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

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ABSTRACT

Background: The bursting pattern of thalamocortical (TC) pathway dampens nociception. Whether brain stimulation mimicking endogenous patterns can engage similar sensory gating processes in the cortex and reduce nociceptive behaviors remains uninvestigated.
Objective: We investigated the role of cortical parvalbumin expressing (PV) interneurons within the TC circuit in gating nociception and their selective response to TC burst patterns. We then tested if transcranial magnetic stimulation (TMS) patterned on endogenous nociceptive TC bursting modulate nociceptive behaviors.
Methods: The switching of TC neurons between tonic (single spike) and burst (high frequency spikes) firing modes may be a critical component in modulating nociceptive signals. Deep brain electrical stimulation of TC neurons and immunohistochemistry were used to examine the differential influence of each firing mode on cortical PV interneuron activity. Optogenetic stimulation of cortical PV interneurons assessed a direct role in nociceptive modulation. A new TMS protocol mimicking thalamic burst firing patterns, contrasted with conventional continuous and intermittent theta burst protocols, tested if TMS patterned on endogenous TC activity reduces nociceptive behaviors in mice.
Results: Immunohistochemical evidence confirmed that burst, but not tonic, deep brain stimulation of TC neurons increased the activity of PV interneurons in the cortex. Both optogenetic activation of PV interneurons and TMS protocol mimicking thalamic burst reduced nociceptive behaviors.
Conclusions: Our findings suggest that burst firing of TC neurons recruits PV interneurons in the cortex to reduce nociceptive behaviors and that neuromodulation mimicking thalamic burst firing may be useful for modulating nociception.
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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

Abbreviations: cTBS, continuous theta-burst-stimulation; iTBS, intermittent theta-burst-stimulation; PV, parvalbumin expressing; PNNs, Peri-neuronal nets; rTMS, repetitive transcranial magnetic stimulation; S1, primary somatosensory cortex; SOM, somatostatin; TC, thalamocortical; TMS, transcranial magnetic stimulation; TRN, thalamic reticular nucleus; VPL, ventroposterolateral.

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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 suggests 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 excitatory 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/6 J 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, 2015025). 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 μA 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 fluorescent 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 × 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 background 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 delivered (intermittent theta-burst-stimulation: iTBS, continuous theta-burst-stimlation: cTBS, or 'Thalamic burst').

Electronic von Frey (IITC Inc.) was used to measure hind paw withdrawal thresholds. Two measurements were made each for baseline and during stimulations. Measurements were taken at 20 min interval for each paw. Optical stimulation was delivered at 20 Hz (tip power 0.8 mW) with a 473 nm blue laser diode (Shanghai Dream Lasers) just before measuring threshold changes induced by light stimulation and immediately turned off after making a measurement. For the magnetic stimulation, TMS was turned on for 600 pulses before the first measurement and stayed on until a set number of pulses were delivered.

Thermal thresholds were measured with a plantar test apparatus (Ugo Basile). Infrared (IR) intensity was set at 60 and cut off time was 33 s. Hind paw withdrawal latency was measured at 30 min intervals. Two measurements were taken for baseline and during stimulations. Same optical and magnetic stimulation methods as the von Frey experiment was used.

Formalin-induced nociception was induced by injecting 10 μl of 5% formalin solution diluted in saline (0.9%) to the left hind paw with a syringe attached to a customized 28 G needle (Hamilton). Optical stimulation (1 ms at 20 Hz, 473 nm light, 0.8 mW at tip) was turned on for 0–5 min and 15–30 min segments during the formalin test, which corresponds to the two peaks of nociceptive behaviors. Behaviors were videotaped and the duration of licking and shaking were scored in 5 min blocks 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 wounding of 0.3 mm enamel insulated copper wire was used for TMS. A plastic support stand was designed to anchor a solenoid coil to a 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: $NaCl_2$ 130, KCl 3.5, $MgCl_2$ 1, $CaCl_2$ 1.5, NaH_2PO_4 1.25, $NaHCO_3$ 25, glucose 10). Slices were then incubated in ACSF saturated with 95% O_2 and 5% CO_2 for at least an hour before commencing whole cell recordings using a MultiClamp700 B (Molecular Devices). The internal solution of glass recording electrodes contained 130 K-gluconate, 15 KCl, 5 NaCl, 5 Mg-ATP, 1 $MgCl_2$, 5 EGTA, 1 $CaCl_2$, 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),

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.

