Motor cortex stimulation and neuropathic pain: how does motor cortex stimulation affect pain-signaling pathways?

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OBJECTIVE Neuropathic pain is often severe. Motor cortex stimulation (MCS) is used for alleviating neuropathic pain, but the mechanism of action is still unclear. This study aimed to understand the mechanism of action of MCS by investigating pain-signaling pathways, with the expectation that MCS would regulate both descending and ascending pathways.

METHODS Neuropathic pain was induced in Sprague-Dawley rats. Surface electrodes for MCS were implanted in the rats. Tactile allodynia was measured by behavioral testing to determine the effect of MCS. For the pathway study, immunohistochemistry was performed to investigate changes in c-fos and serotonin expression; micro–positron emission tomography (mPET) scanning was performed to investigate changes of glucose uptake; and extracellular electrophysiological recordings were performed to demonstrate brain activity.

RESULTS MCS was found to modulate c-fos and serotonin expression. In the mPET study, altered brain activity was observed in the striatum, thalamic area, and cerebellum. In the electrophysiological study, neuronal activity was increased by mechanical stimulation and suppressed by MCS. After elimination of artifacts, neuronal activity was demonstrated in the ventral posterolateral nucleus (VPL) during electrical stimulation. This neuronal activity was effectively suppressed by MCS.

CONCLUSIONS This study demonstrated that MCS effectively attenuated neuropathic pain. MCS modulated ascending and descending pain pathways. It regulated neuropathic pain by affecting the striatum, periaqueductal gray, cerebellum, and thalamic area, which are thought to regulate the descending pathway. MCS also appeared to suppress activation of the VPL, which is part of the ascending pathway.

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NEUROPATHIC pain is often severe, and it can afflict patients for their entire lifetime. It can be caused by direct damage to the nervous system, and by neuronal degeneration. Patients with degenerative diseases such as Parkinson’s disease or dementia may experience neuropathic pain as a complication. Many studies have found that neuropathic pain can be induced by indirect nerve damage, but the mechanisms have not yet been clearly established. The symptoms of neuropathic pain differ among patients, making treatment difficult. Although many studies of neuropathic pain and its control have been conducted, the mechanisms behind neuropathic pain are still not clear.

Motor cortex stimulation (MCS) is a treatment modality used to modulate neuropathic pain by electrical stimulation. It was introduced in 1991 by Tsubokawa et al. and has since been used in clinical practice. MCS has advantages for clinicians and patients: MCS is simpler and easier to implement for pain modulation than other surgical methods, such as direct nerve stimulation and neurectomy, and can be considered an alternative for pain control. Among the many modalities involving electrical
stimulation, the main advantage of MCS is its relatively low degree of invasiveness: the stimulation electrodes for MCS are placed in the epidural space. Other treatment modalities have weaknesses compared with MCS: pharmacological treatment could cause addiction or require increasing dosages, and direct stimulation of peripheral nerves or neurectomy may cause irreversible nerve damage. MCS could provide a safer approach to pain modulation and offer clinicians and patients another option for the treatment of pain. However, the action mechanism of MCS is still unclear, and its effectiveness for pain modulation is not as high as other methods. MCS probably acts via the primary motor cortex (M1), which modulates the thalamic area or zona incerta (ZI) via direct or indirect pathways, and the thalamic area and the ZI modulate the secretion of opioids or serotonin. Detailed studies of the action mechanism of MCS and its ability to modulate pain are urgently required.

Therefore, we designed our study to investigate MCS using experimental animals. We induced neuropathic pain in rats and applied MCS to these animals. We used immunohistochemistry, micro-PET (mPET) imaging, and electrophysiological methods to observe any changes resulting from MCS. Specifically, we wanted to understand how disease alters brain activity and how electrical stimulation modulates brain activity and pain symptoms. Therefore, we set a region of interest (ROI) for observing changes in neuronal activity in the brain and measured acquired brain activities using electrophysiology. However, MCS produces electrical stimulation artifacts that can obscure real differences in the activity that occur with and without electrical stimulation. To solve this problem, we removed stimulation artifacts using an artifact removal method that we devised.

