of the forearm for 4 weeks and observed that transient movement of the elbow, wrist, and carpometacarpal joints provoked pain and increased the skin temperature on the pulp of each finger, along with mechanical and cold hypersensitivities in the distal parts of the immobilized extremity after cast removal.

Although the mechanism underlying immobilization-induced hypersensitivity remains unclear, Nakano et al suggested that cast immobilization induces epidermal thinning and increases peripheral nerve profiles in the upper dermis, which may in part be responsible for this phenomenon. In addition, immobilization-induced hypersensitivity has been reported to relate to plasticity in the central nervous system. Ushida and Willis reported that immobilization of the rat forearm for 4 weeks induced an increase in the number of neurons with a wide dynamic range and movement-responsive neurons and further demonstrated that these changes induced mechanical allodynia. Another recent study in rats reported that cast immobilization for 5 weeks induced phenotypic changes associated with alterations in the size of calcitonin gene-related peptide (CGRP)-positive neurons in the dorsal root ganglion (DRG) and increased CGRP distribution in the spinal dorsal horn. Calcitonin gene-related peptide has been suggested to play a role in the processing of nociceptive information in primary afferents and the spinal cord. Furthermore, the release of CGRP into the spinal dorsal horn induces mechanical hyperalgesia. Increased CGRP expression and changes in the size distribution of CGRP-positive neurons in the DRG also have been observed in various pain models.

We previously examined mechanical and thermal hypersensitivities in the rat hind paw during cast immobilization of the hind limbs for 4 and 8 weeks. Mechanical and thermal hypersensitivities were defined as a decreased paw withdrawal threshold to heat and mechanical stimulation. Sensitization was analyzed by measuring the distribution of CGRP in the spinal dorsal horn. Sensitization causes hypersensitivity via activation of peripheral and central neurons. Our results suggested that immobilization induced central sensitization in the spinal cord. Pain caused by changes in peripheral tissues and central sensitization (ie, immobilization-induced hypersensitivity) may disrupt the progress of rehabilitation in patients who require cast immobilization owing to injuries such as fracture or sprain.

Meanwhile, vibration has been used, although not frequently, in exercise therapy and physical therapy to enhance muscle strength and power and to improve balance function. Some studies have evaluated the effect of vibration therapy in patients with chronic stroke and Parkinson disease. Vibration therapy also was reported to noninvasively affect blood circulation in the skin. Previous studies demonstrated that vibration accelerates
Vibration Therapy and Hypersensitivity

blood flow via venule vasodilation in human skin. However, in addition, vibration has been applied as a nonpharmacological technique used to reduce pain. Vibration has been reported to relieve pain in the musculoskeletal system both in clinical and experimental studies. Kosek and Hanson demonstrated a reduction in mechanosensitivity after 15 minutes of sustained vibration at 100 Hz to muscles of the forearm. Vibration also reduced pain in patients with acute and chronic musculoskeletal pain. Broadbent et al. reported that vibration therapy reduced muscle soreness and associated inflammatory markers after downhill running in healthy individuals. Evidence also shows that whole-body vibration training effectively reduces chronic back pain, probably by relaxing the back muscle.32,33 In a rat model, vibration was shown to inhibit nociceptive receptors in dorsal horn neurons. However, although many studies have demonstrated that vibration therapy can reduce various types of pain, whether vibration reduces immobilization-induced pain remains unclear.

The purpose of this study was to investigate the preventive and therapeutic effects of vibration therapy on immobilization-induced hypersensitivity. We examined the mechanical hypersensitivity and epidermal thickness of the hind paws of rats that received vibration therapy during immobilization. Moreover, sensitization in the primary afferents and spinal cord was examined by immunohistochemical analyses of CGRP expression in the spinal cord and DRG.

Materials and Method

Animals
Thirty-five 8-week-old male Wistar rats were obtained from Kudo Laboratories (Tokyo, Japan). The rats were housed 2 or 3 per cage at 24°C, with water and food available ad libitum.

