Brain stimulation patterns emulating endogenous thalamocortical input to parvalbumin-expressing interneurons reduce nociception in mice

Yeowool Huh a, b, Dahee Jung a, b, c, Taeyoon Seo d, Sukkyu Sun d, Su Hyun Kim c, e, Hyewhon Rhim e, Sooyoung Chung c, e, Chong-Hyun Kim c, e, Youngwoo Kwon d, Marom Bikson f, Yong-an Chung g, Jeansok J. Kim h, Jeiwon Cho a, b, *

a Translational Brain Research Center, Catholic Kwandong University International St. Mary’s Hospital, Incheon, South Korea
b Dept. of Medical Science, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do, South Korea
c Department of Neuroscience, University of Science and Technology, Daejeon, South Korea
d Department of Electrical and Computer Engineering, Seoul National University, Seoul, South Korea
e Center for Neuroscience, Korea Institute of Science and Technology, Seoul, South Korea
f Department of Biomedical Engineering, The City College of the City University of New York, NY, USA
g Department of Radiology, Incheon St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, South Korea
h Department of Psychology, University of Washington, Seattle, WA, USA

1. Introduction

The investigation of electrical brain stimulation to control central pain is long-standing and often empirical [1–3]. Generally, the mechanism of pain relief is based on interfering with neuronal circuits responsible for pain processing or perception [4,5] with synthetic brain stimulation patterns intended to override
endogenous activity. Could novel brain stimulation strategies that correct circuit pathology with patterns emulating endogenous activity enhance therapeutic efficacy? Such an approach derives from a precise hypothesis on disease etiology to identify 1) firing patterns correlated with suppression of pain and, 2) the cellular targets of that patterned activity that mediate pain processing.

Nociception serves vital protective functions against bodily injury. As part of the TC circuit, the sensory thalamus plays a critical role in gating transmission of peripheral nociceptive information to the somatosensory cortex, where representation and perception of pain is assumed to occur [6–10]. This sensory gating function of the thalamus has been suggested to be mediated by the ability of individual TC neurons to fire in tonic and burst firing modes via interconnections with the cortex and thalamic reticular nucleus (TRN) [11–14]. Specifically, the γ-aminobutyric acid (GABAergic) projection from TRN to TC neurons de-inactivates T-type calcium channels, inducing strong inhibition that, in turn, leads to low threshold calcium spike “rebound” bursts [15]. Subsequent in-vivo studies suggest that the tonic firing of TC neurons correlates with nociceptive responses [16–18] while the burst firing of TC neurons correlates with suppression of pain responses [16,19–21].

Although studies suggested differential roles for TC tonic and burst firing in pain processing, how the dual firing modes of TC neurons contribute to differential pain processing in the somatosensory cortex, which should be a crucial part of an ascending pain control mechanism, is currently unknown. The sensory cortex is a highly organized structure with layer specific input/outputs and the sensory TC neurons, which directly receive peripheral sensory inputs, primarily synapse onto layer 4 of the cortex [22]. Of the two firing modes of TC neurons, burst firing, compared to tonic firing, has been shown to have greater potency to activate inhibitory interneurons in the cortex [23,24].

Among the interneuron types expressed in the cortex, PV expressing inhibitory interneurons are especially suited to exert feed-forward inhibition to excitation pyramidal neurons. Of the two main type of GABAergic interneurons expressed in layer 4 of the cortex, PV interneurons are more abundant (constituting 60% of GABAergic interneurons) than somatostatin (SOM) expressing interneurons (constituting 20–30% of GABAergic interneurons) [25,26]. PV interneurons are fast-spiking and synapse onto proximal dendrites or somatic regions of pyramidal neurons [27,28]. Cortical PV interneurons are directly innervated by thalamic projections [29,30] while SOM interneurons only have weak connections with thalamic inputs [31,32]. Together, these properties make PV interneurons ideal for implementing feed-forward inhibition [29] that can be driven by high frequency TC burst firing.