Methods
General Surgical Procedures

We designed 3 different experiments for studying the relationship between neuropathic pain and pain suppression by MCS. We used 47 Sprague-Dawley rats. The rats weighed 150–170 g at delivery to our animal facility. After the initial week of adaptation to the environment (see below), their weight was 190–200 g. The first experiment was conducted to observe behavioral changes induced by MCS, the second was an mPET imaging study conducted to observe changes in brain activity, and the last employed electrophysiology to measure functional changes in small brain structures.

In the first experiment, 3 groups of animals were used: normal (Normal, n = 10), neuropathic pain (Pain, n = 10), and neuropathic pain + MCS (MCS, n = 11). The mPET experiment comprised 3 similar groups: normal (Normal, n = 3), neuropathic pain (Pain, n = 3), and neuropathic pain + MCS (MCS, n = 3). Additionally 2 groups were established for the electrophysiological study: normal (Normal, n = 2) and pain + MCS (MCS, n = 5).

This study was conducted according to the guidelines of the Ethics Committee of the International Association for the Study of Pain and the Institutional Animal Care and Use Committee of Yonsei University. It was approved by the Institutional Animal Care and Use Committee of Yonsei University.

Before the induction of neuropathic pain, rats were housed for 1 week in the rat facility for environmental adaptation. After this initial week, the rats (at that time weighing 190–200 g) were anesthetized using phenobarbital sodium (50 mg/kg). To induce neuropathic pain, we followed a protocol for the spared nerve injury model. We exposed the rat’s left sciatic nerve by means of a small incision through the skin and muscle. Under a surgical microscope (Olympus), the 3 major divisions of the sciatic nerve (tibial, sural, and common peroneal nerves) were separated carefully. Before transection of the tibial and common peroneal nerves, we ligated these 2 nerves using silk suture thread proximal to the cutting point. Finally, we removed a 2-mm section from each of these 2 nerves. Hemostasis was achieved, and the cut was closed with sutures.

Electrical Stimulation

We fabricated a custom surface electrode for MCS using liquid crystal polymer (LCP, Fig. 1) that covers the hindlimb area of the right primary motor cortex. One week after pain induction surgery, rats were anesthetized using pentobarbital sodium (50 mg/kg, intraperitoneally) and fixed using a stereotactic frame (Narishige). We then opened each rat’s scalp to expose the skull. To place the electrode on the region of the primary motor cortex that controls the right hindlimb area, we made a small rectangular opening to expose the dura (2.0 mm × 2.0 mm). The coordinates were −0.2 to +1.8 mm from bregma and 0.5 to 2.5 mm from the midline. The electrode was placed on the dura mater and was firmly fixed using bolts and glue. After all the procedures had been performed, the scalp was secured with sutures.

For the electrical stimulation of the motor cortex, we stimulated each rat’s primary motor cortex at a frequency of 130 Hz, for 60 μsec, at an amplitude of 2.5 V, and the stimulation parameters were simultaneously monitored using an oscilloscope (HDS1022 M-N, Lilliput Technol-
ogy Co., Ltd.). Behavioral tests for pain threshold were performed. We measured tactile allodynia during electrical stimulation (Fig. 2). Each rat’s mechanical stimulation (pain) threshold was measured 3 times during each electrical stimulation phase: 30 minutes before electrical stimulation to measure baseline levels (Pre); 30 minutes after the start of electrical stimulation (ON); and 30 minutes after electrical stimulation ceased (OFF).

**Behavioral Testing**

Tactile allodynia was measured by placing each rat inside an acrylic cage (8 × 10 × 20 cm) on a wire mesh grid that allowed the clinician to reach the rat’s hind paw using von Frey filaments. After 1 hour of adaptation, innocuous mechanical stimulation was applied to the ipsilateral hind paw using von Frey filaments with 0.4 g, 0.6 g, 1 g, 2 g, 4 g, 6 g, 8 g, and 15 g of bending force by the up-down test method. After obtaining the von Frey filament response results, we calculated each rat’s pain threshold using the 50% threshold calculation formula.6

During electrophysiological recording, we applied mechanical stimulation using von Frey hairs (300 g, 180 g, 15 g, 4 g, 2 g, and 0.4 g of bending force) and applied pinching pressure stimulation using a clamp (Moria MC43, F.S.T.). Each stimulus was applied to the rat’s hind paw for 20 seconds.

