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Motor Cortex Stimulation Activates the Incertothalamic Pathway in an Animal Model of Spinal Cord Injury

Myeounghoon Cha[*], **Yadong Ji**[*], and **Radi Masri**[*,†]

[*]Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Baltimore, School of Dentistry, Baltimore, Maryland [†]Department of Anatomy and Neurobiology, University of Maryland Medical School, Baltimore, Maryland

Abstract

We have shown previously that electrical stimulation of the motor cortex reduces spontaneous painlike behaviors in animals with spinal cord injury (SCI). Because SCI pain behaviors are associated with abnormal inhibition in the inhibitory nucleus zona incerta (ZI) and because inactivation of the ZI blocks motor cortex stimulation (MCS) effects, we hypothesized that the antinociceptive effects of MCS are due to enhanced inhibitory inputs from ZI to the posterior thalamus (Po)—an area heavily implicated in nociceptive processing. To test this hypothesis, we used a rodent model of SCI pain and performed in vivo extracellular electrophysiological recordings in single well-isolated neurons in anesthetized rats. We recorded spontaneous activity in ZI and Po from 48 rats before, during, and after MCS (50 μA, 50 Hz; 300-ms pulses). We found that MCS enhanced spontaneous activity in 35% of ZI neurons and suppressed spontaneous activity in 58% of Po neurons. The majority of MCS-enhanced ZI neurons (81%) were located in the ventrorateral subdivision of ZI—the area containing Po-projecting ZI neurons. In addition, we found that inactivation of ZI using muscimol ($GABA_A$ receptor agonist) blocked the effects of MCS in 73% of Po neurons. Although we cannot eliminate the possibility that muscimol spread to areas adjacent to ZI, these findings support our hypothesis and suggest that MCS produces antinociception by activating the incertothalamic pathway.

Perspective—This article describes a novel brain circuit that can be manipulated, in rats, to produce antinociception. These results have the potential to significantly impact the standard of care currently in place for the treatment of patients with intractable pain.

Keywords

Analgesia; neuropathic pain; zona incerta; posterior thalamus; central pain

A common consequence of spinal cord injury (SCI) is the development of severe, debilitating neuropathic pain. The pain is spontaneous and persistent in the absence of an insult, but it can also present as hypersensitivity to painful stimuli (hyperalgesia) and hypersensitivity to normally innocuous stimuli (allodynia).[12,13] Unfortunately, current treatments cannot produce complete relief of the pain and are not effective in all patients.[2,14] One potential treatment, motor cortex stimulation (MCS), was serendipitously found to

Address reprint requests to Radi Masri, University of Maryland Baltimore, School of Dentistry, Department of Endodontics, Prosthodontics and Operative Dentistry, 650 West Baltimore St, 4th floor, Baltimore, MD 21201. Radi.Masri@gmail.com

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reduce neuropathic pain in patients.[55,56] Since then, MCS has been proposed for the treatment of other chronic neuropathic pain conditions including SCI pain,[48–51,58] and several noninvasive MCS protocols have been developed; these include transcranial magnetic stimulation and transcranial direct current stimulation.[23,28] Some authors report that MCS results in pain relief in approximately 50% of the patients[24]; however, evidence from placebo-controlled trials to support these claims is lacking.[10] Pain relief occurs progressively after the onset of MCS[19] and persists after the stimulation has stopped. This poststimulation effect can last from minutes to days in some patients[21,22] and suggests that MCS could potentially serve as a therapy for treatment-resistant neuropathic pain. However, the mechanisms by which MCS alleviates pain remain poorly understood.

Recently, we described a novel system for the regulation of nociceptive processing in the thalamus, the incertothalamic system.[31] In this system, the zona incerta (ZI; a GABAergic nucleus located in the diencephalon) inhibits the flow of nociceptive and somatosensory information in the posterior thalamus (Po),[31,54] and this inhibition is regulated by the cholinergic system.[32] In animals with SCI, we demonstrated that hypersensitivity is associated with an abnormally reduced inhibition from ZI.[31] The reduced inhibition results in enhanced spontaneous and evoked activity in higher order thalamic nuclei (Po) and cortical structures involved in nociceptive processing.[31,43,45] In the same animals, we found that MCS reverses spontaneous painlike behaviors and hypersensitivity without having an effect on motor performance.[11,27] We also found that electrical stimulation of ZI mimics MCS effects and that reversible inactivation of ZI blocks antinociception produced by MCS, suggesting an integral role for the ZI in mediating MCS effects.[27] Because the main input to ZI is from frontal cortical areas, and in particular the motor cortex (M1),[33,34] and because ZI projects densely upon Po,[3,53] we hypothesized that antinociceptive effects of MCS are due to the activation of the incertothalamic pathway.

Methods

General Surgical Procedures

All procedures were conducted according to Animal Welfare Act regulations and Public Health Service guidelines. Strict aseptic surgical procedures were used, according to the guidelines of the International Association for the Study of Pain, and approved by the institutional Animal Care and Use Committee. Forty-eight adult male Sprague Dawley rats weighing 250 to 300 g were used in this study. Animals were anesthetized with either isoflurane (initial, 5%; during surgery, 1.5%) for SCI surgery or urethane (1.5 g/kg, intraperitoneally) for in vivo electrophysiological recording experiments. The animals were attached to a stereotaxic frame and placed on a thermoregulated heating pad. The respiratory rate, corneal reflex, and tail pinch response were monitored and used to ensure that animals were sufficiently anesthetized. Local anesthetic (2% lidocaine) was applied to incision sites before surgery began.

