Published in final edited form as:

Pain. 2011 June ; 152(6): 1398–1407. doi:10.1016/j.pain.2011.02.025.

Motor Cortex Stimulation Reduces Hyperalgesia in an Animal Model of Central Pain

Jessica M Lucas^{1,2,3}, Yadong Ji³, and Radi Masri^{1,2,3}

¹Program in Neuroscience

²Department of Anatomy and Neurobiology, University of Maryland School of Medicine

³Department of Endodontics, Prosthodontics, and Operative Dentistry, Baltimore College of Dental Surgery

Abstract

Electrical stimulation of the primary motor cortex has been used since 1991 to treat chronic neuropathic pain. Since its inception, motor cortex stimulation (MCS) treatment has had varied clinical outcomes. Until this point, there has not been a systematic study of the stimulation parameters that most effectively treat chronic pain, or of the mechanisms by which MCS relieves pain. Here, using a rodent model of central pain, we perform a systematic study of stimulation parameters used for MCS and investigate the mechanisms by which MCS reduces hyperalgesia. Specifically, we study the role of the inhibitory nucleus zona incerta (ZI) in mediating the analgesic effects of MCS. In animals with mechanical and thermal hyperalgesia, we find that stimulation at 50 μ A, 50 Hz, and 300 μ s square pulses, for 30 minutes is sufficient to reverse mechanical and thermal hyperalgesia. We also find that stimulation of the ZI mimics the effects of MCS and that reversible inactivation of ZI blocks the effects of MCS. These findings suggest that the reduction of hyperalgesia may be due to MCS effects on ZI.

Keywords

Chronic pain; Zona Incerta; Posterior Thalamus

Introduction

Central pain is defined as pain resulting from primary lesion or dysfunction in the central nervous system [34]. The pain is unrelenting, and refractory to pharmacologic treatments [1]. Electrical stimulation of the primary motor cortex was introduced in 1991 for the treatment of central pain syndrome (CPS) [61]. Since then its use has been extended for the treatment of several neuropathic pain conditions. These include trigeminal deafferentation pain [18], postherpetic neuralgia [5], brachial plexus, and phantom limb pain [53].

© 2011 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

Corresponding author: Dr. Radi Masri, Department of Endodontics, Prosthodontics and Operative, Dentistry, Baltimore College of Dental Surgery, 650 West Baltimore Street, 4th floor N. Baltimore, MD 21201, Tel: (410) 706-8133, rmasri AT umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare no conflicts of interest related to this work.

Stimulation of other brain structures has also been used for the treatment of neuropathic pain. These include the internal capsule [37]; the periaqueductal gray-periventricular gray complex [25]; and the thalamus [38]. However, motor cortex stimulation (MCS) is more effective and more advantageous because of the low occurrence of complications [9], the lower propensity to cause seizures [2,15], and the ability to apply it non-invasively using repetitive transcranial magnetic stimulation [27].

Human studies report mixed outcomes after MCS and success rates vary. MCS relieves pain in approximately 50% of patients [21,31] while studies involving only patients with CPS report success rates as high as 77% [60,61]; but see [14]. The mixed success rates and the mixed outcomes after MCS are a reflection of the complexity and variability of neuropathic pain conditions. Adding to the variability of MCS efficacy is the lack of standardized surgical, stimulation, and treatment protocols [20]. In human studies, stimulation parameters vary (intensities: 1 to 8 V, frequencies: 15 to 130 Hz, pulse duration: 60–500 μ s), as do stimulation protocols (MCS on range: 15 min–3 hours, MCS off: 2–24 hours; reviewed in: [31]). Because of this variability it is not clear which stimulus parameters are critical for MCS to be successful.

Pain relief occurs almost immediately after onset of MCS and persists after the stimulation has stopped. Like stimulation parameters, the duration of effect after cessation of stimulation (“post effect”) is rarely systematically examined and reported values vary among studies. In some reports these post effects were minimal and only lasted for 5–10 minutes [54,55,61]. In others, post effects varied from 45 minutes–2 hours [45], 3–5 hours [56], and even up to 1–3 days [40,41]. The variability in post effect duration is also a reflection of the various parameters used, various conditions predisposing for neuropathic pain and different stimulation techniques.

Here, we take advantage of an animal model of central pain to systematically test a large parameter space of stimulus conditions. We determine the stimulus parameters that are effective in reducing hyperalgesia and study the mechanisms of increased inhibition in the thalamus following MCS.

We have recently shown in an animal model of central pain that there is abnormally high neuronal activity in the posterior thalamic nucleus (PO) and that this increased activity and hyperalgesia is correlated with reduced activity in the inhibitory nucleus zona incerta (ZI) [33]. Because the motor cortex sends dense projections to the ventral division of ZI (ZIV), [35,62] we hypothesized that MCS reduces hyperalgesia by increasing activity in ZI.

METHODS

General Surgical procedures

All procedures were conducted according to Animal Welfare Act regulations and PHS guidelines. Strict aseptic surgical procedures were used, according to the guidelines of the International Association for the Study of Pain, and approved by the Baltimore College of Dental Surgery Animal Care and Use Committee. Twenty-six adult female Sprague-Dawley rats weighing 250–300 g were used in this study. Animals were anesthetized with ketamine/xylazine (100/8 mg/kg, ip) and placed into a stereotaxic frame. Animals were then placed on a thermoregulated heating pad and respiratory rate, corneal reflex, and tail pinch response were monitored and used to ensure that animals were sufficiently anesthetized. Additional anesthesia (10 mg/kg, ip, diluted ketamine 1:10 in saline) was administered when needed. Local anesthetic (2% lidocaine) was applied to surgery sites before procedures began.

After the end of the surgical procedure, animals were left to recover on a thermoregulated heated pad and the analgesic buprenorphine (0.05 mg/kg) was administered every 12 h for 24 h postoperatively (2 doses total).

Spinal lesions

A midline, inch-long longitudinal incision overlying the area of C2-T2 was made using #11 scalpel. 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 preformed using rongeurs, and the dura covering the exposed spinal cord was removed. A quartz-insulated platinum electrode (5 μ m tip) was targeted to the anterolateral quadrant on one side of the spinal cord. In our previous publications, we used only one electrolytic lesion to injure the spinal cord [33,49]. To produce larger spinal lesions, we modified our approach and used DC current (10 μ A for 10 s repeated 4 times) to produce two lesions, 0.4mm apart (lesion locations: 0.8 mm and 1.2 mm lateral from midline; depth: 2.1 mm). However, the modification in the protocol had no effect on the consistency or features of the resultant hyperalgesia. After the completion of surgery, the incision sites were approximated and sutured in layers.