3. Results

3.1. Thalamic firing modes differentially affect the activity of cortical PV interneurons

To determine the relationship between thalamic firing patterns and cortical PV interneuron activity, two bipolar stimulating electrodes were implanted into each animal's right ventroposterolateral (VPL) thalamus (Fig. 1A). Mice were assigned to either burst (5 pulses at 333 Hz with 600 ms inter-burst interval; known to produce antinociceptive effects [16,19]), tonic (2 Hz), which does not induce any antinociceptive effect (data not shown), or sham control (no stimulation) groups. Stimulation procedures were carried out under urethane anesthesia to minimize the influence of sensory signals from confounding the results. The number of cFos and PV expressing neurons were quantified in layers 4/5a of the primary somatosensory cortex, corresponding to the hind limb (S1HL), which receives extensive inputs from the ipsilateral primary sensory thalamus [11,22]. While the number of cFos or PV expressing neurons in the right S1HL was similar between the three groups (Supplementary Fig. 1), the number of neurons co-expressing cFos and PV was significantly elevated only in animals that received burst stimulation. Specifically, >40% of PV interneurons in the right S1HL expressed cFos in response to burst stimulation of the right VPL, compared to 9% and 15% of PV interneurons co-expressing cFos in tonic stimulation and sham control groups, respectively (Fig. 1B). Further, the increased cFos/PV co-expression observed in the burst stimulation groups was confined to the ipsilateral hemisphere, which corresponded to antinociceptive effects on the contralateral body regions (see below Fig. 2C). The fact that the total number of cFos expressing neurons in layers 4/5a of the primary somatosensory cortex did not differ following different stimulation patterns to the VPL suggests that more non-PV neurons expressed cFos in tonic stimulation and sham control groups. Presumably, in the burst stimulation group, the increased PV interneuron activity (cFos/PV co-expression) reflects suppression of non-PV neuron activity (cFos only expression). These immunohistochemical results indicate that burst firing activities of the thalamus, which inhibit nociceptive responses, preferentially activate cortical PV interneurons.

3.2. Optogenetic activation of cortical PV interneurons decreases nociceptive behaviors

Next, we examined whether bypassing the VPL and directly activating PV interneurons can modulate nociceptive responses. To do so, double floxed (DIO) Cre-dependent adeno-associated virus (AAV) expressing channelrhodopsin-2 (AAV-DIO-ChR2-eYFP) or control virus (AAV-DIO-eYFP) was injected into the right S1HL of PV-Cre transgenic mice (Fig. 2A). ChR2 was expressed robustly and selectively in PV interneurons ($90 \pm 3\%$) in layers 1–5 of the S1HL region within AP 0.38 mm to AP -1.34 mm range (Fig. 2B and Supplementary Fig. 2). Following confirmation of functional expression of ChR2 via *in vitro* whole cell electrophysiology (Supplementary Fig. 3), PV interneurons in the right S1HL were optogenetically stimulated while performing several pain tests including the von Frey (acute mechanical), plantar (acute thermal) and formalin (lasting inflammatory) tests, to demonstrate that PV interneurons in the cortex are critically involved in modulating pain thresholds. Specifically, nociceptive thresholds of ipsilateral and contralateral paws were compared during optical stimulation to verify contralateralization of peripheral nociceptive information to the VPL-S1HL circuit (Fig. 2C). 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/mm^2 at the tip to 29 mW/mm^2 at 150 μm distance [38,39], the present antinociceptive 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.