SCI

A midline, longitudinal incision (10 mm) overlaying the C2-T2 area was made. The muscles were dissected under a dissecting microscope with blunt scissors to expose vertebrae C6 and C7. A laminectomy to expose the spinal cord immediately rostral to C7 was performed using a rongeur, and the dura mater covering the spinal cord was removed. A quartz-insulated platinum electrode (5-μm tip) was inserted into the anterolateral quadrant of the right side of the spinal cord (2.1 mm lateral to the midline). DC current was passed (10 μA for 10 seconds repeated 5 times) at 2 locations to produce 2 lesions (lesion locations, .8 and 1.2 mm lateral from midline; depth, 1.7 mm) as described previously.[11,27] After the end of SCI surgery, the muscles and skin were approximated and sutured in layers. The animals were

left to recover on a thermoregulated heated pad and the anti-inflammatory drug carprofen (Rimadyl; Pfizer, Rouses Point, NY) 50 mg/mL, subcutaneously, was administered postoperatively every 12 hours for 3 days.

Behavioral Testing

A Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio, Italy) was used to assess bilateral mechanical withdrawal thresholds on both hindpaws. To minimize anxiety, the animals were acclimated to the behavioral apparatus 30 minutes before testing. The filaments were applied on the plantar surface of the paws 3 times and mechanical withdrawal force was defined as the average force at which the animal withdrew the paw. Withdrawal thresholds were assessed on 3 consecutive days before SCI surgery, at day 3 postsurgery, at day 7 postsurgery, and at weekly intervals thereafter.

In Vivo Experiments

Extracellular Electrophysiological Recording—At least 14 days after surgery, rats were anesthetized with urethane (1.5 g/kg, intraperitoneally) and prepared for extracellular recordings as previously described.[31] We selected urethane because it has no, or negligible, effects on glutamatergic and GABAergic transmission and therefore produces only minimal disruption of signal transmission in the neocortex.[44] We monitored electrocorticograms to assess the stage of anesthesia and maintained the rats at stage III/3– 4[16] and administered supplementary injections (150 mg/kg) as needed.

We recorded from the ipsilateral ZI (stereotaxic coordinates: anteroposterior, 3.6; lateral, 2.9) and ipsilateral Po (anteroposterior, 3.4; lateral, 2.8), relative to the SCI site. The bone overlying ZI or Po regions was removed and the dura covering the brain was carefully dissected. Custom-made quartz-insulated platinum electrodes (2–4 MΩ) were used to record from ZI or Po based on stereotaxic coordinates relative to Bregma. In Po, we recorded spontaneous activity from neurons with receptive fields in the hindpaw as determined by manual stimulation using a wooden probe. These neurons typically receive convergent inputs from other parts of the body such as the vibrissa.[31]

MCS—The bone overlying the M1 was removed, and custom-made insulated bipolar platinum electrodes (diameter, 70 μm; exposed tip, 50 μm; distance between electrodes, 500 μm) were applied epidurally above the M1 at stereotaxic coordinates determined from our previous behavioral experiments (anterior, 1.8 mm; lateral, 2 mm)[11,27] ipsilateral to the SCI site. The MCS electrodes were held in place using 2 bone screws and acrylic resin and the electrodes were connected to a stimulator (ISO-Flex and Master-8; A.M.P.I, Jerusalem, Israel).

Experimental Protocol—We recorded spontaneous activity from well-isolated single neurons in ZI and Po before (recorded for 5 minutes), during (recorded for either 5, 15, or 30 minutes), and after MCS until the cell recovered. In all experiments, we stimulated the M1 continuously at 50 μA, 50 Hz, 300-ms pulses because we have shown previously that these parameters are effective in reducing hypersensitivity and spontaneous painlike behaviors.[11,27] However, we varied the duration of stimulation (5, 15, or 30 minutes) to assess the effect of duration of stimulation on neuronal activity in ZI and Po. The waveforms of signals recorded from well-isolated units were digitized (40 kHz) through a Plexon Data Acquisition System (Plexon, Dallas, TX). The recorded units were sorted offline (Plexon's off-line sorter) using dual thresholds and principal component analyses.

Pharmacologic Inactivation of ZI—In a subset of animals (n = 5), a microdialysis probe (CMA11; Microdialysis, Solna, Sweden) was implanted into ZI using stereotaxic

coordinates[38] Muscimol (200 μM, $GABA_A$ agonist; Sigma-Aldrich, St. Louis, MO) was infused using a pump (Genie Plus; Kent Scientific, Kent, CT) at a rate of 2.5 μL/min into ZI 10 minutes before recording from neurons in Po because we found in previous experiments that muscimol infusion requires at least 5 minutes to produce behavioral effects.[27] Muscimol infusion continued for the duration of the experiment, and spontaneous activity of Po neurons was assessed before, during, and after 15 minutes of MCS.

Pyramidotomy—In a subset of animals (n = 5), the pyramidal tract was cut bilaterally at the level of the medulla oblongata before recording from Po neurons to remove any influence of MCS on ascending afferents in the spinal cord. In these animals, the bone covering the medullary pyramids was removed and the dura was dissected. The pyramids were cut 1.5 mm rostral to decussation with a #11 scalpel blade (Butler Schein, Albany, NY) as described in Z'Graggen et al.[62]

Histology—At the end of recording experiments, electrolytic lesions (20 μA for 10 seconds repeated 2 times) were used to mark the recording sites. The animals were then perfused transcardially with buffered saline followed by 4% buffered paraformaldehyde. We obtained coronal brain and spinal sections (80 μm thick) and Nissl-stained each section. The sections were examined under the microscope to identify recording sites, lesion sites, and muscimol injection location in experiments where ZI was inactivated.