Activity of PV interneurons is reduced or disrupted in the somatosensory cortex of mice with neuropathic pain [33,34] and SOM activation can alleviate neuropathic pain associated allodynia [34]. However, the role of cortical PV interneurons within the TC circuit in gating nociception of non-neuropathic conditions remains uninvestigated. In particular, a circuit level mechanism of how the dynamics between TC tonic and burst dual firings modulate nociception at the cortical level is unknown. The present study examined whether burst, but not tonic, firing mode of TC neurons engages cortical PV interneurons to exert inhibitory modulation of pyramidal neurons in the primary (S1) somatosensory cortex and whether activation of cortical PV interneurons could behaviorally suppress nociceptive responses in mice.

Using electrical stimulation and immunohistochemical methods we investigated whether burst stimulation of TC neurons could significantly activate PV interneurons in the sensory cortex compared to tonic stimulation or sham control conditions. Next we tested whether selective activation or inactivation of cortical PV interneurons with optogenetic or patterned transcranial magnetic stimulations could modulate nociceptive behaviors in mice.

2. Materials and methods

2.1. Animals

Optogenetic experiments employed PV-Cre male mice (8–16 weeks; Jackson Laboratories). All other studies used first generation 129/SvJae x C57BL/6J hybrid mice (male, 8–12 weeks). Mice were group-housed and maintained at 12 h light-dark cycle (lights on at 8 a.m.) with free access to food and water. Following a surgery, animals were singly-housed. All experiments were conducted in compliance with the Animal Care and Use Committee (Approval number: AP, 20150225). Mice were randomly assigned to experimental groups and based on histology animals with misplaced electrodes or viral injections were excluded from analyses.

2.2. Surgical procedures

All surgical procedures were performed under anesthesia (30 mg/kg Zoletil, IP) and using a stereotaxic instrument (Kopf Instruments) with brain coordinates based on the Paxinos and Franklin (2001) mouse brain atlas [35]. Animals were given Ketoprofen (5 mg/kg SC) right after surgery and daily for a week for post-operative recovery.

For electrical stimulation of the ventroposterolateral (VPL) thalamus, two bipolar stimulating electrodes (0.6 mm apart; Teflon-coated stainless steel, 0.003” bare 0.055” coated, A-M Systems) were implanted (AP: –1.34 mm, ML: –1.85 mm, DV: –3.2 mm). The electrodes were secured onto the skull with stainless steel screws and dental cement.

For optogenetic experiments, AAV-DIO-Chr2–eYFP and AAV-DIO–eYFP purchased from the University of North Carolina Vector Core were injected into the primary somatosensory cortex corresponding to the hind limb region (S1HL; AP: –0.5 mm, ML: –1.64 mm, DV: –0.5 mm). The virus injections were made slightly lateral to the optic fiber implantation site, avoiding major arteries. Using glass pipettes (tip size 20–38 μm), a total of 200 nl was injected over 10 min using a Nanoliter Injector (World Precision Instruments). After a week of recovery, an optic fiber (GIF 625, Thor Labs) was chronically implanted into the S1HL (AP: –0.5 mm, ML: –1.6 mm, DV: –0.4 mm).

For TMS, a plastic baseplate was permanently affixed to the skull with Loctite 454 and dental cement. Later, a solenoid coil was connected to the baseplate for magnetic stimulation centering on the S1HL (AP: –0.5 mm, ML: –1.6 mm).

2.3. Electrical stimulation

Mice were habituated to tethering, mockup IP injection (using a syringe without needle), and the experimental apparatus for 30 min daily for a week. On the experiment day, mice were anesthetized with urethane (1.5 g/kg IP), connected to a stimulation cable, and after 10 min received either tonic or burst stimulation for 5 min. Mice in the sham control group were attached to the stimulation cable for the same duration without receiving stimulations. All stimulating pulses were biphasic square pulses with 100 μs current amplitude and 100 μs duration. Burst stimulation consisted of 3 ms intervals of 5 burst pulses with 600 ms interval between the 5 burst pulses, while tonic stimulation was 600 pulses at 2 Hz.
2.4. Immunohistochemistry

Mice were anesthetized with urethane (1.5 g/kg IP) and brains were extracted after transcardial perfusion with physiological saline (0.9%) followed by 10% formalin solution diluted in physiological saline at room temperature. Brains were then successively placed in 10% formalin solution for a day at 4°C and 30% sucrose solution for two days at 4°C, before being cut in coronal sections (40 μm) with a cryostat (Microm). Free floating sections were processed for standard immunohistochemical procedures, mounted on microscope slides, and images were acquired with a fluorescence microscope (Zeiss AxioImager M2) and a confocal microscope (Olympus Fluoview FV 1000) for analysis.