**Immunohistochemistry**

Rats were anesthetized using urethane (1.3 g/kg), sacrificed by perfusion with normal saline, and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). The L3–5 section of the spinal cord was carefully dissected. Two days postfixation, spinal cords were processed as frozen sections; first they were cryoprotected in 30% sucrose, and then 30-μm sections were cut on a cryostat. Sections were then subjected to immunohistochemical staining to visualize c-fos and serotonin. Prepared tissue sections were incubated for 18–36 hours in primary antibody. For c-fos or serotonin labeling, an affinity-purified rabbit antibody raised against recombinant human c-fos (1:100, PC05, Calbiochem) or an affinity-purified rabbit antibody raised against recombinant human serotonin (1:100, ab16007, Abcam) was diluted in 10% normal serum and incubated at 4°C for 24 hours. Sections were incubated for 30 minutes in biotinylated secondary antibodies at 37°C (1:100, BA-1000 for rabbit and BA-9200 for mouse, both from Vector). Sections were processed using an ABC kit (DAKO) and diaminobenzidine (DAB) staining, and then rinsed briefly in PBS and distilled water. After dehydration and clearing of sections, slides were mounted in Zylene/Permount (3:1, Permount:Zylene). Microscopic images of laminae I–III of the left dorsal horn were obtained using a microscope (magnification ×400, Olympus).

**PET Scanning**

A 2-deoxy-[18F]fluoro-d-glucose ([18F-FDG]) mPET scan was performed to measure brain glucose metabolism. Each rat was kept in a cage for 30 minutes for adaptation before [18F-FDG] injection. The rat was lightly anesthetized using 2% isoflurane, and [18F-FDG] (2 mCi) was administered intraperitoneally. After administration, the rats were placed inside the cage for 60 minutes. In the Pain and MCS groups, we performed behavioral testing to encourage glucose uptake. The rats were anesthetized with 2% isoflurane before the mPET scan, which was performed for 30 minutes. During the scan, the rat’s body temperature was maintained by means of a heating pad with a temperature sensor.

After acquisition, PET data were reconstructed and aligned using an MRI rat brain template based on the rat brain atlas.36 Each ROI from the thalamic area, cerebellum, and left and right striatum was analyzed using AMIDE software (open-source software).

**Electrophysiology**

We recorded neuronal activity in the ventral posterolateral nucleus (VPL) by extracellular recordings of single unit activity. Rats were anesthetized with urethane (1.3 g/
Artifact Removal

We used custom artifact removal technique to observe changes in the firing rate of VPL neurons during electrical stimulation of the motor cortex (MCS). Stimulation artifacts were removed according to the method of Ryu et al.\(^4^3\) that is briefly described here. To remove stimulation artifact and minimize any residual artifact, a template for artifact subtraction was constructed using a curve-fitting method. First, the artifact waveform was divided into several segments and each segment was curve-fitted with a 2nd to 4th order polynomial. The length of each segment was adjusted according to the shape of the segment; usually a 50- to 100-sample length (2–4 msec) was appropriate in the present study. The order of the polynomial was chosen to avoid curve-fitting the spike waveform in the artifact. After all the segments were curve-fitted, they were assembled to construct a template. For our experimental data, the period of 2 msec from the stimulation onset was flattened because the artifact waveform was saturated, and spikes could not be detected during this period. In the final step, the constructed template was subtracted from the original waveform. As a result, the artifact waveform was effectively removed. This procedure was repeated for all the waveforms and finally the artifact-free neural signals were obtained. After the artifacts were removed, spikes were detected, and the firing rates with and without MCS were calculated and compared.

Statistical Analysis

Data are expressed as a mean ± SEM. To compare the effect of MCS, statistical analysis was performed using paired t-tests. To perform statistical analyses between groups, the Kruskal-Wallis 1-way ANOVA was used, followed by Tukey’s post hoc and Fisher’s least significant difference post hoc tests. When comparing neuron spikes, a Student t-test was performed. In all tests, p values < 0.05 were considered significant (p < 0.001, highly significant). All statistical analyses were performed using SPSS software (version 15, SPSS Inc.).