Motor cortex electrode implantation (n=18)

Concurrent with spinal lesion surgery, a midline longitudinal incision was made along the midline of the skull to expose bregma and lambda. The bone overlying the primary motor cortex (M1) was removed contralateral to the spinal lesion site. Custom made epidural bipolar insulated platinum electrodes (diameter: 70 μ m, exposed tip: 50 μ m, distance between electrodes: 500 μ m) were targeted to the M1 contralateral to the site of spinal lesion using stereotaxic coordinates (A: 1.8 mm, L: 2 mm [43]). These coordinates were obtained from pilot experiments using electrical microstimulation and from data obtained from previously published motor cortex mapping work done by our collaborator Dr. Asaf Keller [70]. This allowed us to reliably target the hindpaw representation of M1, especially since the location of major subdivisions such as the forelimb or hindlimb areas in the rat motor cortex are somatotopic and consistent from animal to animal [39]. MCS electrodes were attached to amphenol pins to facilitate connection to the isolated pulse stimulator (A-M Systems, WA). Electrodes were fixed in place using four bone screws and acrylic resin.

Zona incerta electrode implantation (n=8)

Concurrent with spinal lesion surgery, eight rats received custom made bipolar insulated stainless steel electrodes (diameter: 139 μ m, exposed tip: 75 μ m, distance between electrodes: 280 μ m) implanted in ZI contralateral to the spinal lesion, targeted using stereotaxic coordinates (A: -3.6 mm, L: 2.8 mm, D: 7.3 mm, [43]). Briefly, a longitudinal incision was made along the midline of the skull to expose bregma and lambda. Bone overlying ZI was removed and the electrodes lowered 7.3 mm over the course of twenty minutes. Electrodes were fixed in place using 4 bone screws and acrylic resin.

Behavioral confirmation of hyperalgesia

To minimize anxiety the animals were habituated for one week before behavioral testing and surgery. The animals were trained to stand upright with forepaws on the experimenter's hand as described by Ren [50]. Testing of mechanical thresholds and thermal withdrawal latencies was performed on three consecutive days before the spinal lesion surgery, and on postsurgical days 3, 7, 14, and 21 to confirm the development of hyperalgesia.

To assess mechanical thresholds, calibrated von Frey filaments (Stoelting, IL) were applied in ascending order to the hindpaws. We applied the filaments to the *dorsal* surface of the

paws based on studies demonstrating that the dorsal approach more reliably and consistently detects threshold changes [50]. Mechanical withdrawal threshold was defined as the force at which the animal withdrew the paw to three of five stimuli delivered.

To assess thermal withdrawal latency, an analgesia meter with a moveable infrared heat source (IITC, Life Science Inc., CA) was used to apply radiant heat to the ventral surface of the hindpaws as described in Hargreaves *et al.*, [26]. Rats were acclimated to the test chambers (plexiglass boxes 17 (d) × 69 (l) × 14 (h) cm) for 30 minutes before testing. Latency to withdraw was recorded and thermal thresholds were computed as the average latency to withdraw across three trials.

Motor cortex stimulation

Before MCS was initiated, animals (n = 15) were habituated in stimulation chambers and handled to minimize anxiety. Baseline mechanical thresholds and thermal withdrawal latencies were obtained (see above) before MCS was performed. The stimulating electrodes were connected to a stimulator (A-M Systems) and stimulation parameters were varied. Stimulation intensity ranged from 0–75 μ A, frequency from 0–75 Hz, and duration from 0–90 minutes. A 300 μ s square pulse was used for all experiments. MCS effects were tested with von Frey filaments and a radiant heat source immediately after the end of MCS stimulation and every 30 minutes thereafter until mechanical thresholds returned to baseline values. Each animal was stimulated no more than once daily, and stimulation was repeated at least 3 times. For sham stimulation, animals were connected to the stimulator and placed in the test chambers but no current was passed.

During electrical stimulation animals remained in the test chamber and were under constant observation. Stimulation did not produce any visible muscle twitches and the animals showed no signs of distress. We obtained all behavioral responses after the end of MCS stimulation because previous studies in humans suggest that the neural mechanisms responsible for long-term analgesia occur (and are most readily observable) after MCS, rather than during the stimulation period [44].

Zona incerta stimulation

Eight animals with mechanical and thermal hyperalgesia received electrical stimulation in ZI. Similar to MCS, the animals were handled and habituated in test chambers before stimulation. Post-spinal lesion thresholds were obtained using the behavioral metrics described above. Over the course of two weeks, each animal was tested immediately after the termination of incertal stimulation on each of at least three days (intensity: 25 μ A, frequency: 50 Hz, duration: 15 minutes, 300 μ s square pulse) and three days of sham stimulation in a randomized fashion. After completion of ZI stimulation experiments electrolytic lesions were made at the site of the electrode to confirm placement.

Zona incerta inactivation

In a subset of animals (n=6) and concurrent with M1 electrode implantation, a microdialysis probe (CMA Microdialysis, Solna, Sweden) was implanted into the ventral portion of ZI (stereotaxic coordinates: A: −3.6 mm, L: 2.8mm, D: 7.1 mm [43]). After recovery, behavioral testing to confirm the development of hyperalgesia, and behavioral testing to obtain reliable baseline values for efficacy of MCS, the microdialysis probe was used to administer lidocaine (2%), muscimol (200 μ M), or saline to ZI. A total of 50 μ L was administered over 20 minutes (beginning 5 minutes before MCS and continuing through the first 15 minutes of MCS) at a rate of 2.5 μ L / min. Over the course of two weeks, infusion of drugs or saline was repeated three times per animal and the data reported represent the average of these trials. In all animals, testing for changes in mechanical thresholds was

performed after the termination of MCS. At the end of the experiments, the animals were perfused to identify the location of the microdialysis probes.

Histology

The animals were deeply anesthetized with sodium pentobarbital (60 mg/kg). The animals were perfused transcardially with buffered saline followed by 4% buffered paraformaldehyde. We obtained coronal brain sections (80 μ m thick) and Nissl-stained them. The sections were examined under the microscope to identify stimulation sites, lesion sites and probe implant location.

Data Analysis

Confirmation of Hyperalgesia—To determine that mechanical and thermal thresholds dropped significantly after spinal lesion a Wilcoxon Signed Rank test was performed using the average of three pre-surgical baseline trials and the average of at least two post-surgical trials. All lesioned animals exhibited significant reductions in withdrawal thresholds and thermal withdrawal latencies following spinal cord lesions.

Examining various stimulation protocols and parameters—The effects of differing stimulation trains (theta burst, intermittent theta burst and continuous pulse stimulation) were examined using a Friedman test followed by a modified Dunnett's post hoc.

The effect of varying MCS parameters (intensity, frequency and duration) was tested using the Kruskal-Wallis test followed by a Dunn's post hoc test. To test for correlation between stimulus duration and the duration of post effects, Spearman's rho test was performed.

ZI stimulation and inactivation—Pre and post ZI stimulation thresholds were tested using a Wilcoxon signed rank test, while the effect of ZI inactivation was tested with a Kruskal-Wallis test.

In all experiments performed, we determined the appropriate sample size by performing a power analysis using $\alpha = 0.05$ and power = 0.85. All data were analyzed using SigmaStat (Aspire Software International, VA) and presented as means $\pm$ SD. In all experiments a $p < 0.05$ was considered significant.