Data Analysis

Mechanical Hypersensitivity—To determine if mechanical thresholds were changed significantly after spinal injury, analysis of variance (ANOVA) on ranks was performed followed by Dunn's multiple comparison test using the average of 3 presurgical baseline trials and the average of each postsurgical trial at days 3, 7, and 14. All the animals included in this study exhibited significant reductions in mechanical withdrawal thresholds. Changes in mechanical withdrawal thresholds were used only as an assay to demonstrate that hypersensitivity occurs after spinal cord injury. We demonstrated previously, in these animals, that reduction in mechanical withdrawal thresholds is associated with the development of a tonic aversive state suggesting the presence of ongoing spontaneous painlike behavior.[11]

The Effect of MCS on Spontaneous Activity of Individual Neurons in ZI and Po —For each cell, spontaneous activity before, during, and after MCS was divided into 1-minute blocks. The mean firing rate during each minute (or block) was calculated, and repeated measures ANOVA (RM ANOVA) followed by Dunn's multiple comparison test was used to test if mean firing rate in each block after MCS has changed significantly from baseline values. Data obtained during electrical stimulation were not included in the statistical analysis because we could not reliably eliminate the stimulus artifact. Neurons with at least 2 consecutive significantly changed blocks ($P < .05$; in the same direction, either enhanced or suppressed) were considered significantly changed and subsequently classified into neurons with enhanced or suppressed spontaneous activity. Neurons that did not meet these criteria were not affected by MCS.

The Magnitude and Duration of MCS Effects in ZI and Po—The mean firing rate across significantly changed blocks, for each cell, was calculated before and after MCS (separately for enhanced and suppressed units) and then averaged across neurons to assess enhanced or suppressed spontaneous activity in neuronal populations. The Wilcoxon signed-rank test was used to compare the averaged mean firing rate for neuronal populations before and after MCS. In addition, Spearman rank order test was used to test for correlation

between the change in mean firing rate in neuronal population after MCS and the duration of MCS (5, 15, and 30 minutes).

The length of time that spontaneous activity was enhanced or suppressed after MCS (duration of MCS effects) was also assessed by summing the duration of significantly changed blocks for each unit after MCS. The duration of MCS effects was averaged across neurons to assess the duration of MCS effects in ZI and Po neurons as a group. In some units, we were unable to record from the neurons until they completely recovered, and therefore these units were not included in our analysis of the duration of effects. Spearman rank order test was used to test for correlation between the duration of enhanced or suppressed spontaneous activity after MCS and duration of electrical stimulation (5, 15, and 30 minutes).

Histological Analysis—Nissl-stained slides were examined under the microscope to identify recording tracts and electrolytic lesions in ZI and Po. Electrolytic lesions and electrode tracts were cross-referenced with the stereotaxic coordinates of penetrations recorded during the recording experiments. The approximate location of each neuron within ZI or Po was determined and plotted on a drawing obtained from the Paxinos and Watson Atlas.[38] Postmortem histological analysis was performed blindly (blinded to the result of MCS effect on individual neurons).

In all experiments, data were analyzed using Sigma-Stat program (Aspire Software International, Ashburn, VA). A $P < .05$ was considered significant.

Results

We and others have previously shown that rats with SCI develop mechanical hypersensitivity caudal to the lesion site.[11,31,61] Consistent with the literature, animals in this study showed a significant reduction in mechanical hindpaw withdrawal thresholds bilaterally within 7 days of the injury. On the ipsilateral hindpaw (relative to the injury) mechanical thresholds decreased from 35.2 ± 1.3 g (median, 35.5 g; range, 27.0–47.4 g) to 22.9 ± 3.7 g (median, 21.9 g; range, 17.1–36.4 g; $P < .001$, ANOVA on ranks). We observed similar results on the contralateral hindpaw and mechanical withdrawal thresholds decreased from 36.3 ± 1.3 g (median, 35.3 g; range, 31.2–48.8 g) to 25.6 ± 3.8 g (median, 25.3 g; range, 18.1–33.1 g; $P < .001$).

Effects of MCS on ZI Activity

We recorded from 72 well-isolated single units in ZI and assessed spontaneous activity before and immediately after continuous MCS (see Methods). Figs 1A–C shows representative examples of ZI neurons exhibiting enhanced activity after 5, 15, or 30 minutes of MCS. In these examples, MCS enhanced spontaneous activity by 264% after 5 minutes of MCS (Fig 1A) and by 366% and 281% after 15 and 30 minutes stimulation, respectively (Figs 1B and 1C). In individual neurons, MCS significantly enhanced spontaneous activity in 35% of ZI units (25/72; $P < .02$, RM ANOVA). As a group, spontaneous activity was enhanced on average by 220% ($P = .0007$, Wilcoxon) after 5 minutes of MCS, by 439% ($P < .001$) after 15 minutes of stimulation, and by 363% ($P = .02$) after 30 minutes of stimulation (Figs 1D–F). As a group, there was no correlation between the change in spontaneous activity and the duration of MCS (rho = .18, $P = .39$, Spearman; Fig 1G). However, there was a significant positive correlation between the duration of enhanced spontaneous activity after MCS and the duration of MCS (5-minute MCS: 10.3 ± 1.7 minutes; median, 11.5 minutes; range, 3.0–14.0 minutes; 15-minute MCS: 11.3 ± 1.5 minutes; median, 16.0 minutes; range, 2.0–18.0 minutes; 30-minute MCS: 20.2 ± 4.6 minutes; median, 25.0 minutes; range, 4.0–30.0 minutes; rho = .5, $P = .01$, Spearman; Fig