Results

Effect of MCS on Neuropathic Pain

In our behavioral test, the mechanical threshold for the Pain group was 0.39 ± 0.02 g, which is significantly lower than that of the Normal group (18.0 ± 0.0 g). The mechanical threshold for the MCS group was higher than that of the Pain group (3.56 ± 0.56 g) but did not exceed that of the Normal group (Fig. 3).

The pain threshold was decreased by electrical stimulation of the primary motor cortex. The baseline pain threshold was 0.40 ± 0.02 g (Fig. 4, Pre). During stimulation, the pain threshold increased significantly to 3.56 ± 0.56 g (Fig. 4, ON). We then stopped the electrical stimulation to determine whether MCS increased the threshold directly. The threshold decreased to 0.52 ± 0.06 g (Fig. 4, OFF).

We measured the pain threshold for both ipsilateral and contralateral pain stimuli to verify the effect of electrical stimulation. In our behavioral tests for ipsilateral pain stimuli, tactile allodynia was changed by MCS. For ipsilateral paws, the pain threshold was increased from 0.40 ± 0.02 g to 3.56 ± 0.56 g by electrical stimulation, and decreased to 0.52 ± 0.06 g after the electrical stimulation ceased (Fig. 5, ipsilateral). However, for contralateral paws, the pain threshold was not changed by MCS. All the thresholds from contralateral paws remained at 18.0 ± 0.00 g (Fig. 5, contralateral).

Immunohistochemistry

We labeled c-fos and serotonin in sections from the L3–5 levels of the spinal cord to observe any changes due to the pain state and the state in which electrical stimulation modulated pain. The c-fos labeling in laminae I–III of the left dorsal horn was increased by the onset of neuropathic pain. The c-fos expression decreased slightly with electrical stimulation (Fig. 6A–C). With serotonin labeling, serotonin immunoreactivity was slightly increased in the Pain group. In the MCS group, serotonin labeling was increased to a greater extent than that observed in the Pain group (Fig. 6D–F).

mPET Scan

We observed changes in glucose metabolism in the striatum, thalamic area, and cerebellum. In the striatum, glucose uptake was decreased by neuropathic pain, while...
Changes in tactile allodynia in response to electrical stimulation (MCS). "Pre" represents the response before electrical stimulation (baseline), "ON" represents the response during MCS application, and "OFF" represents the response 30 minutes after cessation of electrical stimulation. The pain threshold was significantly increased by MCS; on termination of the stimulation, the threshold reverted to a level close to baseline. Values on the y-axis indicate pain threshold (g). ***p < 0.001, paired t-tests.

Comparison of the behavioral test results between ipsilateral and contralateral pain stimuli for verifying the adequacy of MCS. To determine whether the MCS effects were ipsilateral or bilateral, we compared the behavioral results from both ipsilateral and contralateral stimuli. "Pre" represents the period before electrical stimulation (baseline), "ON" represents the response during MCS application, and "OFF" represents the response 30 minutes after ceasing the electrical stimulation. For ipsilateral stimuli, the pain threshold was significantly increased by MCS and was reversed after the stimulation ceased. However, on the contralateral side, the thresholds were at the highest level and were not changed by electrical stimulation. Values on the y-axis indicate pain threshold (g). ***p < 0.001, paired t-tests.

Neuronal Activity in the VPL During MCS

Figure 9A shows the change in neuronal activity caused by mechanical stimulation of the rat hindlimb. Neuronal firing was rapidly increased by mechanical stimulation and was higher than in the resting state. Figure 9B shows artifacts caused by MCS. We removed these stimulation artifacts using our custom artifact removal technique (Fig. 9C) and calculated the neuronal firing rate during electrical stimulation (Table 1).