Experimental Design
To investigate the preventive and therapeutic effects of vibration therapy on immobilization-induced hypersensitivity, 30 rats were immobilized for 8 weeks and divided randomly into an immobilization-only group (Im group, n = 10) and 2 immobilization plus vibration groups (Im + Vib1 and Im + Vib2, n = 20). In the Im + Vib1 group (n = 10), vibration therapy was initiated just after the onset of immobilization and continued throughout the immobilization period (8 weeks); this group was used to examine whether vibration therapy, as a primary prevention, inhibits immobilization-induced hypersensitivity. In the Im + Vib2 group (n = 10), vibration therapy was initiated 4 weeks after the onset of immobilization, at the midpoint of the immobilization period. The data from this group were compared with those from the Im + Vib1 group to determine whether vibration therapy is effective as a secondary prevention for immobilization-induced hypersensitivity and to determine the more effective starting point and duration of vibration therapy. The remaining 5 rats were used as the control group (Fig. 1A).

Immobilization
The rats in the Im, Im + Vib1, and Im + Vib2 groups were anesthetized with sodium pentobarbital (40 mg/kg); subsequently, their right ankle joints were fixed in full plantar flexion using plaster casts (ipsilateral side). The plaster cast, which was positioned from above the knee joint to the distal foot, was changed every day to prevent progressive loosening of the cast consequent to muscle atrophy. The period of cast immobilization was 8 weeks. The rats in both the Im and control groups were anesthetized to control for the influence of anesthesia. One rat in the Im + Vib2 group was excluded from analysis due to the induction of edema.
Vibration Therapy and Hypersensitivity

Vibration Therapy
A vibrator (Mediacraft Inc, Saitama, Japan) with a 3.5-cm probe diameter was used to apply vibration at a frequency of 80 Hz and amplitude of 5 mm to the plantar surface of the ipsilateral (right) hind paw (Fig. 1B). Vibration therapy was performed for 15 minutes, once daily, 5 days per week. Vibration was initiated just after the onset of immobilization in the Im+/Vib1 group and 4 weeks after the onset of immobilization in the Im+/Vib2 group. During vibration, the plaster cast was removed temporarily, and the rats were placed in a cloth restrainer because ankle joint contracture prevented those in the 3 Im groups from walking using their hind limbs. The restrainer allows the animal to dance safely, with the legs positioned to be free and under no loading.4

Behavioral Testing
The paw withdrawal response (PWR) was tested to confirm whether the immobilized (Im, Im+/Vib1, and Im+/Vib2 groups) and control rats exhibited mechanical hyperalgesia and allodynia. The PWR was determined using von Frey filaments (4 and 15 g). The von Frey filament, which was used to assess mechanical allodynia, as previously described.57 In an identical manner, withdrawal response frequency was approximately 21% upon application of the 15-g von Frey filament, which was used to assess mechanical hyperalgesia.

Sampling and Preparation
At the end of the immobilization period, the rats were deeply anesthetized with sodium pentobarbital (40 mg/kg), and transcardial perfusion was performed using saline followed by 4% paraformaldehyde in a 0.1-M phosphate buffer. Subsequently, the ipsilateral (right) and contralateral (left) glabrous skin tissues of the bilateral hind paw (10 × 10 mm), the L4–L5 segments of the spinal cord, and the associated DRG were removed. After soaking in 10% and 20% sucrose for 24 hours, the tissues were embedded in tragacanth gum, frozen, and stored at −80°C. The frozen tissue sections (10 μm) were cut using a cryostat (CM1950, Leica Mikrosysteme Vertrieb GmbH, Leica, Germany). The tissue samples were handled randomly for blinding in all the analyses.

Histological Analysis of Skin
Five sections from each skin tissue sample (1 mm apart) were stained with hematoxylin-eosin (H&E) to identify morphological characteristics and signs of pathological changes such as inflammation in skin tissue. Skin tissue from the central domain in each footpad was photographed (×200) by section. Epidermal thickness was measured using Image J software (Scion Corp, Frederick, Maryland), as in a previous study.4 Briefly, the measurement was made by drawing a vertical bar from the lower edge of the basal layer to the top of the granular layer at 7 random points in the skin tissue images (×200). The measurements were performed in 5 sections from each sample.