1H). These results are consistent with our previous findings that MCS reduces spontaneous painlike behaviors in animals with SCI and that the duration of stimulation correlates positively with the duration of antinociception produced.[11,27]

However, not all ZI neurons were enhanced after MCS. In 26% (19/72) of ZI cells spontaneous activity was significantly suppressed ($P < .001$, RM ANOVA), and in the remaining neurons MCS had no effect on spontaneous activity (Table 1). The ZI contains a heterogenous collection of cells and is compromised of several sectors[33,42,46]; therefore, the mixed results we observe after MCS could be due to the heterogeneity of the sampled ZI neurons. To test this notion, we performed a postmortem histological analysis to identify the location of recorded ZI neurons. We divided the ZI into 4 sectors by drawing a horizontal line bisecting ZI into ventral and dorsal portions and another vertical line in the middle of ZI, perpendicular to the horizontal line. We found that the majority of neurons exhibiting enhanced activity after MCS (81%, 13/16) were located in the ventrolateral portion of ZI (Fig 2), an area shown previously to provide inhibitory inputs to Po.[3,54] All other neurons (suppressed or no change in activity after MCS) were distributed evenly in ZI. These findings support our overarching hypothesis that antinociceptive effects of MCS are due to enhanced ZI inputs to Po.

Effects of MCS on Po Activity

MCS suppressed spontaneous activity in the majority of Po neurons (58%, 32/55 cells; $P < .001$, RM ANOVA). Only a small percentage (15%, 8/55) of Po neurons exhibited enhanced spontaneous activity after MCS ($P < .02$), and in 27% (15/55 cells) MCS had no significant effects ($P > .05$) (Table 2). In Figs 3A–C we show representative examples of the effects of MCS on Po activity. MCS suppressed spontaneous activity by 19% after 5 minutes of MCS (Fig 3A) and by 53% and 37% after 15 and 30 minutes stimulation, respectively (Figs 3B and 3C). As a group, spontaneous activity was suppressed by 51% after 5 minutes of MCS (range, 1.7–84.7%; $P = .007$, Wilcoxon), by 50% after 15 minutes of MCS (range, .3–74%; $P = .001$), and by 25% after 30 minutes of MCS (range, 6.1–54%; $P = .014$) (Figs 3D–F). There was no correlation between the change in spontaneous activity and the duration of stimulation (rho = .28, $P = .13$, Spearman; Fig 3G). Similar to the effect of MCS on ZI, the duration of suppressed activity in Po after MCS was positively correlated with the duration of MCS (5-minute MCS: mean, 11.7 ± 2.2 minutes; median, 12.0 minutes; range, 3.0–26.0 minutes; 15-minute MCS: mean, 15.2 ± 2.3 minutes; median, 17.0 minutes; range, 3.0–30.0 minutes; 30-minute MCS: mean, 22 ± 3.6 minutes; median, 22.5 minutes; range, 13.0–30.0 minutes; rho = .4, $P = .03$, Spearman; Fig 3F). The locations of recorded Po units are shown in Fig 4.

It has been suggested that MCS directly inhibits nociceptive neurons in the dorsal horn,[1,25] and therefore suppressed activity in Po could be due to MCS inhibition of ascending nociceptive inputs from the spinal cord rather than due to MCS-enhanced ZI activity. To allay this concern, in a subset of animals (n = 5), we performed bilateral pyramidotomy at the level of the caudal medulla and recorded Po responses to 15 minutes of MCS (see Methods). Cutting the pyramidal tract did not block the effects of MCS, and spontaneous activity of the majority of Po neurons (75%) was suppressed after stimulation. A representative example is shown in Fig 5A where spontaneous activity was significantly suppressed from a mean of 2.5 ± .2 spikes/second to .2 ± .0 spikes/second after 15 minutes of MCS ($P < .001$, RM ANOVA). These findings suggest that MCS effects are not due to direct influence of MCS on ascending spinal afferents.

To determine if the suppressed activity in Po is mediated through MCS activation of ZI, in a subset of animals (n = 8), we pharmacologically inactivated ZI and assessed the effects of MCS (15 minutes) on Po activity. In the representative example shown in Fig 5B, MCS had

no effect on spontaneous activity of a Po neuron when muscimol was infused in ZI (11.7 ± .8 spikes/second to 10.7 ± .4 spikes/second; $P = .987$, RM ANOVA). The infusion of muscimol blocked the effects of MCS in the majority (73%) of Po neurons studied. Although we cannot dismiss the possibility that muscimol diffused beyond the boundary of ZI, these findings suggest that MCS suppresses activity in Po by activating ZI.