To demonstrate sensitization by stimulation intensity and its changes in response to MCS in the Pain group, we performed a series of mechanical stimulations: first, application of pinch pressure and, then, stimulation with von Frey filaments at 300 g, 180 g, 15 g, 2 g, and 0.4 g of bending force (Fig. 10, No MCS, Table 1). For stimulation with 2 g and 0.4 g of bending force, the number of spikes was not significantly different. We assumed that 2 g and 0.4 g of stimulation were too light to evoke a significant increase in action potentials in the VPL. During MCS, all neuronal activity significantly decreased for every trial (resting state, 300 g, 180 g, 15 g, 2 g, and 0.4 g). In the resting state, the number of spikes was decreased (to 0.28 ± 0.41) by MCS.

Discussion

We have previously published several papers on the use of...
Mechanism of MCS in neuropathic pain

of electrical stimulation for the modulation of neuropathic pain, but it is only with the present set of experiments that we are beginning to get a clear sense of how electrical stimulation suppresses neuropathic pain. Although many studies have reported pain modulation using electrical stimulation, none have adequately explained the mechanism of pain suppression. MCS is an electrical stimulation modality for pain modulation, and there are many studies on MCS for neuropathic pain, but the pain modulation mechanism still needs clarification. The only plausible explanation that has been suggested for the effect of MCS is that it acts as a descending modulator. More studies are needed to fully understand the mechanism of pain modulation by electrical stimulation.

Fig. 6. Immunohistochemistry results for c-fos and serotonin labeling. A–C: Labeling for c-fos in rat spinal cord sections from the Normal group (A), the Pain group (B), and the MCS group (C). D–E: Labeling for serotonin in rat spinal cord sections from the Normal group (D), the Pain group (E), and the MCS group (F). The arrowheads show c-fos or serotonin labeling. Original magnification ×400. Figure is available in color online only.

Fig. 7. Representative axial rat brain images acquired by mPET scan showing ROIs in the striatum (A–D), thalamic area (E–H), and cerebellum (I–L). A, E, and I are from the Normal group; B, F, and J are from the Pain group; and C, G, and K are from the MCS group. D, H, and L are images that subtract ROIs in the MCS group from those of the Pain group. Figure is available in color online only.
required to understand the relationship between MCS and neuropathic pain. We hypothesized that MCS acts as not only a descending modulator but also an ascending modulator of pain pathways.

Therefore, to clarify how MCS changes the sensation of pain and how it affects each node in the brain network or pain-signaling system, we used behavioral testing, immunohistochemistry, imaging (mPET), and electrophysiological methods on experimental animals.

**How Does MCS Affect the Descending Pathway?**

To investigate how MCS can suppress neuropathic pain, we established animal models of peripheral neuropathic pain. The pain threshold was decreased after surgery but was reversed to a nearly normal state by MCS. We demonstrated that MCS can effectively suppress neuropathic pain (Fig. 4).

In the comparison of sensory thresholds from ipsilateral and contralateral pain stimuli, the threshold for the contralateral side always showed the highest value and was not changed by electrical stimulation, while the threshold for the ipsilateral side was changed by pain surgery and MCS (Fig. 5). Therefore, we conclude that MCS can only modulate neuropathic pain.

We hypothesized that MCS acts as a descending modulator. The primary motor cortex that we targeted is the center for the planning and execution of movement activity with other motor areas and projects to the internal capsule and spinal cord through the corticospinal tract. Because of these features of the primary motor cortex, we hypothesized that electrical stimulation of the primary motor cortex could be closely related to descending modulation.

Fonoff et al. and Lucas et al. hypothesized that MCS stimulates serotonin secretion in the periaqueductal gray (PAG) directly, or indirectly via the zona incerta (ZI). Therefore, we expected to observe changes in serotonin levels in the spinal cord. To verify this, we used immunohistochemical methods for observing changes in serotonin reactivity. First, to verify whether neuropathic pain had been established, we observed changes in c-fos in the spinal cord, as c-fos is used as a marker for determining the onset of neuropathic pain. Any type of external stimulation increases c-fos expression, and its level depends on the strength of the stimuli. Therefore, expression would be increased by painful peripheral stimulation. We observed an increase in c-fos expression with neuropathic pain, which was decreased by MCS (Fig. 6A). Therefore, we suggest that MCS effectively suppresses neuropathic pain. Second, we measured changes in serotonin expression in the spinal cord. Serotonin is known to be one of the descending pain modulators, like opioids. It is released by painful stimulation. In our results, the amount of serotonin was slightly increased in the Pain group. This is a normal feature of neuropathic pain, because opioids and serotonin are released automatically on painful stimulation as a form of self-protection, but the amount is not usually high. In the MCS group, the amount of serotonin released was more than in the Pain group. Therefore, we have demonstrated that MCS activated the PAG and finally increased the amount of serotonin; this is consistent with the hypothesis stated in previous reports. From these findings, MCS appears to be a descending modulator of neuropathic pain.