3.3. TMS protocol mimicking thalamic bursts reduces 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

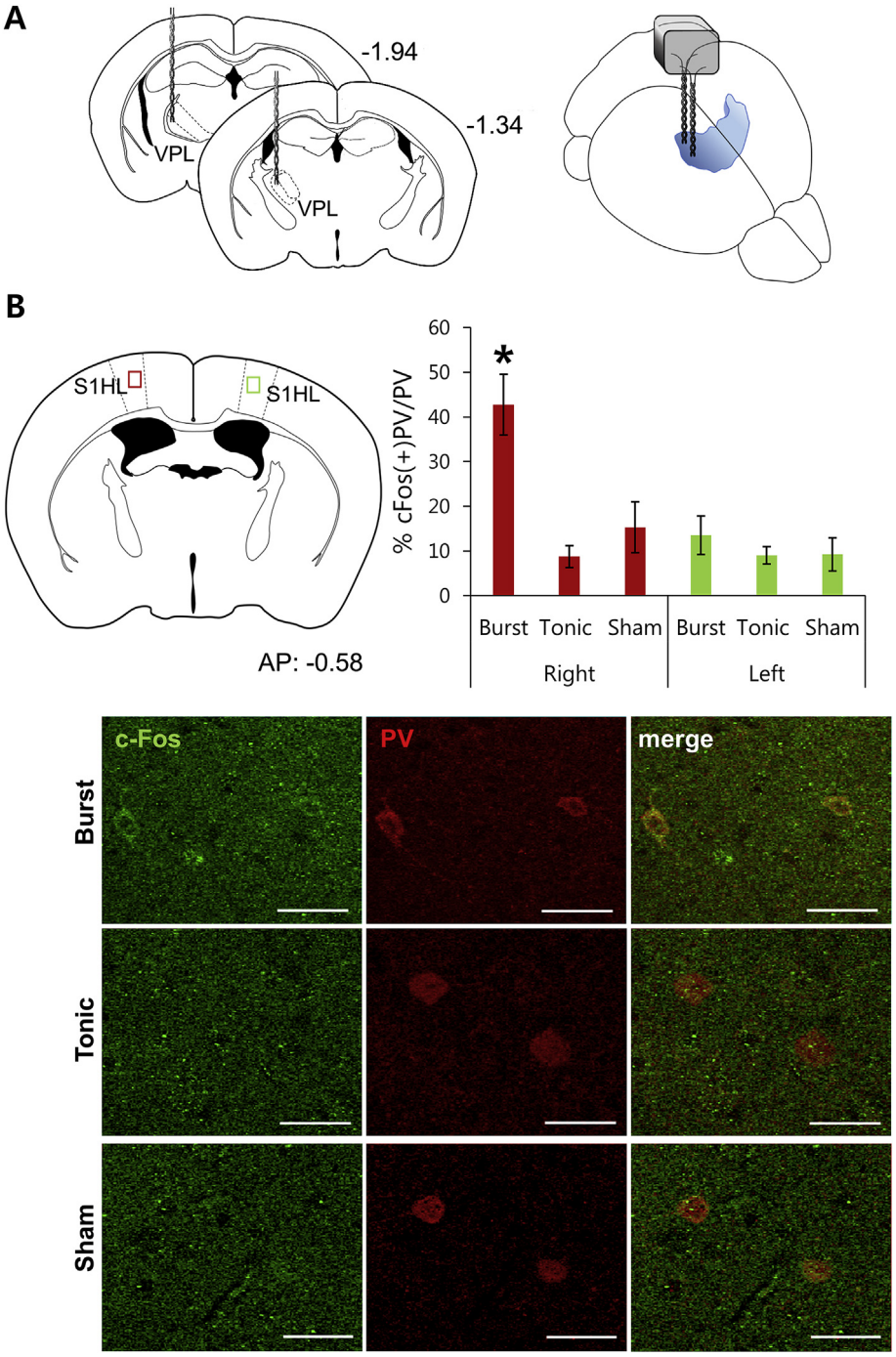

Fig. 1. Activation of cortical PV interneurons to electrical stimulation of the thalamus. (A) Schematic drawings of stimulating electrodes placed in the right VPL. (B) The red and green squares show the locations of the somatosensory cortex examined for cFos and PV double staining after electrical stimulation of the thalamus (top left). They also represent ipsi- and contralateral hemispheres to the stimulation site, respectively. Bar graph shows the percentage of cFos positive PV neurons to different types of electrical stimulations (top right). Bars indicate mean ± SEM * $P < 0.05$, Welch's ANOVA with Games-Howell post hoc indicated significant difference between burst and other groups. Bottom images shows representative samples of cFos and PV staining for 3 groups of electrical stimulations. (n = 3 mice per group, 3–4 slices per mouse).