Immunohistological Analysis for CGRP
Calcitonin gene-related peptide expression in the dorsal horn of the spinal cord and CGRP-positive cells in the DRG were identified using fluorescent immunohistochemical staining with anti-CGRP antibody in 5 sections of each spinal cord and each DRG sample. To inhibit endogenous peroxidases, the sections were incubated for 30 minutes at room temperature with 0.3% H2O2 dissolved in methanol. Next, the sections were blocked for 20 minutes with 5% bovine serum albumin dissolved in phosphate-buffered saline (PBS), followed by incubation with an anti-CGRP polyclonal antibody (1:500 rabbit, Immunostar Inc, Hudson, Wisconsin) overnight at 4°C. The sections underwent three 5-minute washes in PBS. Subsequently, they were incubated with goat anti-rabbit immunoglobulin G conjugated to Texas Red (1:500, Vector Laboratories Inc, Burlingame, California) diluted in PBS for 1 hour at room temperature. Quantitative evaluation of CGRP expression was performed using image analysis software (NIS-Element version 3, Nikon Instruments Inc, New York, New York). Quantity fluorescence was defined as the intensity of the CGRP expression in the superficial layers (laminae I and II) and deep layers (laminae III–VI) of the spinal dorsal horn in 5 sections per tissue, as in a previous study.7 The CGRP-positive cells were counted in 5 unbiased images (×200) covering the entire DRG area, and the number of CGRP-positive cells per unit area (1 mm2) was reported. Next, the size of the cross-sectional area of CGRP-positive neurons was measured.
Data Analysis
All the data are presented as means and standard errors (SEs). Statistical analysis was performed using multiple comparisons after repeated-measures analysis of variance. The Scheffé post hoc test was used for comparison of the 4 groups in each week and between each group and baseline. Differences were considered significant at $P<.05$.

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Results

Mechanical Hypersensitivity
In the Im group, the PWR of the ipsilateral hind paw began to increase significantly 3 weeks after immobilization, when measured using the 4-g von Frey filaments (Fig. 2A). Similarly, the PWR with 15-g von Frey filaments began to increase significantly 2 weeks after immobilization in the ipsilateral hind limb (Fig. 2C). The increase in PWR in the Im group persisted for the entire immobilization period measuring with both the 4-g and 15-g von Frey filaments (Figs 2A and 2C). In the Im + Vib1 group, the PWR to 15-g von Frey filaments began to increase significantly 2 weeks after immobilization, but this effect was not seen with the 4-g von Frey filaments (Figs. 2A and 2C). The PWR to 15-g von Frey filaments in the Im + Vib1 group was reduced significantly relative to

Figure 2.
Time course of changes in the mechanical sensitivity of the rat hind paw. Paw withdrawal response (PWR) was measured during 10 repetitive stimulations with 4- and 15-g von Frey filaments in the rats’ ipsilateral paws (A, C) and contralateral paws (B, D). The data are presented as mean ± standard error. Im = immobilization-only group; Im + Vib1 = vibration therapy group 1, in which vibration therapy was initiated immediately after the onset of immobilization; Im + Vib2 = vibration therapy group 2, in which vibration therapy was initiated 4 weeks after the onset of immobilization. *$P<.05$, significantly different from the control group. **$P<.05$, significantly different from the Im group. †$P<.05$, significantly different from the baseline in each group.
the Im group at 3 weeks after immobi-
lization (Fig. 2C). In the Im+Vib2 group, the PWR to the 4-g von Frey filaments began to increase significantly 3 weeks after immobilization (Fig. 2A). Similarly, the PWR to the 15-g von Frey filaments began to increase significantly 2 weeks after immobilization (Fig. 2C). After initiation of vibration therapy (4 weeks after immobilization), the PWR in the Im+Vib2 group continued to increase, similar to that in the Im group (Figs. 2A and 2C). Ultimately, the PWR results with 4- and 15-g von Frey filaments in the Im+Vib2 group were similar to those in the Im group (Figs. 2A and 2C). The PWR of the contralateral hind paw in the Im, Im+Vib1, and Im+Vib2 groups was not significantly different from that in the control group during the immobilization period (Figs. 2B and 2D).