**Using mPET to Determine How MCS Changes the Brain Network**

To study how MCS works in pain modulation, we performed mPET imaging using $^{18}$F-FDG. We demonstrated that neuropathic pain decreased glucose metabolism in the cerebellum, striatum, and the thalamic area (Fig. 7B, F, and J). During electrical stimulation, the activity of the cerebellum, striatum, and thalamic area was increased. Specifically, the increase in glucose metabolism in the right striatum...
and right thalamic area was higher with MCS than that on the left side (Fig. 7C, G, and K).

Regarding the metabolic changes in the striatum, this structure is known to modulate neuropathic pain by releasing dopamine, and it has been established that activation of the dopamine D2 receptor in the striatum attenuates neuropathic pain. Like opioids, dopamine is released in response to painful stimuli. Dopamine also seems to be implicated in placebo-induced analgesia. Therefore, the striatum has been regarded as a descending modulator in pain suppression. We observed decreased activation of the striatum in the absence of electrical stimulation, and the activation increased in response to MCS. This may demonstrate that the striatum plays a key role as a descending modulator in pain attenuation.

The changes observed in the cerebellum, in which glucose metabolism was decreased in the absence of MCS and increased by MCS, are interesting because it is difficult to find evidence for a relationship between the cerebellum and neuropathic pain. The cerebellum is known as the main center for movement control. It receives sensory signals from the spinal cord and participates in precise control of movement. In pain-signaling pathways, noxious signals evoked by painful mechanical stimulation propagate to the brain through the spinothalamic tract. Most of the noxious signals arrive at the sensory part of the thalamic nuclei, but the spinothalamic tract branches into the cerebellum. This suggests that the cerebellum is not a direct modulator of pain, but we suggest that the changes in activity in the cerebellum result from a relationship between pain and an animal’s movements or responses. Animals could tend to minimize their movements when in

![Fig. 9. Changes in VPL neuronal activity during MCS. A: Raw waveform recorded in the VPL during mechanical stimulation. From 30 to 50 seconds, the neuronal firing rate increased considerably with mechanical stimulation (pinching pressure). B: Waveform recorded during MCS. From 20 to 80 seconds, the recorded waveform is obscured by large stimulation artifacts. C: Waveform after removal of the stimulation artifacts (in panel B). Although residual artifacts remain both at the beginning and the end of the MCS period, neuronal activity was clearly revealed in the time period during which mechanical stimulation was applied (indicated by a solid line). The red bar indicates the time of electrical stimulation (MCS), and the blue bar indicates the time of pain stimulation. Figure is available in color online only.](image)

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<th>Condition</th>
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<th>MCS</th>
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<tr>
<td>Resting</td>
<td>3.56 ± 2.08</td>
<td>0.28 ± 0.41</td>
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<tr>
<td>Pinch pressure</td>
<td>11.09 ± 6.35</td>
<td>1.53 ± 1.32</td>
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<tr>
<td>300 g</td>
<td>8.37 ± 5.07</td>
<td>1.24 ± 1.48</td>
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<td>180 g</td>
<td>6.96 ± 4.77</td>
<td>1.73 ± 1.39</td>
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<td>15 g</td>
<td>4.45 ± 2.92</td>
<td>0.71 ± 0.94</td>
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<tr>
<td>2 g</td>
<td>3.41 ± 2.57</td>
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<td>0.4 g</td>
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pain. However, if they no longer feel pain with MCS, they could try to move energetically. Therefore, the cerebellum is not a direct modulator of neuropathic pain, but it could be involved in assessing the extent of neuropathic pain.