Discussion

Our overarching hypothesis is that MCS ameliorates pain by activating the incertothalamic pathway. This hypothesis arises from our previous findings that reduced hypersensitivity after MCS can be mimicked by electrical stimulation of ZI and that antinociception is blocked by inactivating ZI.[27,30] In agreement with this hypothesis, we demonstrate that MCS enhances spontaneous activity in putative Po-projecting ZI neurons and suppresses activity in the majority of Po neurons. We also demonstrate that suppressed activity in Po remains even when the pyramidal tract is cut. The effects of MCS on ZI and Po activity outlasted the stimulation period and were dependent on the duration of stimulation. These findings are in agreement with previous reports in animals and humans that the behavioral effects of MCS outlast the stimulation period and are dependent on the duration of stimulation.[22,27]

Enhanced Activity in ZI

GABAergic, Po-projecting neurons are located in the ventrolateral portion of ZI (see Fig 1 in Trageser et al[54]) and M1 fibers innervating ZI terminate densely in the ventral portion (ZIv).[34,57] Despite their proximity within ZIv, Po-projecting ZI neurons are not directly influenced by M1 inputs.[57] Because ZIv neurons are GA-BAergic and because they have extensive local axon collaterals, it was suggested that excitatory input from M1 may result in increased intranuclear inhibition and therefore suppress rather than enhance activity in Po-projecting ZI neurons.[57] However, we find that neurons in the lateral portion of ZIv (putative Po-projecting ZI neurons) are enhanced after MCS. A possible explanation is that continuous MCS of ZIv neurons that receive direct input from M1 depletes GABA. This will result in disinhibition of Po-projecting ZI neurons and enhanced inhibitory inputs from ZI to Po. Consistent with this notion, we find that longer MCS periods result in prolonged enhanced activity in ZI and suppressed activity in Po.

Furthermore, it is possible that inhibitory inputs other than those from ZI are enhanced by MCS. An important source of inhibitory inputs to the thalamus is the GABAergic reticular nucleus (TRN). Unlike ZI, the major source of excitatory input to TRN is from the somatosensory cortex.[26] Further, GABAergic terminals in Po that originate from ZI differ from those of TRN by their larger size, the presence of multiple release sites, and multiple filamentous contacts, all features suggesting that ZI exerts significantly more potent inhibition upon Po.[3,4] Another source of GABAergic input to Po that may be influenced by MCS is the anterior pretectal nucleus.[4,35] The anterior pretectal nucleus is implicated in regulating nociceptive responses.[59,60] It receives direct inputs from M1[5] and is a target that we will explore in the future.

Suppressed Activity in Po

In addition to ZI, M1 projects directly to Po and MCS may result in enhanced activity in Po rather than suppression. However, only a small percentage of Po neurons (3.9%) are activated directly by electrical stimulation of M1,[47] which suggests that the suppressed activity of Po neurons observed in our study is due to the indirect effects of MCS on ZI. In agreement with this, we found that the infusion of muscimol into ZI blocked the effects of MCS on the majority of, but not all, Po neurons. The partial effects of muscimol are possibly

due to incomplete block of ZI. At the same time, it is possible that muscimol may have diffused beyond the boundaries of ZI and affected neighboring structures such as the internal capsule or the ventroposterior thalamus; therefore, results from inactivation experiments should be interpreted with caution.

Specificity of MCS Effects

We focused our efforts on studying the ZI and PO because they have been shown to play a major role in the pathogenesis and the development of SCI pain, especially when compared to other brain structures involved in nociceptive processing such as the ventroposterior thalamus.[31] Nonetheless, it is important to note that M1 projections are diffuse and target numerous cortical and subcortical structures, and therefore antinociceptive effects of MCS may not only be due to the activation of the incertothalamic pathway. MCS effects could be due to influences on other brain structures such as the ventroposterior thalamus, primary somatosensory cortex, and periaqueductal gray. Additional studies are needed to test the effect of MCS on activity in these structures. Similarly, in addition to Po, ZI innervates the mediodorsal thalamus, nucleus submedius, and other higher order thalamic nuclei involved in nociceptive processing. Activity in these nuclei may also be suppressed by MCS.

Mechanisms of Pain Relief After MCS

Several hypotheses have been proposed to explain how MCS causes pain relief. Some authors hypothesized that MCS reduces pain by suppressing nociceptive inputs in the spinal cord either directly[1,25] or indirectly by activating the descending inhibitory systems.[15,29,36,37] However, manipulations that activate endogenous opioid release, such as deep brain stimulation of the periaqueductal gray, are especially poor for the treatment of pain resulting from an injury to the central nervous system.[20,52] In addition, we found that suppressed activity in Po remains even when the M1 inputs to the spinal cord were eliminated, suggesting that suppressed activity is not due to direct inhibition of ascending spinal afferents but is likely due to effects of MCS on supraspinal structures.

Evidence from imaging studies further supports a supraspinal mechanism of pain relief after MCS. It has been shown that MCS increases regional cerebral blood flow in several regions including the thalamus, striatum, and anterior cingulate cortex.[17,18,39–41] Some authors have hypothesized that MCS activates corticothalamic connections, and these in turn inhibit nociceptive processing in the thalamus.[6,7] In support of this hypothesis, it was argued that patients responsive to GABA or barbiturate treatment are more likely to benefit from MCS.[8,9] However, the specific role of the thalamus is still debatable,[19,39] and the source of altered inhibition, the mechanisms for engagement of inhibition, and the specific nuclei affected by MCS remain to be elucidated.

Here, we describe a novel inhibitory brain circuit that can be manipulated to produce pain relief. At the center of this circuit is the ZI that potently regulates the flow of nociceptive information from the posterior thalamus to the cortex. We have demonstrated previously that activating or inhibiting this circuit is causally related to the development of hypersensitivity and spontaneous pain-like behavior in animals after SCI.[11] Along with our present findings, these results support the conclusion that MCS ameliorates pain by activating the incertothalamic pathway. Increased inhibition within the thalamus will hinder the flow of nociceptive information to cortical areas involved in nociceptive processing such as the somatosensory cortex, the anterior cingulate cortex, and the insula, and will result in reduced pain.