**Epidermal Thickness**

In the ipsilateral skin surface, the rough and scaling appearance of the stratum corneum was confirmed in the Im, Im+Vib1, and Im+Vib2 groups, whereas inflammation was not observed in any of the groups (Figs. 3A–D). The mean ipsilateral epidermal thickness in the Im, Im+Vib1, and Im+Vib2 groups was thinner than that in the control group. No differences in epidermal thickness were observed across the 3 Im groups (Fig. 3E). No significant difference in epidermal thickness was observed in the contralateral hind paw across all 4 groups (Fig. 5E).

**CGRP Expression Intensity in the Spinal Cord**

The CGRP immune response in the ipsilateral superficial layer (laminae I and II) of the dorsal horn in the Im, Im+Vib1, and Im+Vib2 groups was stronger than that in the control group (Figs. 4A–D). In particular, CGRP-positive neural fibers were clearly observed in the deep layer (laminae III–VI) of the dorsal horn in the Im and Im+Vib2 groups but not in the control group (Figs. 4A–D). Analyses of CGRP expression intensity in the superficial layer of the dorsal horn revealed higher values in the Im, Im+Vib1, and Im+Vib2 groups than in the control group (Fig. 4E). This increase in CGRP expression intensity was stronger in the Im and Im+Vib2 groups than in the Im+Vib1 group (Fig. 4E). The same results were obtained from the deep layer of the dorsal horn as from the superficial layer (Fig. 4F). The CGRP expression intensity in the contralateral spinal dorsal horn in the Im, Im+Vib1, and Im+Vib2 groups was not significantly different from that in the control group (Figs. 4E and 4F).

**Histological Change in the Glabrous Skin of the Hind Paw**

In the ipsilateral skin sections obtained from representative animals in the control (A), Im (B), Im+Vib1 (C), and Im+Vib2 (D) groups are shown. Scale bar=50 μm. Note the rough and scaling appearance of the stratum corneum (sc) and the epidermal thinning in the Im (B), Im+Vib1 (C), and Im+Vib2 (D) groups. The epidermal thickness of the ipsilateral and contralateral glabrous skin of the hind paw was measured (E). The data are presented as mean±standard error. Im=immobilization-only group; Im+Vib1= vibration therapy group 1, in which vibration therapy was initiated immediately after the onset of immobilization; Im+Vib2= vibration therapy group 2, in which vibration therapy was initiated 4 weeks after the onset of immobilization; ep=epidermis; der=upper dermis. *P<.05, significantly different from the control group.

**CGRP-Positive Neurons in the DRG**

In the ipsilateral DRG, no marked immunohistological change in the CGRP-positive cells was observed (Figs. 5A–D). When histograms of the size distribution of CGRP-positive cells were compared with the control group, the peak of the histogram shifted to the right (toward the larger size) in the Im (Fig. 5F) and Im+Vib2 (Fig. 5H) groups but not in the Im+Vib1 group (Fig. 5G). The mean (±SE) area of CGRP-positive cells in the ipsilateral DRG was significantly larger in the Im (499.0±10.6 μm²) and Im+Vib2 (506.4±11.0 μm²) groups than in the control group (434.8±9.8 μm²) and Im+Vib1 (461.1±10.4 μm²) groups (Fig. 5I). The area of CGRP-positive neurons in the contralateral DRG was not significantly different from that in the control group (Fig. 5I). Moreover, no significant differences in the number of CGRP-positive cells per unit area in the ipsilateral and contralateral DRGs were observed across the 4 groups (Fig. 5J).