Glucose metabolism in the thalamic area was decreased by pain, and this was reversed by MCS. A previous study has reported that the activation of the thalamic area was decreased by pain, and this was reversed by MCS.\(^{41}\) The thalamic area receives the initial sensory input from the spinothalamic tract, and it is in charge of processing sensory inputs, including pain. Therefore, it could be considered that the thalamic nucleus is located at the middle of the ascending pathway. In previous reports, decreased activation in the thalamic area is explained by the following 2 hypotheses. One is that a compensatory mechanism in the spinal dorsal horn or the spinothalamic tract attenuates noxious signals from the periphery if the pain signal is abnormal and has robust firing.\(^3,4,17\) This compensatory mechanism may block the abnormal signal caused by painful stimulation at the spinal level. Another possibility is that the inhibition of painful inputs by a self-defense mechanism attenuates the activity of the thalamic area itself. An increase in gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter, results in a decrease in glucose metabolism in the thalamic area.\(^{17}\) This is consistent with our serotonin results, by producing descending modulation of the PAG. Iadarola et al. have suggested that afferent input needs a feedback mechanism against external sensory input. A feedback circuit in the sensory system inevitably consists of an ascending and descending processor and modulator.\(^{17}\) We suggest that serotonin participates in this feedback mechanism as one of the descending modulators, like GABA and dopamine. Therefore, GABA and serotonin could directly inhibit the upward pain signal, resulting in decreased activity of the thalamic area.\(^2,3,5\)

In our mPET results, we saw some evidence for descending modulation by MCS. MCS generates dopamine release by the striatum. MCS simultaneously affects the PAG directly or indirectly, to cause the PAG to release serotonin. Dopamine and serotonin can attenuate neuropathic pain, ultimately suppressing the input signal evoked by painful stimulation.\(^{12–14,28,34,35}\)

We used isoflurane for anesthetizing the rats during mPET imaging. There are many controversies regarding the relationship between isoflurane and brain function.\(^{23–25,42}\) Previous reports suggest that isoflurane causes neurodegeneration in young or infant rats.\(^{92}\) This neurodegeneration could be accelerated when isoflurane used for anesthesia is combined with nitrous oxide or benzodiazepines.\(^{37}\) In addition, isoflurane appears to be related to Alzheimer’s disease through reaction with amyloid-beta plaques.\(^{24}\) Therefore, isoflurane may be an inappropriate anesthetizing agent because it could be blocking the activity of the brain network. However, mPET imaging is an indirect way of scanning whole-brain activity by measuring the radioactivity of a tracer that is selected for tracking a specific target. We selected \(^{18}\)F-FDG as a glucose tracer. \(^{18}\)F-FDG requires 30–60 minutes for uptake by neurons in the brain. The rats administered \(^{18}\)F-FDG were awake for 60 minutes, and we performed behavioral testing and electrical stimulation at that time. Isoflurane was used only during the mPET scan. Given that mPET measures the level of spatial radioactive distribution and the radioactivity of \(^{18}\)F-FDG had already accumulated over time during the uptake period, we suggest that isoflurane could not have affected our mPET results.

**MCS as an Ascending Modulator**

Our mPET and immunohistochemical studies have some limitations for understanding pain and its modulation. First, mPET has low spatial resolution and needs time to acquire imaging data. The time required to obtain an image depends on the isotope. Because of this, some isotopes require as much as 1 hour for image acquisition. Second, immunohistochemical studies inevitably require the sacrifice of experimental animals. Because of this, immunohistochemistry is not an appropriate method for demonstrating a time-course change in an animal. Therefore, the methods that we have mentioned above are not suitable for the long-term or real-time study of neuropathic pain and electrical stimulation. Electrophysiological methods, however, can demonstrate real-time changes in neuronal activity in a specific brain area that is considered particularly relevant to neuropathic pain. Therefore, we carried out extracellular recording in the brain\(^8\) and targeted the ventral posterolateral nucleus (VPL). The VPL receives somatosensory input via the spinothalamic tract and is related to the processing of painful sensations.\(^{16}\) In our previous study, we electrically stimulated the VPL in rats with induced neuropathic pain and demonstrated that VPL stimulation was effective for pain modulation.\(^{20,21}\) Therefore, we thought that monitoring neuronal activity in the VPL would be beneficial for understanding the action mechanism of MCS.