For the electrical stimulation experiment, mice were perfused 90 min after stimulation and processed for cFos and parvalbumin double fluorescence labeling. Free floating brain sections were blocked with 10% normal donkey serum (NDS) in PBS containing 0.3% Triton X-100 for 2 h at room temperature and incubated in a mixture of 1:1000 rabbit anti-parvalbumin (Abcam; ab11427) and 1:100 goat anti-cFos (Santa Cruz Biotechnology; sc-52-G) in 3% NDS diluted with PBS for 72 h at 4°C. The brain sections were then incubated for 1 h with 1:200 AlexaFluor 568 anti-rabbit (Invitrogen; A10042), and 1:200 AlexaFluor 488 anti-goat (Invitrogen; A11055).

For verification of expression location and specificity of PV neurons expressing ChR2, PV-Cre mice injected with AAV-DIO-Chr2–eYFP virus were double labelled for PV and eYFP. Coronal brain sections (40 μm) cut through the S1HL region were blocked with 10% NDS in PBS containing 0.1% Triton X-100 for 2 h at room temperature and incubated in a mixture of 1:1000 rabbit anti-parvalbumin (Abcam; ab11427) and 1:1000 chicken anti-YFP (Abcam; ab13970) overnight at room temperature. The brain sections were then incubated for 2 h with 1:200 Alexa 488 anti-chicken (Jackson ImmunoResearch; 103-545-155) and 1:200 Alexa 594 anti-rabbit (Vector Laboratories; DI-1594).

2.5. Analysis of immunolabeled neurons

Images (317 x 317 μm size) of the S1HL region from AP -0.46 mm to 0.82 mm in layer 4 and 5 were obtained with a confocal microscope using FluoView FV 1000 (Olympus). Laser settings were kept constant throughout the whole experiment. The number of cFos and PV labeled cells were manually counted by two investigators blind to groups using the Olympus FV10-ASW ver.4.1a Viewer.

2.6. Behavioral nociception tests

Electronic von Frey, plantar and formalin tests were used to gauge acute and tonic nociception changes to optical and magnetic stimulations. All brain stimulations were given in the right hemisphere. Before behavioral tests, mice were handled and habituated to the experimental environment for 30 min/day for a week. All experiments were performed under blind conditions to the background of mice (ChR2 or eYFP expression) and the type of magnetic stimulation. The number of cFos and PV labeled cells were manually counted by two investigators blind to groups.

2.7. TMS coil design and rTMS protocol

A solenoid coil (4 mm diameter x 10 mm height) made of 113 winding of 0.3 mm enamel insulated cooper wire was used for TMS. A plastic support stand was designed to anchor a solenoid coil to the baseplate. The weight of a baseplate, a support stand, and a coil was approximately 2 g. High frequency alternating current (125 kHz, 800 mA) was used for repetitive TMS (rTMS). TMS protocols used were, iTBS and cTBS, adapted from Haung et al. (2006) [36], and ‘Thalamic burst’, a new stimulation protocol devised based on thalamic burst firing patterns. For iTBS, 3 pulses at 50 Hz were repeated every 200 ms for 2 s with 8 s pause between bursts (Fig. 3b). For cTBS, 3 pulses at 50 Hz were repeated every 200 ms for 40 s with a 160 s pause between stimulations. For ‘Thalamic burst’, 5 pulses at 333 Hz (3 ms between pulses) were repeated every 314 ms for 37.44 s with a 162.56 s pause between stimulations. Equal number of pulses were delivered for iTBS, cTBS, and ‘Thalamic burst’: a total of 4200 pulses were delivered during the 25 min stimulation time in von Frey tests and a total of 6000 pulses were delivered during the 35 min stimulation time in plantar and formalin tests. Pulse duration was 10 ms for iTBS and cTBS, while pulse duration for ‘Thalamic burst’ was 2 ms, due to a short interval between stimulation pulses.