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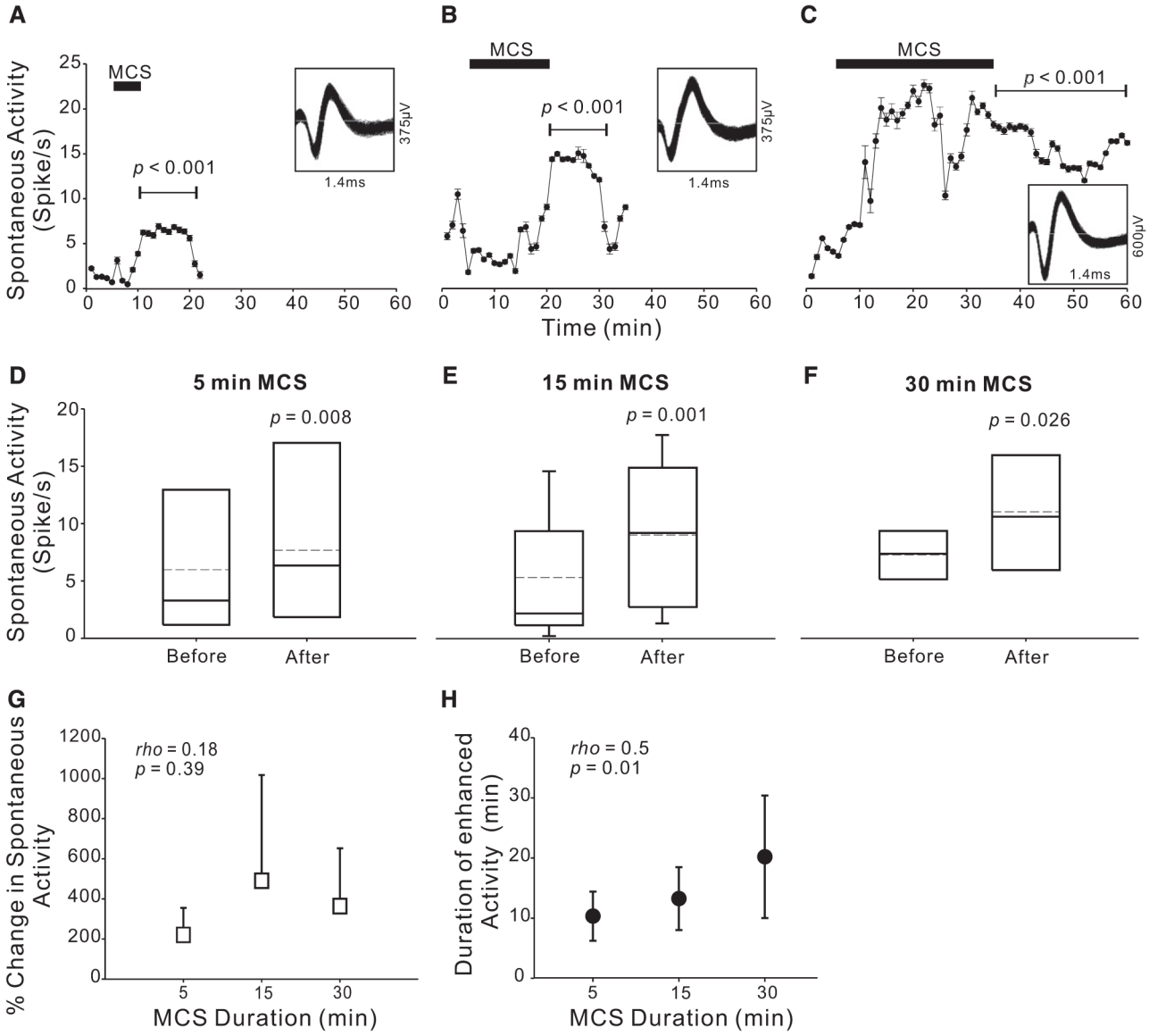

Figure 1.

MCS effects on spontaneous activity in ZI. **(A–C)** Representative examples of ZI response to 5 **(A)**, 15 **(B)**, and 30 **(C)** minutes of MCS (solid horizontal bar). Change in mean firing rate for each cell was calculated and plotted during 1-minute blocks. Statistical analysis was performed using RM ANOVA. Insets represent waveforms of recorded units sorted using a spike matching algorithm (Plexon, Offline Sorter). **(D–F)** Box plots of the mean firing rate of ZI neurons exhibiting enhanced spontaneous activity before and after 5 **(D)**, 15 **(E)**, 30 **(F)** minutes of MCS. The solid horizontal line in the box plots represents the median value and the dashed horizontal line represents the mean value. In this figure and the following figures, the box plots represent the 25th and 75th percentile and the error bars represent the 10th and 90th percentile. Statistical analysis was performed using Wilcoxon signed-rank test. **(G)** The correlation between the change in mean firing rate for ZI neurons exhibiting enhanced activity after MCS and the duration of stimulation. **(H)** The correlation between the duration of enhanced activity in ZI neurons after MCS and the duration of stimulation.

Correlation analysis was performed using the Spearman's Rho test. $P < .05$ was considered significant.