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

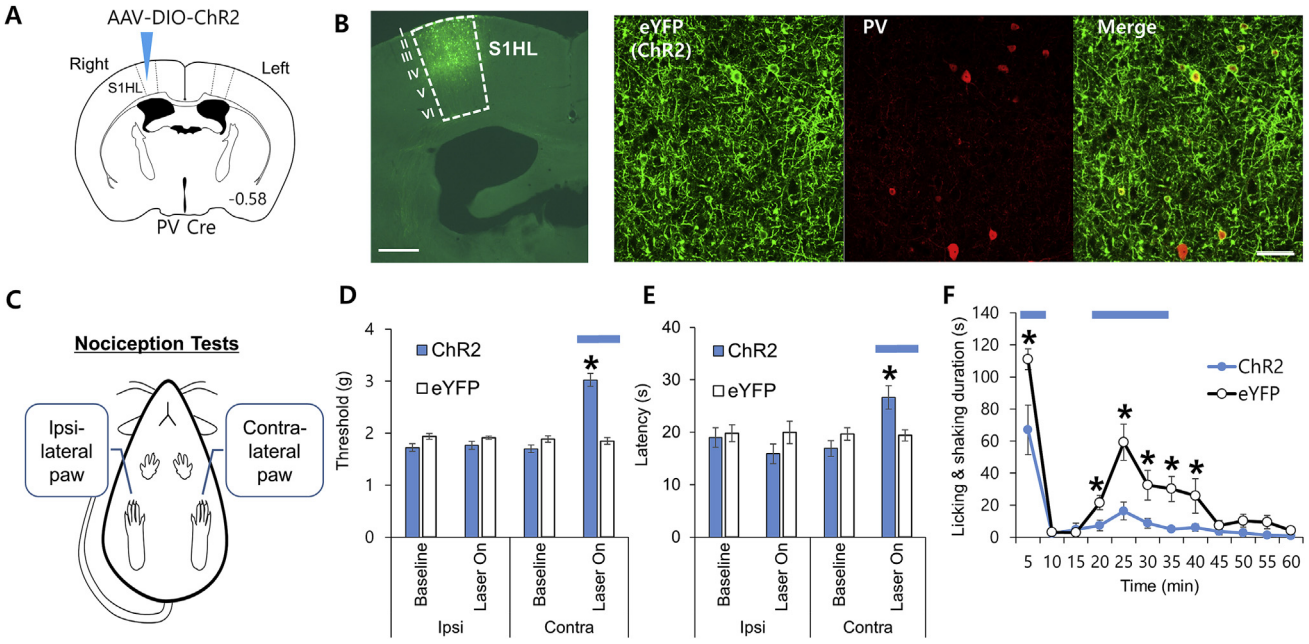

Fig. 2. The effects of optogenetic manipulation of PV interneurons in the primary sensory cortex on various nociception tests. (A) Schematic drawing of AAV-DIO-ChR2 injected into the right hemisphere of PV Cre mice. (B) Images showing ChR2 expressions in the upper layers of the S1HL region (left-most panel, 500 μm scale; three panels, 50 μm scale). (C) Nociceptive threshold changes to PV interneuron activation were measured for both paws in acute pain tests. Behavioral nociception was measured only for the left paw during the longer lasting formalin induced nociception. (D–F) Blue horizontal lines indicate blue light stimulation in the right hemisphere which affected the left paw, since nociceptive signals are transmitted contralaterally. (D) Mechanical threshold changes triggered by blue light stimulation were measured via von Frey (Welch's ANOVA with Games-Howell post hoc; ChR2, n = 6 mice; eYFP, n = 4 mice). (E) Acute thermal nociception changes triggered by blue light stimulation were measured via plantar test (Kruskal-Wallis test with Mann-Whitney *U* test; ChR2, n = 6 mice; eYFP, n = 4 mice). (F) Response to inflammatory nociception induced by formalin injection in the left paw. Blue light stimulation was given during the 0–5 min and 15–30 min segments as indicated with blue horizontal bars (repeated measures ANOVA followed by Games Howell post hoc; ChR2, n = 8 mice; eYFP, n = 8 mice). Data are presented as mean ± SEM, *P < 0.05.

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 insignificantly affecting the activity of PV interneurons (Fig. 4A). In contrast, high frequency burst firing of TC neurons is predicted to preferentially activate cortical PV interneurons to decrease nociception (Fig. 4B).