**Discussion**

Here we examined the effect of vibration therapy on immobilization-induced hypersensitivity in rats. To achieve the study objective, the hind
The limb of rats was immobilized with a cast for 8 weeks, and they received vibration therapy during the immobilization period. The induction of immobilization-induced hypersensitivity similar to that observed in previous studies was confirmed in all the immobilized rats. The Im+Vib1 group, which received vibration therapy throughout the immobilization period, showed a decrease in PWR to the 4- and 15-g von Frey filaments, demonstrating that vibration, when used as a primary prevention, inhibits immobilization-induced hypersensitivity. Notably, there was no significant difference in PWR to the 4-g von Frey filaments between the Im+Vib1 and control groups at each week. We believe that this lack of statistical difference between the Im+Vib1 and control groups was not due to a small sample size (control group, n=5; Im+Vib1 group, n=10), because a power analysis indicated that the sample size was sufficient to detect a difference of at least 1 point in PWR with 80% power (alpha level=5%). However, the Im+Vib2 group, which received vibration therapy 4 weeks after the onset of immobilization, did not show any change in PWR to the 4- and 15-g von Frey filaments compared with the Im group. At 4 weeks after the onset of immobilization, immobilization-induced hypersensitivity had already occurred. Thus, applying vibration therapy as a secondary prevention after 4 weeks of immobilization was not effective in treating immobilization-induced hypersensitivity. These results show that the initiation of vibration therapy in the early phase of immobilization may be important for the treatment of immobilization-induced hypersensitivity.

Cast immobilization of the hind limb may give rats stress. For example, when their bodies are wrapped with soft wire mesh and adhesive tape, rats develop mental and physiological stress, and this stress triggers mechanical hypersensitivity by causing changes in neural systems. Stress-induced hypersensitivity is thought to develop bilaterally in the limbs. However, cast immobilization in the present study induced mechanical hypersensitivity of the immobilized hind paw but not the contralateral paw. Considering that the immobilization-induced hypersensitivity in these rats was unilateral, this change could not be attributed to changes induced by stress.

Histological analysis of skin tissue, spinal cord, and DRG samples shed some light on the possible underlying mechanism of immobilization-induced hypersensitivity. Nakano et al suggested that epidermal thinning induced by cast immobilization may be one of the mechanisms of immobilization-induced hypersensi-
We also found that cast immobilization resulted in epidermal thinning in the immobilized rats. However, the epidermal thinning was not inhibited by vibration therapy in the Im\/H11001 Vib1 and Im\/H11001 Vib2 groups. Therefore, we speculate that the effect of vibration therapy on immobilization-induced hypersensitivity does not act on the skin tissues.

A convincing mechanism of immobilization-induced hypersensitivity reported in previous studies is central sensitization based on changes in the dorsal horn of the spinal cord and DRG. Calcitonin gene-related peptide expression in the dorsal horn of the spinal cord was reported to increase greatly in immobilized rats. Calcitonin gene-related peptide is a neurotransmitter of nociceptive primary afferents, is produced in a selective subpopulation of CGRP neurons in the dorsal root ganglion (DRG).

Figure 5.
The number and cross-sectional area of calcitonin gene-related peptide (CGRP)-positive cells in the dorsal root ganglion (DRG). Representative photomicrographs of the CGRP immunostaining of the ipsilateral DRG in the control (A), Im (B), Im+Vib1 (C), and Im+Vib2 (D) groups are shown. Scale bar=100 μm. Histograms of the size distribution of CGRP-positive ipsilateral DRG neurons in the control (E), Im (F), Im+Vib1 (G), and Im+Vib2 (H) groups are shown. The mean of size of CGRP-positive cells (I) and number per unit area were measured in the ipsilateral and contralateral DRGs (J). The data are presented as mean ± standard error. Im=immobilization-only group; Im+Vib1=vibration therapy group 1, in which vibration therapy was initiated immediately after the onset of immobilization; Im+Vib2=vibration therapy group 2, in which vibration therapy was initiated 4 weeks after the onset of immobilization. *P<.05, significantly different from the control group. #P<.05, significantly different from the Im group. §P<.05, significantly different from the Im+Vib1 group.
of sensory neurons, peptidergic neurons, of small and medium size in the DRG.\textsuperscript{42,45} It is mainly produced in small neurons with nonmyelinated axons (C-fibers) and medium-sized neurons with myelinated axons (A\textdelta-fibers) that release it to the superficial (laminae I and II) and deep layers (laminae III–VI) of the dorsal horn of the spinal cord, respectively. Hence, the increases in CGRP expression in both the superficial layers (laminae I and II) and deep layers (laminae III–VI) of the dorsal horn in the Im group indicate overexpression of CGRP by both small and medium-sized sensory neurons in the DRG. The increase of secreted CGRP in the dorsal horn of the spinal cord causes enhanced excitability for afferent stimulation via a release of substance P in both layers; this central sensitization in the spinal cord leads to an increase in PWR to von Frey filaments.\textsuperscript{44–46} The increase in PWR to the 15-g von Frey filament (noxious stimulation) in the Im group, which indicates the development of mechanical hyperalgesia, might be supported by this mechanism of central sensitization induced by CGRP overexpression.