2.8. In vitro electrophysiology

Under isoflurane anesthesia, the mouse brains were rapidly extracted, and coronal sections (300 μm) were made using a vibratome (Leica) in ice cold ACSF (in mM: NaCl2 130, KCl 3.5, MgCl2 1, CaCl2 1.5, NaH2PO4 1.25, NaHCO3 25, glucose 10). Slices were then incubated in ACSF saturated with 95% O2 and 5% CO2 for at least an hour before commencing whole cell recordings using a Multi-Clamp700 B (Molecular Devices). The internal solution of glass recording electrodes contained 130 K-glucurate, 15 KCI, 5 NaCl, 5 Mg-ATP, 1 MgCl2, 5 EGTA, 1 CaCl2, and 10 HEPES, pH 7.2 (300 mOsm). All recordings were done at room temperature.

2.9. Data analysis

Statistical significances were assessed using repeated measures ANOVA followed by Games Howell post hoc test when comparing changes over time, and one-way ANOVA with Tukey HSD when comparing more than two means, except where noted. For data with normal distribution and unequal variance, Welch's ANOVA and Games–Howell post hoc test were performed. In cases where Levene's Test of Homogeneity of Variance was significant, Kruskal–Wallis and Mann-Whitney tests were used to test for group differences. Significance was determined at *p < 0.05. Appropriate group sizes were determined by a power analysis (G*Power 3.1),
interneurons in the cortex are critically involved in modulating pain. The increased PV interneuron activity (cFos/PV co-expression) relative to the sham control groups. Presumably, in the burst stimulation group, following different stimulation patterns to the VPL suggests that in layers 4/5a of the primary somatosensory cortex did not differ to the ipsilateral hemisphere, which corresponded to anti-Cre transgenic mice (Fig. 2A). ChR2 was expressed robustly and control virus (AAV-DIO-eYFP) was injected into the right S1HL of PV- expressing interneurons (Supplementary Fig. 2). Following con region within AP 0.38 mm to AP -1.34 mm range (Fig. 2B and C). A light stimulation protocol of 1 ms pulses of blue light (473 nm) delivered at 20 Hz significantly elevated mechanical and thermal nociceptive thresholds in the contralateral paw compared to the ipsilateral control paw (Fig. 2D and E). Inflammatory nociceptive responses induced by formalin injection in the contralateral paw were also significantly reduced by activation of cortical PV interneurons (Fig. 2F). Although ChR2 was expressed across layers 1–5 in S1HL, because the optic fiber tip was in layer 4 and because the estimated light density drops from 257 mW/mm² at the tip to 29 mW/mm² at 150 μm distance [38,39], the present anti-nociceptive effects are largely due to stimulation of PV interneurons in the layer 4. The fact that selective activation of PV interneurons can suppress diverse pain behaviors, strongly support the hypothesis that PV interneurons in the S1 are essential in gating nociception.

3.2. Optogenetic activation of cortical PV interneurons decreases nociceptive behaviors

Since thalamic burst firings reduced nociceptive behavior by affecting the activity of cortical inhibitory interneurons, we tested whether TMS protocol mimicking thalamic bursts, ‘Thalamic burst’, could also reduce nociceptive behaviors. To test this, the ability of a newly developed ‘Thalamic burst’ protocol to modulate nociceptive behaviors were compared with those of established protocols, iTBS and cTBS. A customized miniature TMS coil (Fig. 3A) was secured above the right hemisphere to apply magnetic stimulation. Nociceptive thresholds were measured before and after delivering identical number of pulses to keep the total number of stimulating pulses equal among stimulation protocols (see methods for details). ‘Thalamic burst’, iTBS, or cTBS was applied to separate groups of animals to assess whether non-invasive method could actually modulate nociceptive behaviors. ‘Thalamic burst’ significantly decreased nociceptive responses of the contralateral paw in von Frey (mechanical) and plantar (thermal) tests (Fig. 3C and D). Formalin induced nociceptive behaviors were also significantly reduced by ‘Thalamic burst’ (Fig. 3E). The first phase behavioral responses (0–5 min) did not differ, but the second phase responses (20–25 min) significantly differed from the other groups (Fig. 3E, bar graph). In contrast, iTBS, which was reported to decrease the activity of cortical PV interneurons [40,41], significantly enhanced nociceptive behaviors in both the first (0–5 min) and second phases (25–30 min), while cTBS has no significant effect (Fig. 3C–E). These rTMS results support that nociceptive behaviors could be differentially modulated by TMS protocols designed to mimic endogenous thalamocortical input.