In this study, neuronal activity in the VPL was drastically increased by painful stimulation (Fig. 9A), and the increased activity remained for a while. It diminished gradually, in a process known as afterdischarge. This result is the same as that obtained in our earlier study on cold

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**FIG. 10.** Firing rate of VPL neurons with various forms of mechanical stimulation. Firing rates of VPL neurons were strongly affected and increased according to the intensity of the mechanical stimulation. The firing rate was significantly decreased by MCS for mechanical stimulation by pinching and stimulation with 300 g, 180 g, and 15 g (**p < 0.05, ***p < 0.001, Student t-test). However, the differences were not significant for 2 g and 0.4 g. Pinch = pinch pressure. Figure is available in color online only.
allodynia, and previous reports have shown that neuronal activity is increased in the thalamic area, including the VPL. We therefore considered this particular change in activity to be a discriminative marker of neuropathic pain. A key characteristic of this electrophysiological study is artifact removal. Electrical stimulation confounds the monitoring of neuronal activity by causing stimulation artifacts, because electrical pulses obscure neuronal activity, which is represented by the voltage difference between the electrical pulses for stimulation and neuronal activities. However, our artifact removal method can reveal the buried neuronal signals. We found that MCS effectively suppressed the ectopic discharge resulting from painful stimulation (Fig. 9). Therefore, we have demonstrated that MCS acts not only as a descending modulator of the striatum and PAG but also as an ascending modulator by directly suppressing abnormal sensory input signals in the VPL.

**MCS Suppresses Activity of the VPL: Observing the Clues Using Electrophysiology**

We demonstrated how sensitization appeared in response to neuropathic pain during our electrophysiological study. We prepared 6 types of mechanical stimulation of different intensity (pinch pressure, and 300 g, 180 g, 15 g, 2 g, and 0.4 g of bending force delivered by von Frey hairs). In mechanical stimulation without MCS, neuronal activity in the Pain group was significantly higher in response to pinch pressure, 300 g, 180 g, and 15 g of bending force than the Normal group, but there was no statistically significant difference for 2 g and 0.4 g between the Normal and Pain groups. During MCS, neuronal firing rates for pinch pressure, 300 g, 180 g, 15 g, 2 g, and 0.4 g of bending pressure were significantly decreased in the Pain group, but were not prominent for 2 g and 0.4 g (Fig. 10). The most interesting aspect is that the amount of neuronal firing depends on the strength of the mechanical stimulation for pinch pressure, 300 g, 180 g, and 15 g (Fig. 10). On the basis of our sensitization study, we think that neuropathic pain increases the neuronal firing elicited from peripheral sensations by decreasing the sensory threshold. This accounts for the onset of mechanical allodynia in neuropathic pain. We believe that MCS modulates the ascending pathway by suppressing neuronal activation and directly regulates the tactile threshold.

**Conclusions**

In conclusion, electrical stimulation of the primary motor cortex effectively decreased neuropathic pain in this rat model. MCS seems to modulate both the ascending and descending pathways of pain signaling. In the ascending pathway, MCS suppressed peripheral pain input by stimulating the striatum and the PAG. The thalamic area appears to participate in both ascending and descending pathways. In the descending part of the thalamic area, MCS appears to regulate the level of opioids or GABA, and in the ascending part, MCS directly suppresses ectopic discharges sent from the dorsal horn and regulates the tactile threshold. In our electrophysiological study, we devised a method for removing stimulation artifacts to demonstrate changes in neuronal activity caused by MCS. Painful stimulation rapidly increased the neuronal activity of the VPL, which was directly suppressed by MCS. Therefore, MCS appears to be an effective ascending modulator of neuropathic pain. These results revealing the action mechanisms of MCS could be used to develop a feedback-based closed-loop technique for modulating neuropathic pain in this particular field of neurosurgery.

**References**

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Author Contributions
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