Additionally, central sensitization can occur by functional plasticity of sensory neurons. In the Im group, the mean area of CGRP-positive cells in the ipsilateral DRG was significantly larger than in the control group, but the number of CGRP-positive cells did not change. This phenomenon may be explained by a phenotype switch of primary sensory neurons.\textsuperscript{47,48} When peripheral tissue and nerve are exposed to an abnormal situation such as lack of sensation or inflammation, nonpeptidergic medium-sized neurons, which have myelinated axons (A\textdelta-fibers), become peptidergic neurons and begin to express CGRP. By contrast, peptidergic small neurons change to nonpeptidergic neurons and stop expressing CGRP; therefore, the total number of CGRP-positive cells in the DRG does not change. In the context of abnormal neuropeptide expression, nonnoxious stimulation to peripheral tissue may be conveyed as noxious stimulation to the central nervous system. These changes also may influence the CGRP expression in the dorsal horn of the spinal cord. Although the details have not yet been fully confirmed, the phenotype switch of neurons in the DRG is considered one of the mechanisms of allodynia, for which CGRP is an important marker.\textsuperscript{6,47,48} Thus the increase in PWR to the 4-g von Frey filament (nonnoxious stimulation) in the Im group might be supported by central sensitization induced by a phenotype switch of sensory neurons.

The intensity of CGRP expression in the ipsilateral dorsal horn and the size of the CGRP-positive neurons in the ipsilateral DRG were decreased significantly in the Im+Vib1 group compared with the Im group. Vibration therapy inhibited central sensitization and immobilization-induced hypersensitivity via the prevention of abnormal CGRP expression. We previously confirmed that no signs to indicate inflammation were apparent in biochemical blood analysis of rats exposed to the same immobilization model.\textsuperscript{7} Additionally, no histological change to indicate inflammation was observed in skin tissues from any group in the present study. Therefore, we believe the effect of vibration therapy on immobilization-induced hypersensitivity did not depend on peripheral tissue changes such as inflammation.

There are 2 possible mechanisms for the effect of vibration therapy on immobilization-induced hypersensitivity. First, Pavel et al\textsuperscript{49} demonstrated that the repeated vibration directly affected retrograde axonal transport in the primary sensory neurons and decreased in the production of CGRP via decrease of nerve growth factor in the DRG in normal rats. They suggested that the changes induced by vibration trigger health problems such as hand-arm vibration syndrome.\textsuperscript{49} Vibration also was reported to induce a decrease in the firing rate of nociceptive neurons via depression of neurotransmission in the dorsal horn of the spinal cord in cats, and this vibration-induced inhibition is considered one of the causes of analgesia.\textsuperscript{55,50} However, for immobilization-induced hypersensitivity characterized by an increase in CGRP, vibration might produce a therapeutic effect in sensory neurons.

Second, the lack of sensory input to peripheral tissue due to cast immobilization might induce changes to the nervous system, and vibration stimulation might provide enough sensory input to prevent the change in CGRP expression. A similar effect of vibration therapy also was reported in other disorders. For example, Gay et al\textsuperscript{51} showed that vibration therapy can reduce pain in patients with complex regional pain syndrome (CRPS) type 1. This disorder is thought to be caused, in part, by disturbance of sensorimotor integration due to a loss of sensory input by immobilization or disuse.\textsuperscript{52–54} Vibration therapy might be an effective technique to supplement the loss of sensory input and to inhibit hypersensitivity and pain. To further clarify the mechanism underlying the effect of vibration therapy on immobilization-induced hypersensitivity, further studies are needed to determine its causes.