The exact mechanisms of how TC tonic firing could preferentially activate pyramidal neurons over inhibitory interneurons are unclear, but nociceptive signals may trigger TC neurons to fire in a synchronized tonic mode to excite excitatory neurons in cortical layer 4. Indeed, a previous study showed that synchronously active tonic firings of TC neurons were able to excite excitatory neurons in the cortical layer 4 [61]. Conversely, high frequency stimulation of TC neurons was reported to depress TC synapses [62]. Although direct evidence linking TC tonic firing and cortical pyramidal activity is missing, independent studies showed that increasing TC tonic firing enhanced pain [17,18] and that allodynia in neuropathic pain models was associated with increased activity of pyramidal neurons in the somatosensory cortex [34]. These studies suggest that increased TC tonic activity and cortical excitation could both contribute to enhanced pain behavior.

Burst firing of TC neurons, on the other hand, is likely to preferentially activate cortical PV interneurons to decrease nociception (Fig. 4B). Our data showed that burst stimulation of TC neurons significantly increased activation of PV interneurons in the somatosensory cortex and selective activation of them significantly reduced nociceptive behaviors. Although the role of TC burst firing in pathological pain is still controversial [6], recent papers suggest that burst firing of TC neurons acts to diminish nociceptive pain in non-pathological conditions [16,19–21], and our data provides evidence on how thalamic burst firing could attenuate nociception at the cortical level by a specific type of inhibitory interneuron: PV interneurons.

Among the various types of interneurons expressed in the cortex, PV interneurons are most suitable to exert powerful inhibition onto cortical pyramidal neurons to reduce nociceptive signals. Not only are they the most abundantly expressed inhibitory interneuron in layer 4 of the somatosensory cortex [25,63] which directly receives sensory information, but also their fast-spiking properties make them ideal for feed-forward and feed-back inhibition [29] to attenuate excessive excitatory activity. They are also interconnected via gap junctions, enabling these neurons to exert a synchronized inhibition onto pyramidal neurons in the sensory cortex [64]. Enhanced excitation in the somatosensory cortex was reported in chronic pain models [34,65,66], suggesting that reduced inhibition on pyramidal neurons could lead to pathological pain symptoms. More specifically, PV interneuron connectivity was found to be disrupted in the primary somatosensory cortex of multiple sclerosis chronic neuroinflammatory model [33]. Perineuronal nets (PNNs), a specialized extracellular matrix that enables PV interneurons to fire 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