RESULTS

Animals with spinal lesions develop hyperalgesia

In this project, we adopted a rodent model of central pain induced by spinal cord lesions [33,69]. In these animals, fourteen days after spinal lesions mechanical thresholds significantly reduced from 137.40 ± 45.73 g (mean $\pm$ SD; hindpaw ipsilateral to lesion) and 127.30 ± 37.11 g (contralaterally) before surgery to 58.29 ± 15.89 g and 56.02 ± 22.13 g respectively ($p < 0.001$, Wilcoxon). Latency to withdraw from a radiant heat source fell from 11.29 ± 1.51 s (ipsilateral to the lesion) and 11.41 ± 1.98 s (contralaterally) before spinal lesion to 9.40 ± 0.06 s and 9.74 ± 1.73 s respectively after spinal lesion ($p = 0.019$ ipsilateral to the lesion, $p = 0.014$ contralaterally, Wilcoxon; $n=11$).

Motor cortex stimulation reduces hyperalgesia in animals with spinal cord injury

In our animal model of central pain, motor cortex stimulation (50 μ A, 50 Hz, 300 μ s square pulse, 30 minute duration, "continuous pulse MCS") significantly reduced mechanical hyperalgesia measured immediately after the termination of MCS stimulation and every 30 minutes thereafter (Fig. 1). On the hindpaw ipsilateral to spinal lesion, MCS increased

mechanical thresholds from $60.00 \pm 0.00\text{g}$ to $92.73 \pm 16.18\text{g}$ and from $53.82 \pm 13.75\text{g}$ to $80.64 \pm 20.10\text{g}$ on the contralateral hindpaw ($p < 0.001$ both sides, Friedman test; $n=11$; Fig. 1A). Mechanical thresholds remained elevated for at least 30 minutes after the stimulation ceased and returned to prestimulation values within 60 minutes after stimulation.

Continuous pulse MCS also significantly reduced thermal hyperalgesia. Latency to withdraw from a radiant heat source increased immediately after MCS from $7.65 \pm 0.45\text{s}$ to $11.12 \pm 1.16\text{s}$ ipsilateral to lesion and from $7.7 \pm 0.72\text{s}$ to $10.73 \pm 1.11\text{s}$ contralateral to lesion ($p < 0.05$ both sides, Friedman test; $n=6$; Fig. 1B). Withdrawal latencies returned to baseline values within 30 minutes after the end of stimulation.

MCS significantly increased mechanical withdrawal thresholds and thermal withdrawal latencies in 94% (14/15) of the animals on the ipsilateral hindpaw (ipsilateral to the lesion site). On the contralateral hindpaw, MCS was effective in 87% (13/15) of the animals.

Reduction in hyperalgesia is dependent on stimulation parameters

Clinicians are hesitant to prescribe MCS for patients suffering from neuropathic pain because of the varied success rate and mixed outcomes of MCS treatment (see Introduction). This is further compounded by the lack of consensus on what constitutes an effective stimulation protocol and how various stimulation parameters affect the analgesia produced. Studies using repetitive transcranial magnetic stimulation in healthy individuals report that theta burst stimulation (TBS) protocols can produce powerful effects on motor cortex outputs, with intermittent TBS (iTBS) being most effective [27]. Because of this, we tested whether TBS is effective in reducing hyperalgesia in our animal model of central pain.

TBS (3 stimuli at 50 Hz repeated every 200 ms [27]) had no effect on hyperalgesia measured immediately after MCS in animals with spinal cord injury ($n=6$; $p=1.0$; Friedman test; Fig. 1C). iTBS (2 sec trains of TBS repeated every 10 seconds) appeared to increase mechanical withdrawal thresholds on the hindpaw ipsilateral to the lesion immediately after stimulation; however, these threshold changes were not significant ($p = 0.075$, Friedman test; $n=6$; Fig. 1D). No changes in withdrawal threshold were found on the contralateral side. These results indicate that changes in mechanical hyperalgesia are dependent on the stimulation protocol used and that our stimulation protocol, continuous pulse MCS, was more effective in reducing hyperalgesia than the TBS protocols.

Next, using continuous pulse MCS, we examined which stimulation parameters were most effective at reducing hyperalgesia in our animal model spinal cord injury pain. We varied either the intensity, the frequency, or the duration of stimulation while keeping all other parameters constant and evaluated changes in mechanical thresholds immediately after the end of MCS and at 30 minute interval thereafter. In Figure 2A, we show the effects of varying the intensity of stimulation on mechanical thresholds (**constant parameters**: 50 Hz, 30 minutes, 300 μs). Increasing stimulation current resulted in an intensity dependent increase in mechanical thresholds on both the ipsilateral (ipsilateral to the spinal lesion) and contralateral hindpaws. While on the ipsilateral hindpaw stimulation at the lowest intensity, 10 μA ($n=7$), had no effect on the mechanical thresholds, stimulation at higher intensities did significantly increase thresholds [**prestimulation**: $60.00 \pm 0.00\text{g}$; **10 μA** : $71.43 \pm 19.52\text{g}$ ($n=7$); **25 μA** : $82.86 \pm 21.38\text{g}$ ($n=7$); **50 μA** : $93.33 \pm 15.57\text{g}$ ($n=12$); **75 μA** : $88.57 \pm 19.52\text{g}$ ($n=7$); $p < 0.001$, Kruskal-Wallis followed by Dunn's post hoc; Fig 2A].

On the contralateral hindpaw, however, only stimulation at 50 μA was able to significantly raise mechanical thresholds while 10 μA , 25 μA , or 75 μA of stimulation had no significant effect on hyperalgesia [**prestimulation**: $53.82 \pm 13.75\text{g}$; **10 μA** : $55.14 \pm 13.23\text{g}$; **25 μA** : $55.14 \pm 12.85\text{g}$; **50 μA** : $77.19 \pm 24.34\text{g}$; **75 μA** : $65.71 \pm 15.12\text{g}$; $p = 0.013$; Fig. 2A].

Therefore, stimulation at 50 μ A was most effective at reducing hyperalgesia on both hindpaws.

In Figure 2B we show the effect of varying the frequency of stimulation (**constant parameters:** 50 μ A, 30 minutes, 300 μ s) on mechanical hyperalgesia. Stimulating at both 50 Hz (n=11) and 75 Hz (n=6) resulted in a significant reduction of hyperalgesia in the ipsilateral hindpaw (ipsilateral to the lesion), but stimulation at 10 Hz (n=6) had no effect [**prestimulation:** 60.00 $\pm$ 0.00g; **10 Hz:** 60.00 $\pm$ 0.00g; **50 Hz:** 92.73 $\pm$ 16.18g; **75 Hz:** 86.67 $\pm$ 20.66g; $p < 0.001$ Kruskal-Wallis followed by Dunn's post hoc; Fig. 2B).