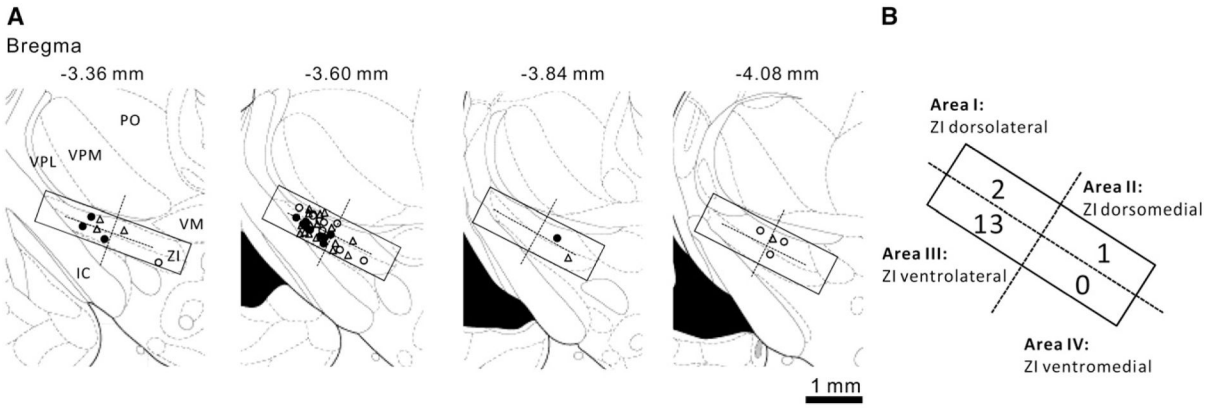

Figure 2.

Postmortem analysis of ZI recording sites. **(A)** Locations of recorded ZI neurons were determined and identified on corresponding drawings obtained from the Paxinos and Watson Atlas.[38] The ZI was divided into 4 sectors by drawing a horizontal line bisecting ZI into ventral and dorsal portions and another vertical line in the middle of ZI, perpendicular to the horizontal line (●, enhanced cell; ○, suppressed cell; Δ, no significant change). **(B)** The number of enhanced ZI units within each sector of ZI. Abbreviations: VPM, ventroposteromedial nucleus; VPL, ventroposterolateral nucleus; VM, ventromedial nucleus; IC, internal capsule.

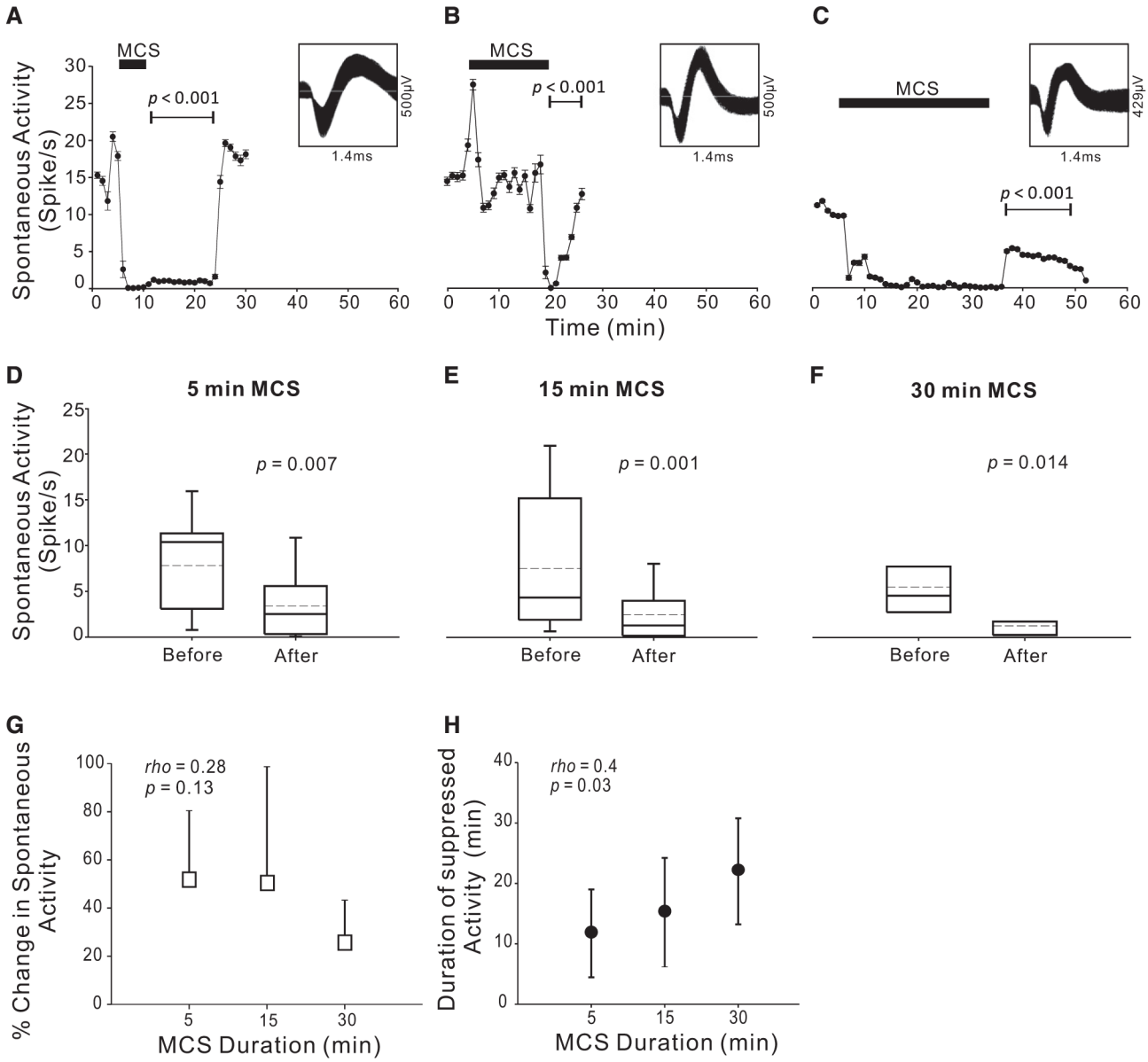

Figure 3.