4. Discussion

4.1. Circuit based neuromodulation

The treatment of pain disorders is among the most long-standing, technologically diverse, and prevalent applications for neuromodulation [42–44]. Brain stimulation anatomical targets have been justified by contemporaneous theories of pain for example gate-control by activation of peripheral and central afferents, stimulated release of endogenous opioids by stimulation of deep nuclei, modulation of sensory integration by thalamic stimulation, modulation of sensory perception by motor cortex stimulation, or modulation of pain by frontal cortex stimulation [3,45,46]. Technological advancement has similarly focused on new anatomical targets (e.g., invasive and non-invasive forms of current delivery) including new implanted leads [47,48], magnetic induction [49,50], and transcranial electrical targeting [51]. The exploration of waveforms has been relatively limited, often exploring using an alpha of 0.05, power of 0.8, and effect size (Cohen’s f) of 0.4 for F tests and 0.8 for t tests [37]. Analyses were performed with SPSS 13.0 and graphs were plotted with Microsoft Excel.
variations in the frequency of tonic stimulation or adopting canonical patterns demonstrated to produce plasticity in human and animal neurophysiology (e.g., theta burst, direct current). Approaches using more customized waveforms are investigated [52,53] including closed-loop approaches [54–58].

A circuit based approach to neuromodulation [59,60] involves consideration of both the anatomical target and waveform, in the context of pathological network activity. The approach taken here was to characterize a network associated with dampening of nociceptive responses and to design brain stimulation strategies to engage this same network activity.

4.2. TC circuit mechanism of nociceptive gating

We believe the present study provides direct evidence of a novel TC circuit mechanism of nociceptive signal gating that involves PV...
interneurons in the somatosensory cortex (Fig. 4). To implement the thalamic sensory gating role, tonic and burst firing of TC neurons are likely to activate different cellular substrates in the cortex. Specifically, our model predicts that tonic firing of TC neurons (conveying pain information) will produce excitatory post-synaptic responses on pyramidal neurons in the sensory cortex while GABAergic activity itself was insufficient to overcome neuropathic pain models was associated with increased nociceptive behavior, enhanced nociception in high frequencies [67,68], were also found to be significantly reduced in the same study [33], emphasizing the importance of functionally active PV interneurons in controlling pain.

4.3. Cell-type specificity

Although increased excitatory activity was reported to be associated with increased nociceptive behavior, enhanced GABAergic activity itself was insufficient to overcome neuropathic
pain symptoms, since the activities of both excitatory and inhibitory cortical neurons were enhanced in a chronic pain model [66]. This may in part be due to the difference in activation pattern of different types of inhibitory interneurons in the cortex, which have different roles. In support of this prediction, a recent study showed that the activities of cortical inhibitory interneurons that express SOM and PV were reduced while those expressing vasoactive intestinal polypeptide (VIP) was enhanced [34]. When SOM neurons were selectively activated, mechanical allodynia induced by neuropathic pain was reduced, supporting the importance of targeting a specific type of neurons for treating pain.

Understanding the cellular targets of neuromodulation (which cellular elements are stimulated [69]) is pivotal to mechanism-based interventions [70,71]. Long-standing efforts to optimize targeting of specific cell types is intended to enhance specificity in outcomes [72,73]; while peripheral stimulation focuses on selecting axon types [74,75], selectivity in the CNS is complicated by the diversity of morphology and interconnectivity of neurons [76]. iTBS was suggested to reduce the activity of cortical PV interneurons while cTBS reduces the activity of cortical SOM interneurons [40,41]. The present finding that iTBS to the somatosensory exacerbated nociceptive behaviors in mice further supports the role of cortical PV interneurons in gating nociceptive signals.