On the hindpaw contralateral to the lesion only stimulation at 50 Hz (n=11) was effective in reducing hyperalgesia while neither stimulation at 10 Hz nor stimulation at 75 Hz significantly increased mechanical thresholds [**prestimulation:** 53.82 $\pm$ 13.75g; **10 Hz:** 48.67 $\pm$ 17.56g; **50 Hz:** 77.55 $\pm$ 25.49g; **75 Hz:** 66.55 $\pm$ 16.33g; $p = 0.024$, Fig. 2B]. These data indicate that stimulation at 50 Hz is most effective at reducing hyperalgesia in both hindpaws.

We next examined the effect of changing stimulation duration on mechanical thresholds. Over the course of two weeks animals were tested using calibrated von Frey filaments immediately before and immediately after 1, 15, 30, 60, or 90 minutes of MCS (50 μ A, 50 Hz, 300 μ s).

In the hindpaw ipsilateral to the lesion, 15 (n=10), 30 (n=11), 60 (n=6), and 90 (n=10) minutes of stimulation significantly increased mechanical thresholds after the end of MCS, but stimulation lasting 1 minute (n=6) failed to significantly reduce hyperalgesia [**prestimulation:** 60.00 $\pm$ 0.00g; **1 min:** 60.00 $\pm$ 0.00g; **15 min:** 88.00 $\pm$ 19.32g; **30 min:** 92.73 $\pm$ 16.18g; **60 min:** 100 $\pm$ 43.82g, **90 min:** 88.00 $\pm$ 19.32g; $p < 0.001$, Kruskal-Wallis followed by Dunn's post hoc; Fig. 2C].

On the contralateral hindpaw (contralateral to the lesion), 30 minutes and 90 minutes of stimulation increased mechanical thresholds significantly after the end of stimulation while stimulation lasting 1, 15, or 60 minutes failed to cause significant changes in hyperalgesia after MCS [**prestimulation:** 53.82 $\pm$ 14.08g; **1 min:** 56.12 $\pm$ 9.39g; **15 min:** 68.3 $\pm$ 24.08g; **30 min:** 80.64 $\pm$ 20.11g; **60 min:** 74.33 $\pm$ 30.74g; **90 min:** 76.00 $\pm$ 20.66g; $p = 0.02$; Fig. 2C]. Therefore, 30 minutes of stimulation was most effective at reducing hyperalgesia in both hindpaws.

Reduction in hyperalgesia outlasts duration of stimulation

Human studies report that MCS not only can provide immediate relief from pain in patients, but that it can also produce analgesia that lasts long after stimulation ceases (see Introduction). Therefore, we investigated the duration during which mechanical thresholds remained elevated after MCS ("post effects") in our animal model of central pain. To this end, we stimulated the motor cortex for varying durations (as described in Methods) and obtained mechanical thresholds from animals with hyperalgesia immediately after the end of stimulation and again at 30 minute intervals until mechanical thresholds returned to prestimulation values.

Stimulation duration positively correlated with the duration of post effects (**ipsilateral to the lesion:** $\rho = 0.61$, $p < 0.0001$; **contralateral:** $\rho = 0.526$, $p < 0.0001$, Spearman's; Fig. 2D). With increased duration, mechanical thresholds in the hindpaw ipsilateral to the lesion remained elevated after the end of stimulation for the following lengths of time [see Fig. 2D; **no stim (n=15):** 0.00 $\pm$ 0.00 min; **1 min stim:** 0.00 $\pm$ 0.00 min; **15 min stim:** 36.00 $\pm$ 30.98 min; **30 min stim:** 50.00 $\pm$ 31.40 min; **60 min stim:** 57.00 $\pm$ 38.60 min; **90 min stim:** 60.00

± 46.90 min]. We obtained similar results on the hindpaw contralateral to the lesion [**no stim**: 0.00 ± 0.00 min; **1 min stim**: 0.00 ± 0.00 min; **15 min stim**: 21.00 ± 14.49 min; **30 min stim**: 36.00 ± 28.23 min; **60 min stim**: 18.60 ± 20.48 min; **90 min stim**: 51.00 ± 37.55 min]. Taken together, these findings suggest that the following stimulation parameters: 50 μ A, 50 Hz, 300 μ s for a duration of at least 15 minutes (continuous pulse MCS) are effective at reducing hyperalgesia bilaterally in rats with spinal cord lesions.

Zona incerta stimulation mimics the effects of MCS

The data presented here demonstrate that continuous pulse MCS significantly reduces hyperalgesia in this model of central pain. We have demonstrated previously that the development of hyperalgesia is associated with reduced activity in the inhibitory nucleus zona incerta (ZI) in rats [33]. Because the motor cortex sends dense projections to the ventral division of ZI (ZIV) [35,62], we hypothesized that MCS reduces hyperalgesia by increasing activity in ZI. This hypothesis predicts that electrical stimulation of ZI will also reduce hyperalgesia.

To test this prediction we implanted bipolar stimulating electrodes in ZI of 8 animals concurrent with spinal lesion surgery (see Methods). In animals that developed hyperalgesia, we stimulated ZI and tested mechanical and thermal thresholds immediately following the termination of stimulation. Electrical stimulation in ZI (25 μ A, 50 Hz, 300 μ s square pulse, 15 minutes) caused a significant increase in mechanical thresholds immediately after the end of stimulation in both hindpaws (**ipsilateral to the lesion**: from 63.33 ± 8.16 g to 95.55 ± 18.21 g; $p = 0.03$; **contralateral**: from 66.67 ± 10.31 g to 102.22 ± 13.12 g; $p = 0.03$, Wilcoxon; $n=6$; Fig. 3A). In addition, ZI stimulation significantly increased thermal withdrawal latencies in both hindpaws after the end of stimulation (**ipsilateral to the lesion**: from 6.13 ± 0.92 s to 10.05 ± 0.95 s; $p = 0.01$; **contralateral**: from 6.28 ± 0.44 s to 9.94 ± 1.28 s; $p = 0.02$, Wilcoxon; $n=8$; Fig. 3B). Therefore, consistent with our hypothesis, increasing activity in ZI reduces hyperalgesia.

Inactivation of ZI prevents MCS induced reduction in hyperalgesia

To further test our hypothesis that MCS reduces hyperalgesia by activating ZI, we investigated whether reversible inactivation of ZI occluded the effects of MCS. We implanted microdialysis cannulae in ZI as well as MCS electrodes above M1. Animals received infusions of either saline ($n=4$), or 2% lidocaine into ZI during MCS and changes in mechanical withdrawal thresholds were assessed immediately after the end of stimulation and at 30 minute interval thereafter (see Methods). Lidocaine infusion in ZI completely blocked the effects of MCS (pre stim: 60.00 ± 0.00 g; end of stim: 60.00 ± 0.00 g; $p = 1.00$, Friedman test; Fig 3C). Because lidocaine inactivates sodium channels, it is possible that infusion of lidocaine inactivated fibers of passage traveling through ZI. Therefore, we repeated the experiments using GABAA agonist muscimol (200 μ M, $n=4$) for more specific inactivation. Muscimol, like lidocaine, blocked MCS effects (pre stim: 60.00 ± 0.00 g; end of stim: 60.00 ± 0.00 g; $p = 1.00$, Friedman). Similar results were seen in the hindpaw contralateral to spinal lesion (data not shown). Importantly, infusion of the same volume of saline into ZI did not disrupt the MCS induced reduction in hyperalgesia (pre stim: 60.00 ± 0.00 g; end of stim: 100.00 ± 0 g; 30 min post stim: 84.00 ± 21.91 g; 60 min post stim: 60.00 ± 0.00 g; $p = 0.01$, Friedman test followed by Dunnett's post hoc; Fig. 3C). In all animals, we performed postmortem histological analysis to confirm correct placement of cannula. Figure 3D shows a small lesion in the ventral portion of ZI at the site of drug infusion and Figure 3E is a schematic representation of ZI and adjacent structures with reconstruction of the injection sites.