MCS effects on spontaneous activity in Po. **(A–C)** Representative examples of Po response to 5 **(A)**, 15 **(B)**, and 30 **(C)** minutes of MCS (solid horizontal bar). Change in mean firing rate for each cell was calculated and plotted during 1-minute blocks. Statistical analysis was performed using RM ANOVA. Insets represent waveforms of recorded units sorted using a spike matching algorithm. **(D–F)** Box plots of the mean firing rate of Po neurons exhibiting suppressed spontaneous activity before and after 5 **(D)**, 15 **(E)**, and 30 **(F)** minutes of MCS. Statistical analysis was performed using Wilcoxon signed-rank test. **(G)** The correlation between the change in mean firing rate for Po neurons exhibiting suppressed activity after MCS and the duration of stimulation. **(H)** The correlation between the duration of suppressed activity in Po neurons after MCS and the duration of stimulation. Correlation analysis was performed using the Spearman's Rho test. $P < .05$ was considered significant.

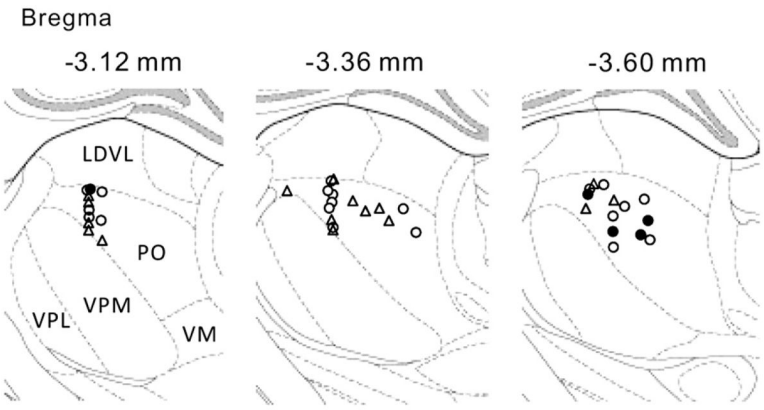

Figure 4.

Postmortem analysis of Po recording sites. Locations of recorded Po neurons were determined and identified on corresponding drawings obtained from the Paxinos and Watson Atlas[38] (●, enhanced cell; ○, suppressed cell; △, no significant change). Abbreviation: LDVL, laterodorsal thalamic nucleus, ventrolateral part.

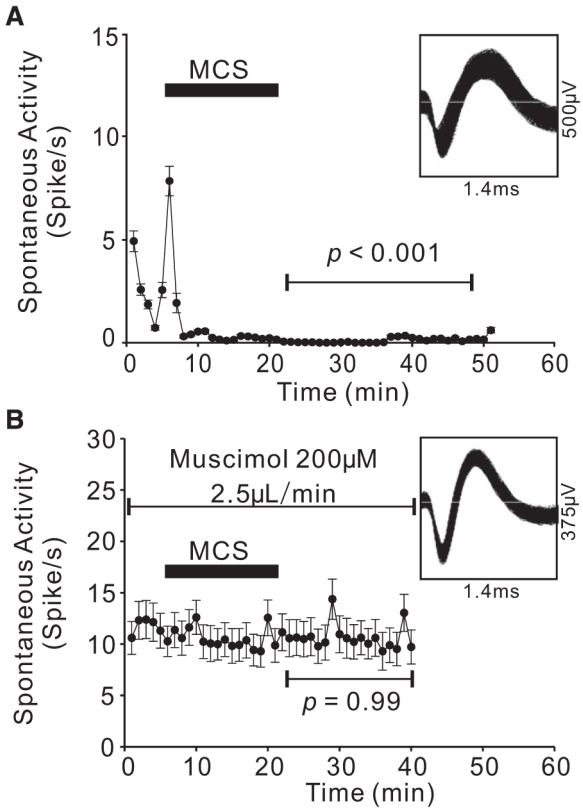

Figure 5.

Suppressed activity in Po is due to MCS effects on ZI. **(A)** A representative example of a Po unit exhibiting suppressed activity after 15 minutes of MCS (horizontal solid line) when the pyramidal tract was cut. **(B)** A representative example of a Po unit exhibiting no change in activity after 15 minutes of MCS (horizontal solid line) when the ZI was inactivated using continuous infusion of muscimol (200 μM, $GABA_A$ agonist; 2.5 μL/min). Insets represent waveforms of recorded units sorted using a spike-matching algorithm.

Table 1

The Effect of MCS on Spontaneous Activity of ZI

Duration of MCS	Enhanced	Suppressed	No Change
5 minutes	42% (6/14)	14% (2/14)	42% (6/14)
15 minutes	31% (14/45)	35% (16/45)	34% (15/45)
30 minutes	38% (5/13)	7% (1/13)	65% (7/13)
Total	35% (25/72)	26% (19/72)	39% (28/72)

Table 2

The Effect of MCS on Spontaneous Activity in Po

Duration of MCS	Enhanced	Suppressed	No Change
5 minutes	17% (3/18)	61% (11/18)	22% (4/18)
15 minutes	11% (3/27)	56% (15/27)	33% (9/27)
30 minutes	20% (2/10)	60% (6/10)	20% (2/10)
Total	15% (8/55)	58% (32/55)	27% (15/55)