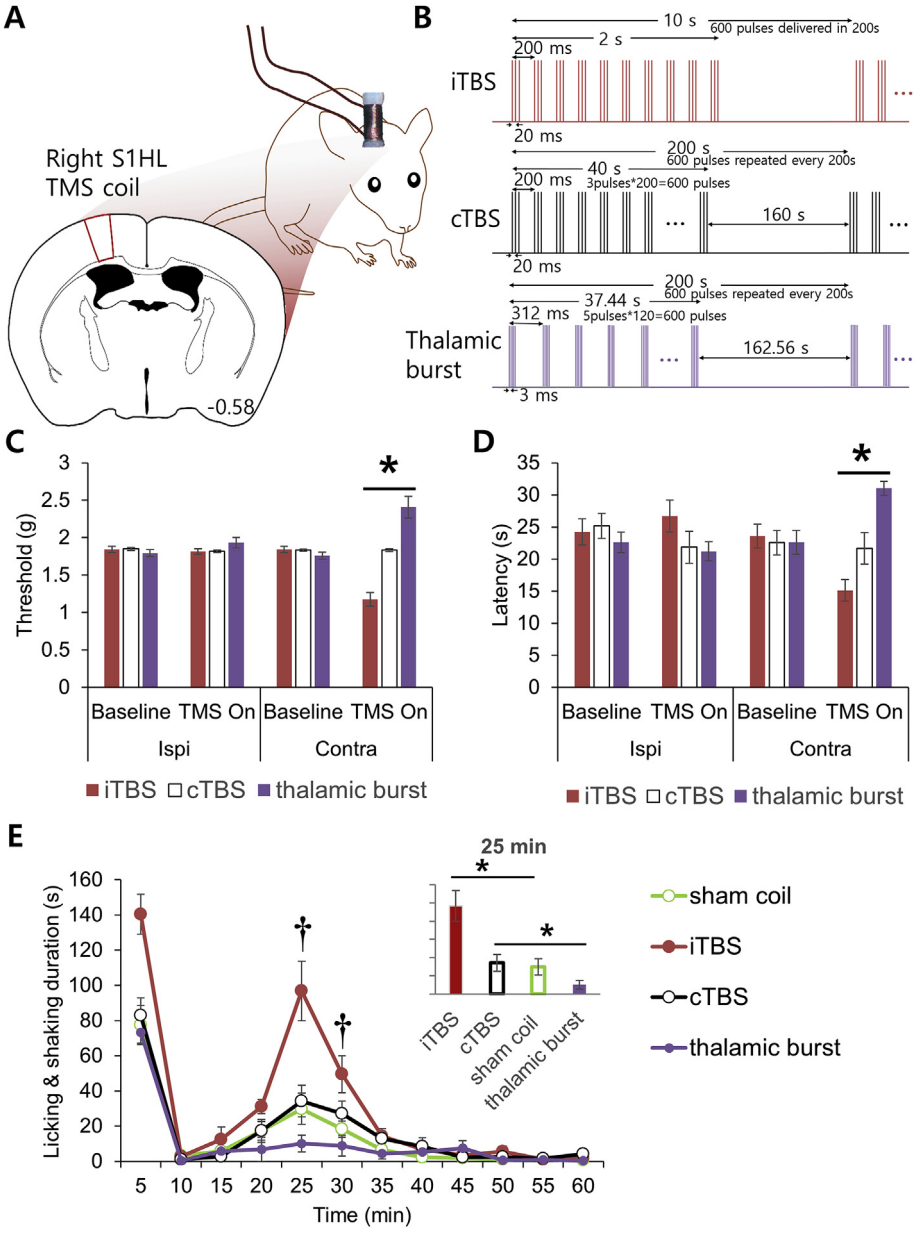

Fig. 3. TMS of the cortex and nociception. (A) Schematic drawing of TMS coil placement. TMS was given in awake behaving mice during nociception tests. (B) The total numbers of iTBS, cTBS, and thalamic burst pulses were matched for each behavioral test (600 pulses/cycle, 1 cycle = 3 min 20 s). Figure 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.

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

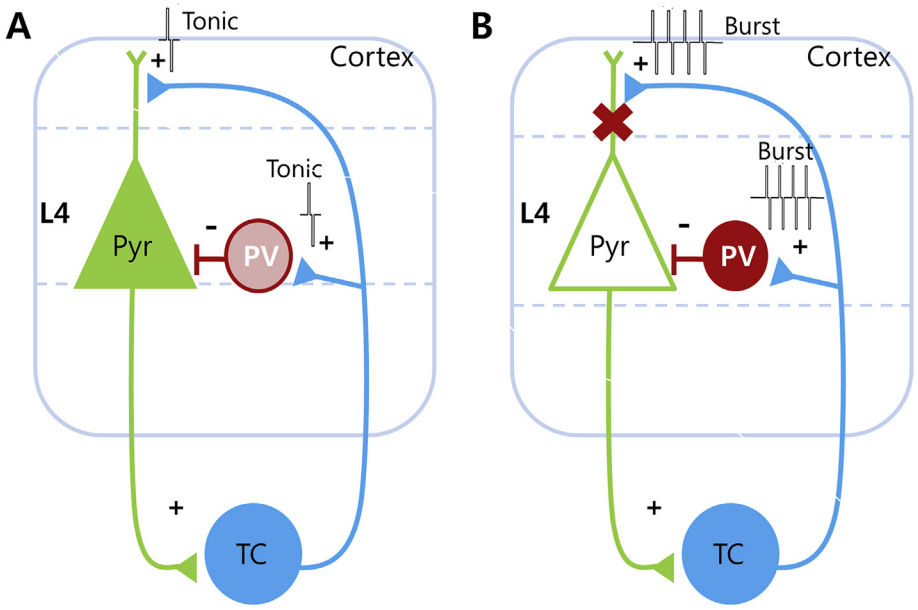

Fig. 4. A putative role of cortical PV interneurons in modulating nociceptive signals within the thalamocortical circuit. (A) Nociceptive signal transmission during TC neuronal tonic firing. (B) Inhibition of nociceptive signal transmission to cortical pyramidal (Pyr) neurons during TC neuronal burst firing. The filled Pyr neuron represents activation while empty Pyr neuron represents inactivation. The gradient of the PV color indicates the level of activation with darker color indicating greater activation.

behaviors. Considering that different stimulation patterns leads 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 active 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.

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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.

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Appendix A. Supplementary data

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

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