These data suggest that MCS reduces hyperalgesia by increasing activity in ZI and suggest that ZI may play an integral role in mediating the reduction in hyperalgesia observed after MCS.

Discussion

Reduced hyperalgesia after MCS

To date, clinical studies have failed to reveal which stimulus parameters are critical for MCS to successfully reduce hyperalgesia (see Introduction). Here, using an animal model of spinal cord injury pain, we systematically varied stimulation parameters to test the effects on hyperalgesia immediately after MCS, an advantage not available in human studies. We found that, in rats, MCS at an intensity of 50 uA and frequency of 50 Hz was most effective at reducing mechanical hyperalgesia bilaterally.

The finding that reduction of hyperalgesia after MCS extends beyond the duration of stimulation is consistent with the reported post effects in humans (see Introduction). These post effects are especially promising, as they offer a potential cure or treatment for intractable pain. Understanding the mechanisms by which MCS induces long lasting pain relief is crucial to increasing the efficacy of MCS treatment in the clinical population.

Specificity of MCS effects

In this study, we stimulated the motor cortex but did not test the effects of stimulating other cortical structures because it has been shown that cortical stimulation in areas other than the motor cortex is not as effective in reducing pain. In humans, stimulation of the pre-frontal and somatosensory cortices did not produce significant analgesia in patients with neuropathic pain [51]. Similarly, in rodents, stimulation of the primary or second somatosensory cortices, or the posterior parietal cortex had little or no effect on nociceptive responses [19,29]. For these reasons, we focused our study on stimulation of the primary motor cortex.

In humans, the consensus is that MCS effects are restricted to areas contralateral to the stimulation site and therefore stimulation is usually applied to the motor cortex contralateral to the painful region, which is normally ipsilateral to the site of the spinal cord injury. However there are some reports that MCS resulted in analgesia on the ipsilateral side [42,58]. In addition, a recent study using repetitive transcutaneous magnetic stimulation demonstrated that MCS reduced laser evoked pain perception bilaterally [48]. Therefore, bilateral effects cannot be ruled out. In the present study, animals developed bilateral mechanical hyperalgesia following spinal cord injury and stimulating electrodes were implanted contralateral to the lesion site. Although this makes it difficult to compare our findings to clinical situations, unilateral MCS in our animal model produced consistent bilateral reduction in mechanical withdrawal thresholds measured after the end of stimulation. The bilateral effects of MCS are consistent with previous reports in rats [66,67] and they could be due to MCS influence on structures that receive somatosensory inputs from bilateral areas of the body such as the posterior thalamus [33,47], or due to trans-callosal activation of the contralateral motor cortex [3].

Animal model of central pain

In humans, a hallmark characteristic of central pain syndrome is severe, spontaneous pain [11,71]. A major limitation of animal models of central pain, including ours, is the inability to convincingly demonstrate that animals suffer from spontaneous pain after injury. Thus far studies attempting to demonstrate spontaneous pain in animals have relied on behaviors such as overgrooming/autotomy, licking, guarding and vocalization [64]. However, these metrics

are not reliable, nor are they specific to pain sensations [36]. Recently, a conditioned place preference paradigm was used to demonstrate that animals with peripheral nerve injury suffer from tonic pain [28]. In the future, a similar strategy to that of the conditioned place preference paradigm may prove useful to test if animals with spinal cord injury develop signs of spontaneous pain and to test if MCS reduces these signs.

A potential limitation to our animal model is that damage to the spinothalamic tract results in bilateral below level hyperalgesia. It is expected that unilateral damage to the spinothalamic tract would result in below level pain that is restricted to areas contralateral to the injury. However, some exceptions are reported in animal and human literature. In rats two different animal models demonstrate that unilateral damage to the spinal cord results in bilateral below level thermal and mechanical hyperalgesia [12,13,65]. Additionally, in monkeys, unilateral cuts of the anterolateral quadrant of the spinal cord result in bilateral increased sensitivity to noxious stimuli [63]. Finally, in humans, below level pain due to spinothalamic tract lesions can be bilateral [24], or even ipsilateral to the site of injury in some instances [4,68]. Although not common, these reports suggest that pain following unilateral spinal cord lesions is not always limited to a contralateral distribution [64]. Development of bilateral hyperalgesia could be due to pathologic changes in distant spinal sites affecting caudal spinothalamic tract projections, or due to changes in supraspinal targets that receive bilateral convergent inputs (eg: [33]).

Mechanisms of pain relief

Several hypotheses have been proposed to explain how MCS provides pain relief. In general, most propose that MCS enhances inhibition in one of three structures along the neural axis: (1) within the neocortex; (2) in the spinal cord; or (3) within the thalamus [8,44,46].

Advocates for a cortical mechanism of pain relief believe that MCS enhances activity of “non-nociceptive” sensory inputs in the primary somatosensory cortex (S1) which, in turn, inhibit nociceptive neurons in S1 that receive inputs from the spinothalamic tract [17,30]. However, this notion may be dismissed by imaging studies demonstrating that MCS was not associated with changes in cerebral blood flow in the primary motor or somatosensory cortices [21–23,44].

There are those who argue that MCS may directly or indirectly inhibit nociceptive inputs in the spinal cord. Direct inhibition is unlikely, though, because M1 does not project to the superficial layers or marginal zone of the dorsal horn [52]. An indirect role is more likely, as MCS may activate descending inhibitory systems and cause endogenous opioid release [19] (but see [66]). Despite limited support for this claim, manipulations that specifically activate endogenous opioid release, such as deep brain stimulation of the periaqueductal gray, are especially poor for the treatment of CPS [25,57].

Some authors hypothesized that MCS activates corticothalamic connections, and these in turn inhibit nociceptive processing in the thalamus [6,7,51]. In support of this hypothesis, it was argued that patients responsive to GABA or barbiturate treatment are more likely to benefit from MCS [8–10]. However, the specific role of the thalamus is still debatable [44], and the source of altered inhibition, the mechanisms for engagement inhibition and the specific nuclei affected by MCS remain to be elucidated.