No effect of vibration therapy on immobilization-induced hypersensitivity was observed in the Im+Vib2 group. The Im+Vib1 and Im+Vib2 groups differed in terms of vibration therapy onset time and duration. In the Im+Vib1 group, vibration therapy was initiated just after immobili-
Vibration Therapy and Hypersensitivity

zation and continued for a period of 8 weeks. By contrast, in the Im+Vib2 group, vibration therapy was initiated 4 weeks after immobilization and continued for a period of 4 weeks. The duration of the therapy in the latter group seems insufficient because the PWR to the 15-g von Frey filaments in the Im+Vib1 group was reduced significantly relative to the Im group at 3 weeks after immobilization. Thus, the effect of vibration therapy will appear within 4 weeks in immobilized rats. We speculate that the lack of inhibition of immobilization-induced hypersensitivity was not due to the delayed initiation of vibration therapy, which was applied after the induction of central sensitization and hypersensitivity in the Im+Vib2 group. We previously showed that the CGRP expression level in the dorsal horn of rats immobilized for 4 weeks was significantly increased compared with that in control rats. We suggest that sensory input by vibration stimulation is ineffective for rats that have already developed spinal cord sensitization. At that point, vibration stimulation may possibly be input as noxious or excessive stimulation. These results suggest that early initiation of vibration therapy is important for the treatment of immobilization-induced hypersensitivity during immobilization.

One limitation of this study is that the results are limited to an animal model and cannot be generalized to humans. In addition, the duration of vibration therapy in the Im+Vib2 group was half that in the Im+Vib1 group. There is a possibility that the lack of effect in the Im+Vib2 group might be due to a lower vibration exposure. However, the PWR in the Im+Vib1 group was decreased significantly compared with the Im group by 3 weeks from the initiation of vibration therapy, whereas the PWR in the Im+Vib2 group was not yet decreased 4 weeks from the initiation of vibration therapy. Therefore, we believe that initiation of vibration therapy during the early phase of immobilization is more effective for reducing immobilization-induced hypersensitivity. Nonetheless, this issue could not be resolved in the current study, and further experimentation is necessary. In future studies, not only the starting point but also the duration of vibration therapy should be considered.

Another possible limitation of this study is that brain samples were not included in the analysis. A supraspinal mechanism possibly contributes to the effect of vibration on immobilization-induced hypersensitivity. In regard to other pain disorders (eg, CRPS), vibration therapy has been proposed to relieve pain via a supraspinal mechanism in a previous study, although the details of this process remain unclear. Sensory input from vibration stimulation also was suggested to affect the remodeling of sensory and motor cortex. We believe that the effect of vibration therapy occurs not only in the spinal cord but also in the brain. This possibility is supported by the phenomenon of vibration stimulation reducing mechanical sensitivity even in healthy individuals. Because the analysis of sensory input using brain samples will be difficult to perform in histological studies, electrophysiological and imaging analyses should be considered in future studies in animals and humans.

In conclusion, cast immobilization causes hypersensitivity and central sensitization, possibly due to the excessive distribution of CGRP expression in the spinal cord, and initiation of vibration therapy at the early phase of immobilization may inhibit these changes. Hence, vibration therapy may be effective for preventing the induction of hypersensitivity in patients undergoing cast immobilization of a limb in clinical rehabilitation.

Dr Nakano provided concept/idea/research design. Dr Nakano and Mr Hamaue provided writing. Mr Hamaue, Dr Sekino, Miss Chuganji, and Dr Sakamoto provided data collection. Mr Hamaue, Dr Sekino, Miss Chuganji, Dr Sakamoto, and Dr Oriuchi provided data analysis. Dr Yoshimura and Dr Okita provided project management. The authors thank Sumihisa Honda, Nagasaki University Graduate School of Biomedical Sciences, for helpful advice on statistical analysis.

The experiments were approved by the Ethics Review Committee for Animal Experimentation of Nagasaki University (approval no. 1305201061).

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