We developed a new stimulation protocol based on thalamic burst firing patterns and targets; ‘Thalamic burst’ TMS, which was shown to have an antinociceptive effect in mice. This is consistent with the approach that stimulation protocols developed based on brain activity patterns may be useful for modulating specific

---

**Fig. 3. TMS of the cortex and nociception.** (A) Schematic drawing of TMS coil placement. TMS was given in awake behaving mice during nociception tests. (B) The total numbers of iTBS, cTBS, and thalamic burst pulses were matched for each behavioral test (600 pulses/cycle, 1 cycle = 3 min 20 s). Figure not drawn to scale. (C) Mechanical threshold changes induced by different TMS protocols. Paw withdrawal thresholds were measure with von Frey filaments (Kruskal-Wallis test with Mann-Whitney U test; n = 6 mice per group). (D) Acute thermal nociception changes triggered by TMS protocols. Paw withdrawal latency to plantar paw IR irradiation (Kruskal-Wallis test with Mann-Whitney U test; n = 6 mice per group). (E) Formalin-induced inflammatory nociceptive behavior changes to different TMS protocols (repeated measures ANOVA followed by Games Howell post hoc; n = 6 mice per group). All data are shown as mean ± SEM. *P < 0.05 between groups indicated by horizontal lines. †P < 0.05 between the ‘thalamic burst’ and the ‘sham coil’ groups.
behaviors. Considering that different stimulation patterns lead to activation of different cell types in the brain [41,77,78], the new stimulation protocol putatively activated cortical PV interneurons, but this remains to be verified.

4.4. Modulatory effect of brain stimulations

Several neuromodulation methods—deep brain stimulation (DBS), motor cortex stimulation (MCS), transcranial direct current stimulation (tDCS), or TMS—have been shown to be effective in modulating pain [4,79,80]. Exact mechanisms of action of these stimulation methods are not completely understood but may activate endogenous (e.g. opioid) regulatory systems [81,82]. Any of these techniques to deliver electricity to the brain may benefit from incorporating endogenous patterns, provided it is symptom etiology and target circuit specific. When stimulation leads to lasting changes (e.g. clinical benefit after a stimulation session) this approach should be linked to identifying underlying neuroplasticity or molecular changes. For example, modulatory effect of brain stimulations may occur partly by influencing glia [83—88], since glial cells in the brain, especially astrocytes and microglia, are closely related to chronic pain [89,90].

4.5. Limitations

The limitations of the present work include those universal to any animal model of diseases. Nonetheless, mechanisms and interventions found relevant in nociception of mice, including the three behaviors tested here, have provided useful translational predictions [91]. There are further inherent limitations in translating the brain stimulation protocols here to clinical use, namely TMS is less focal in rodent models [92—94] even as we developed a specialized coil. However, the differentiations we show in regard to both waveform pattern and laterality, buttress overall conclusions on specificity and targeting. Finally, it is important to recognize that precisely because we suggest matching neuromodulation strategy to endogenous pain networks, diverse pain etiology would suggest distinct interventional strategies.

5. Conclusion

Overall, our findings show that brain stimulations strategies mimicking endogenous TC activity dampen nociceptive behaviors in mice, supporting further investigation of targeted circuit-based neuromodulation interventions for pain.

Funding

This research was supported by the Ministry of Science and ICT through the National Research Foundation of Korea grants: Midcareer Researcher Program (NRF-2015R1A2A2A04005487) and Brain Science Research Program (NRF-2015M3C7A1028392). This study was also funded by NIH grant MH099073.

Declaration of interest

Authors confirm that there were no known conflicts of interest associated with this work and there were no financial support for this work that could have influenced its outcome.

Acknowledgements

We thank Nakajima Ryuichi and Bradley Baker for providing PV-Cre mice, and Frances Cho and Michael M. Morgan for helpful discussion and comments on the manuscript. We also acknowledge Dr. R. Jude Samulski and the UNC Vector Core for preparing gene transfer vectors.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.brs.2018.05.007.
References


Please cite this article in press as: Huh Y, et al., Brain stimulation patterns emulating endogenous thalamocortical input to parvalbumin-expressing interneurons reduce nociception in mice. Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.05.007

ARTICLE IN PRESS

Y. Huh et al. / Brain Stimulation xxx (2018) 1–9