Here we focus on the role of the ZI in mediating the effects of MCS. We found that reversible inactivation of ZI with lidocaine or muscimol blocks the reduction of hyperalgesia observed after MCS. In these experiments it is important to consider the time course of wearing off of lidocaine and muscimol relative to MCS post effects. A previous report [32]

estimated that lidocaine effects last from 30–60 minutes after injection into the cortex at low concentrations (40µg/1µl vs. 1mg/50µl used in this study). The same study [32] found that the effects of muscimol last from 30–120 minutes. Considering that the reduction in hyperalgesia after the end of MCS lasts for 30–60 minutes in control experiments (Fig. 3C) and that drug infusion continues 15 minutes into MCS (see Methods), then the effects of the drugs applied are expected to match or even outlast the post effects of MCS, especially when considering the higher concentrations of lidocaine used in our study. Future experiments using reversible inactivation while varying the concentration and timing of administration of drugs relative to stimulation may prove useful to further test the mechanisms involved in the prolonged after effects observed after MCS. Another possibility is that the applied drugs diffused beyond the boundaries of ZI and affected neighboring structures such as the internal capsule or the ventroposterior thalamus. These structures may be involved in mediating MCS effects and therefore results should be interpreted with caution.

We also found that electrical stimulation of ZI mimics MCS effects and therefore, we hypothesize that MCS produces its effects by enhancing activity of ZI in rats. Enhanced activity in the GABAergic ventral division of ZI may result in increased inhibitory inputs to higher order thalamic nuclei that are involved in nociceptive processing, specifically the posterior thalamic nucleus (PO) [33,59]. It is important to note, however, that the human thalamus is more complex than, and not completely analogous to, the rat thalamus [16]. As such, the human homologue to the rat PO remains to be identified.

In this study, we identify the ZI as a source of inhibition that can be manipulated to produce pain relief and describe a novel system that affects nociceptive transmission within the thalamus through corticothalamic interactions. Identifying the mechanisms involved in the short- and long-term consequences of MCS will shift current research and clinical practice paradigms and lead towards the development of molecular, pharmacologic and physiologic methods for permanent pain relief that target these structures.

Acknowledgments

The authors would like to sincerely thank Dr. Raimi Quiton for her valuable input and Dr. Asaf Keller for his guidance, technical expertise and support. This project was supported by a National Institute of Neurological Disorders Research Grant R01-NS069568 and a Department of Defense Grant SC090126 to R.M.

Reference List

1. Baastrup C, Finnerup NB. Pharmacological management of neuropathic pain following spinal cord injury. *CNS Drugs*. 2008; 22:455–475. [PubMed: 18484790]
2. Bezard E, Boraud T, Nguyen JP, Velasco F, Keravel Y, Gross C. Cortical stimulation and epileptic seizure: a study of the potential risk in primates. *Neurosurgery*. 1999; 45:346–350. [PubMed: 10449080]
3. Bogdanova OG, Sil'kis IG. The effects of high-frequency microstimulation of the cortex on interhemisphere synchronization in the rat motor cortex. *Neurosci Behav Physiol*. 1999; 29:515–522. [PubMed: 10596787]
4. Bowsher D. Contralateral mirror-image pain following anterolateral cordotomy. *Pain*. 1988; 33:63–65. [PubMed: 3380552]
5. Brown JA, Pilitsis JG. Motor cortex stimulation for central and neuropathic facial pain: a prospective study of 10 patients and observations of enhanced sensory and motor function during stimulation. *Neurosurgery*. 2005; 56:290–297. [PubMed: 15670377]
6. Canavero S, Bonicalzi V. Cortical stimulation for central pain. *J Neurosurg*. 1995; 83:1117. [PubMed: 7490637]

7. Canavero S, Bonicalzi V. Resolution of central pain. *J Neurosurg.* 1999; 91:715–716. [PubMed: 10507401]
8. Canavero S, Bonicalzi V. Extradural cortical stimulation for central pain. *Acta Neurochir Suppl.* 2007; 97:27–36. [PubMed: 17691286]
9. Canavero S, Bonicalzi V. Central pain syndrome: elucidation of genesis and treatment. *Expert Rev Neurother.* 2007; 7:1485–1497. [PubMed: 17997698]
10. Canavero S, Pagni CA, Castellano G, Bonicalzi V, Bello M, Duca S, Podio V. The role of cortex in central pain syndromes: preliminary results of a long-term technetium-99 hexamethylpropyleneamineoxime single photon emission computed tomography study. *Neurosurgery.* 1993; 32:185–189. [PubMed: 8437655]
11. Canavero, S.; Bonicalzi, V. *Central Pain Syndrome: Pathophysiology, Diagnosis and Management.* New York: Cambridge Univ Press; 2007. p. 382
12. Christensen MD, Everhart AW, Pickelman JT, Hulsebosch CE. Mechanical and thermal allodynia in chronic central pain following spinal cord injury. *Pain.* 1996; 68:97–107. [PubMed: 9252004]
13. Christensen MD, Hulsebosch CE. Chronic central pain after spinal cord injury. *J Neurotrauma.* 1997; 14:517–537. [PubMed: 9300563]
14. Cruccu G, Aziz TZ, Garcia-Larrea L, Hansson P, Jensen TS, Lefaucheur JP, Simpson BA, Taylor RS. EFNS guidelines on neurostimulation therapy for neuropathic pain. *Eur J Neurol.* 2007; 14:952–970. [PubMed: 17718686]
15. Deuschl G, Herzog J, Kleiner-Fisman G, Kubo C, Lozano AM, Lyons KE, Rodriguez-Oroz MC, Tamma F, Troster AI, Vitek JL, Volkmann J, Voon V. Deep brain stimulation: postoperative issues. *Mov Disord.* 2006; 21 Suppl 14:S219–S237. [PubMed: 16810719]
16. Dostrovsky, JO. Ascending projection systems. In: McMahon, S.; Koltzenburg, M., editors. *Wall and Melzack's Textbook of Pain.* Oxford: Churchill Livingstone; 2005. p. 187-204.
17. Drouot X, Nguyen JP, Peschanski M, Lefaucheur JP. The antalgic efficacy of chronic motor cortex stimulation is related to sensory changes in the painful zone. *Brain.* 2002; 125:1660–1664. [PubMed: 12077014]
18. Ebel H, Rust D, Tronnier V, Boker D, Kunze S. Chronic precentral stimulation in trigeminal neuropathic pain. *Acta Neurochir (Wien).* 1996; 138:1300–1306. [PubMed: 8980733]
19. Fonoff ET, Dale CS, Pagano RL, Paccola CC, Ballester G, Teixeira MJ, Giorgi R. Antinociception induced by epidural motor cortex stimulation in naive conscious rats is mediated by the opioid system. *Behav Brain Res.* 2008; 196:63–70. [PubMed: 18718490]
20. Fontaine D, Hamani C, Lozano A. Efficacy and safety of motor cortex stimulation for chronic neuropathic pain: critical review of the literature. *J Neurosurg.* 2009; 110:251–256. [PubMed: 18991496]
21. Garcia-Larrea L, Peyron R. Motor cortex stimulation for neuropathic pain: From phenomenology to mechanisms. *Neuroimage.* 2007; 37 Suppl 1:S71–S79. [PubMed: 17644413]
22. Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Bonnefoi F, Mauguire F, Laurent B, Sindou M. Positron emission tomography during motor cortex stimulation for pain control. *Stereotact Funct Neurosurg.* 1997; 68:141–148. [PubMed: 9711707]
23. Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Le Bars D, Convers P, Mauguire F, Sindou M, Laurent B. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. *Pain.* 1999; 83:259–273. [PubMed: 10534598]
24. Graf CJ. Consideration in loss of sensory level after bilateral cervical cordotomy. *Arch Neurol.* 1960; 3:410–415.
25. Gybels J, Kupers R. Deep brain stimulation in the treatment of chronic pain in man: where and why? *Neurophysiol Clin.* 1990; 20:389–398. [PubMed: 2092202]
26. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain.* 1988; 32:77–88. [PubMed: 3340425]
27. Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron.* 2005; 45:201–206. [PubMed: 15664172]

28. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci.* 2009; 12:1364–1366. [PubMed: 19783992]
29. Kuroda R, Kawabata A, Kawao N, Umeda W, Takemura M, Shigenaga Y. Somatosensory cortex stimulation-evoked analgesia in rats: potentiation by NO synthase inhibition. *Life Sci.* 2000; 66:PL271–PL276. [PubMed: 10821124]
30. Lefaucheur JP, Drouot X, Menard-Lefaucheur I, Nguyen JP. Neuropathic pain controlled for more than a year by monthly sessions of repetitive transcranial magnetic stimulation of the motor cortex. *Neurophysiol Clin.* 2004; 34:91–95. [PubMed: 15130555]
31. Lima MC, Fregni F. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. *Neurology.* 2008; 70:2329–2337. [PubMed: 18541887]
32. Martin JH. Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neurosci Lett.* 1991; 127:160–164. [PubMed: 1881625]
33. Masri R, Quiton RL, Lucas JM, Murray PD, Thompson SM, Keller A. Zona Incerta: A Role in Central Pain. *J Neurophysiol.* 2009; 102:181–191. [PubMed: 19403748]
34. Classification of Chronic Pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms. Seattle: IASP Press; 1994. p. 240
35. Mitrofanis J, Mikuletic L. Organisation of the cortical projection to the zona incerta of the thalamus. *J Comp Neurol.* 1999; 412:173–185. [PubMed: 10440718]
36. Mogil JS. Animal models of pain: progress and challenges. *Nat Rev Neurosci.* 2009; 10:283–294. [PubMed: 19259101]
37. Namba S, Nakao Y, Matsumoto Y, Ohmoto T, Nishimoto A. Electrical stimulation of the posterior limb of the internal capsule for treatment of thalamic pain. *Appl Neurophysiol.* 1984; 47:137–148. [PubMed: 6334494]
38. Namba S, Nishimoto A. Stimulation of internal capsule, thalamic sensory nucleus (VPM) and cerebral cortex inhibited deafferentation hyperactivity provoked after gasserian ganglionectomy in cat. *Acta Neurochir Suppl (Wien).* 1988; 42:243–247. [PubMed: 3263751]
39. Neafsey EJ, Bold EL, Haas G, Hurley-Glus KM, Quirk G, Sievert CF, Terreberry RR. The organization of the rat motor cortex: a microstimulation mapping study. *Brain Res.* 1986; 396:77–96. [PubMed: 3708387]
40. Nguyen JP, Lefaucheur JP, Le Guerinel C, Eizenbaum JF, Nakano N, Carpentier A, Brugieres P, Pollin B, Rostaing S, Keravel Y. Motor cortex stimulation in the treatment of central and neuropathic pain. *Arch Med Res.* 2000; 31:263–265. [PubMed: 11036176]
41. Nguyen JP, Lefaucheur JP, Decq P, Uchiyama T, Carpentier A, Fontaine D, Brugieres P, Pollin B, Feve A, Rostaing S, Cesaro P, Keravel Y. Chronic motor cortex stimulation in the treatment of central and neuropathic pain. Correlations between clinical, electrophysiological, and anatomical data. *Pain.* 1999; 82:245–254. [PubMed: 10488675]
42. Parrent AG, Tasker RR. Can the ipsilateral hemisphere mediate pain in man? *Acta Neurochir (Wien).* 1992; 117:89.
43. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.
44. Peyron R, Faillenot I, Mertens P, Laurent B, Garcia-Larrea L. Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. *Neuroimage.* 2007; 34:310–321. [PubMed: 17055297]
45. Peyron R, Garcia-Larrea L, Deiber MP, Cinotti L, Convers P, Sindou M, Maguiere F, Laurent B. Electrical stimulation of precentral cortical area in the treatment of central pain: electrophysiological and PET study. *Pain.* 1995; 62:275–286. [PubMed: 8657427]
46. Peyron R, Garcia-Larrea L, Gregoire MC, Convers P, Richard A, Lavenne F, Barral FG, Maguiere F, Michel D, Laurent B. Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. *Pain.* 2000; 84:77–87. [PubMed: 10601675]

47. Poggio GF, Mountcastle VB. A study of the functional contributions of the lemniscal and spinothalamic systems to somatic sensibility. Central nervous mechanisms in pain. Bull Johns Hopkins Hosp. 1960; 106:266–316. [PubMed: 14433641]
48. Poreisz C, Csifcsak G, Antal A, Levold M, Hillers F, Paulus W. Theta burst stimulation of the motor cortex reduces laser-evoked pain perception. Neuroreport. 2008; 19:193–196. [PubMed: 18185107]
49. Quiton RL, Masri R, Thompson SM, Keller A. Abnormal Activity of Primary Somatosensory Cortex in Central Pain Syndrome. J Neurophysiol. 2010 In press.
50. Ren K. An improved method for assessing mechanical allodynia in the rat. Physiol Behav. 1999; 67:711–716. [PubMed: 10604842]
51. Saitoh Y, Shibata M, Hirano S, Hirata M, Mashimo T, Yoshimine T. Motor cortex stimulation for central and peripheral deafferentation pain. Report of eight cases. J Neurosurg. 2000; 92:150–155. [PubMed: 10616094]
52. Schoenen, J.; Grant, G. Spinal cord: connections. In: Paxinos, G.; Mai, JK., editors. The human nervous system. Amsterdam: Elsevier Academic Press; 2004. p. 233-250.
53. Sol JC, Casaux J, Roux FE, Lotterie JA, Bousquet P, Verdier JC, Mascott C, Lazorthes Y. Chronic motor cortex stimulation for phantom limb pain: correlations between pain relief and functional imaging studies. Stereotact Funct Neurosurg. 2001; 77:172–176. [PubMed: 12378072]
54. Son BC, Lee SW, Choi ES, Sung JH, Hong JT. Motor cortex stimulation for central pain following a traumatic brain injury. Pain. 2006; 123:210–216. [PubMed: 16616421]
55. Son UC, Kim MC, Moon DE, Kang JK. Motor cortex stimulation in a patient with intractable complex regional pain syndrome type II with hemibody involvement. Case report. J Neurosurg. 2003; 98:175–179. [PubMed: 12546368]
56. Tani N, Saitoh Y, Hirata M, Kato A, Yoshimine T. Bilateral cortical stimulation for deafferentation pain after spinal cord injury. Case report. J Neurosurg. 2004; 101:687–689. [PubMed: 15481727]
57. Tasker RR. Microelectrode findings in the thalamus in chronic pain and other conditions. Stereotact Funct Neurosurg. 2001; 77:166–168. [PubMed: 12378070]
58. Tasker RR, Parrent AG, Kiss ZHT, Davis K, Dostrovsky JO. Surgical treatment of stroke-induced pain. Stereotact Funct Neurosurg. 1994; 62:311–312.
59. Trageser JC, Keller A. Reducing the uncertainty: Gating of peripheral inputs by zona incerta. J Neurosci. 2004; 24:8911–8915. [PubMed: 15470158]
60. Tsubokawa T, Katayama Y, Yamamoto T, Hirayama T, Koyama S. Treatment of thalamic pain by chronic motor cortex stimulation. Pacing Clin Electrophysiol. 1991; 14:131–134. [PubMed: 1705329]
61. Tsubokawa T, Katayama Y, Yamamoto T, Hirayama T, Koyama S. Chronic motor cortex stimulation in patients with thalamic pain. J Neurosurg Journal of Neurosurgery. 1993; 78:393–401.
62. Urbain N, Deschenes M. Motor cortex gates vibrissal responses in a thalamocortical projection pathway. Neuron. 2007; 56:714–725. [PubMed: 18031687]
63. Vierck CJJ, Greenspan JD, Ritz LA. Long-term changes in purposive and reflexive responses to nociceptive stimulation following anterolateral chordotomy. J Neurosci. 1990; 10:2077–2095. [PubMed: 2376769]
64. Vierck CJJ, Siddall P, Yezierski RP. Pain following spinal cord injury: animal models and mechanistic studies. Pain. 2000; 89:1–5. [PubMed: 11113287]
65. Vierck CJJ, Light A. Effects of combined hemotoxic and anterolateral spinal lesion on nociceptive sensitivity. Pain. 1999; 83:447–457. [PubMed: 10568853]
66. Viisanen H, Pertovaara A. Antinociception by motor cortex stimulation in the neuropathic rat: does the locus coeruleus play a role? Exp Brain Res. 2009; 201:283–296. [PubMed: 19826796]
67. Viisanen H, Pertovaara A. Roles of the rostroventromedial medulla and the spinal 5-HT(1A) receptor in descending antinociception induced by motor cortex stimulation in the neuropathic rat. Neurosci Lett. 2010; 476:133–137. [PubMed: 20398735]
68. Walker AE. The Spinothalamic Tract in Man. Arch Neurol Psychiat. 1940; 43:284–298.

69. Wang G, Thompson SM. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *J Neurosci*. 2008; 28:11959–11969. [PubMed: 19005061]
70. Weiss DS, Keller A. Specific patterns of intrinsic connections between representation zones in the rat motor cortex. *Cerebral Cortex*. 1994; 4:205–214. [PubMed: 8038569]
71. Yezierski RP. Pain following spinal cord injury: pathophysiology and central mechanisms. *Prog Brain Res*. 2000; 129:429–449. [PubMed: 11098709]

Figure 1. Continuous Pulse Motor Cortex Stimulation Reduced hyperalgesia

A. Thirty minutes of continuous pulse MCS significantly increased mechanical thresholds in both ipsilateral and contralateral hindpaws (relative to the lesion) after MCS ($p < 0.001$ Friedman test followed by Dunnett's post hoc; $n=11$). Stim. off values taken immediately before stimulation, time=0 marks the time MCS ended). Horizontal dotted lines indicate average mechanical thresholds after spinal surgery (black: hindpaw ipsilateral to the lesion; gray: contralateral). **B.** Continuous pulse MCS significantly increased latency to withdraw from a radiant heat source in both hindpaws immediately after 30 minutes of stimulation ($p < 0.05$ Friedman test followed by Dunnett's post hoc, $n=6$). **C.** Continuous theta burst stimulation did not significantly increase mechanical thresholds in either hindpaw of animals with hyperalgesia ($n=6$). **D.** Intermittent theta burst stimulation did not significantly increase mechanical thresholds in animals with hyperalgesia (hindpaw ipsilateral to the lesion: $p = 0.075$, Friedman test; $n=6$). Asterisk indicates statistically significant difference ($p < 0.05$).

Figure 2. Effect of MCS Parameters on Hyperalgesia

A. The effect of varying stimulation intensity on mechanical withdrawal thresholds. In the hindpaw ipsilateral to the lesion, 25 μ A (n=7), 50 μ A (n=12) and 75 μ A (n=7) stimulation significantly increased mechanical thresholds after the end of MCS ($p < 0.001$, Kruskal-Wallis followed by Dunn's post hoc). The contralateral hindpaw showed significantly increased thresholds only after 50 μ A stimulation ($p = 0.013$, Kruskal-Wallis followed by Dunn's post hoc). **B.** The effect of varying stimulation frequency on mechanical withdrawal thresholds. Ipsilateral thresholds (ipsilateral to the lesion) were significantly raised when M1 was stimulated at 50 Hz (n=11) and at 75 Hz (n=6) ($p < 0.001$, Kruskal-Wallis followed by Dunn's post hoc). Contralateral thresholds were significantly raised when MCS occurred at 50 Hz ($p = 0.024$, Kruskal-Wallis followed by Dunn's post hoc). **C.** The effect of varying stimulation duration on mechanical withdrawal thresholds. Hyperalgesia in the hindpaw ipsilateral to the lesion was significantly reduced after 15 min (n=10), 30 min (n=11), 60 min (n=6), and 90 min (n=10) of continuous pulse MCS while hyperalgesia in the contralateral hindpaw was significantly reduced after only 30 min and 90 min of MCS ($p < 0.001$ and $p = 0.02$ respectively, Kruskal-Wallis followed by Dunn's post hoc). **D.** Duration of MCS is positively correlated with duration of post effects in both hindpaws ($p < 0.001$; Spearman's).

Figure 3. ZI Mediates the Effects of MCS

ZI stimulation significantly increased (A) mechanical thresholds and (B) thermal thresholds bilaterally in animals with hyperalgesia. C. Inactivation of ZI with lidocaine or muscimol occludes the effects of MCS. The infusion of the same volume of saline had no effect on MCS-induced reduction in hyperalgesia ($p = 0.01$, Friedman test followed by Dunnett's post hoc). Ipsilateral hindpaw (relative to lesion site) shown for clarity; similar effects were seen in the contralateral hindpaw. D. Representative microdialysis cannula placement in ZI. Arrow indicates small lesion produced by drug infusion, scale bar = 150 μm . E. Schematic representation of ZI and adjacent structures and reconstruction of injection site in three animals. The schematic was adopted from [42] and modified. VP: ventral posterolateral thalamus, ZId: zona incerta, dorsal part; ZIv: zona incerta, ventral part, ic: internal capsule.