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Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects

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Abstract: **Pain modulation can be achieved using neuromodulatory tools that influence various levels of the nervous system. Transcranial direct current stimulation (tDCS), for instance, has been shown to reduce chronic pain when applied to the primary motor cortex. In contrast to this central neuromodulatory technique, diffuse noxious inhibitory controls (DNIC) refers to endogenous analgesic mechanisms that decrease pain following the introduction of heterotopic noxious stimuli. We examined whether combining top-down motor cortex modulation using anodal tDCS with a bottom-up DNIC induction paradigm synergistically increases the threshold at which pain is perceived. The pain thresholds of 15 healthy subjects were assessed before and after administration of active tDCS, sham tDCS, cold-water-induced DNIC, and combined tDCS and DNIC. We found that both tDCS and the DNIC paradigm significantly increased pain thresholds and that these approaches appeared to have additive effects. Increase in pain threshold following active tDCS was positively correlated with baseline N-acetylaspartate in the cingulate cortex and negatively correlated with baseline glutamine levels in the thalamus as measured by magnetic resonance spectroscopy. These results suggest that motor cortex modulation may have a greater analgesic effect when combined with bottom-up neuromodulatory mechanisms, presenting new avenues for modulation of pain using noninvasive neuromodulatory approaches.**

Perspective: ***This article demonstrates that both noninvasive motor cortex modulation and a descending noxious inhibitory controls paradigm significantly increase pain thresholds in healthy subjects and appear to have an additive effect when combined. These results suggest that existing pain therapies involving DNIC may be enhanced through combination with noninvasive brain stimulation.***

Key words: *Brain stimulation, transcranial direct current stimulation, tDCS, conditioned pain modulation, descending noxious inhibitory controls, magnetic resonance spectroscopy.*

Pain is mediated by neural activity in multiple networks distributed throughout the central and peripheral nervous systems.[25,52] Accordingly, approaches to treating acute and chronic pain have involved modulating this neural activity through direct modulation of involved cortical and subcortical structures via central neural pathways and indirect modulation via peripheral neural pathways. These 2 modulatory approaches may be viewed as top-down and bottom-up approaches, respectively.

One top-down modulatory approach that has been increasingly investigated involves noninvasive cortical stimulation using transcranial direct current stimulation (tDCS). tDCS is a safe and inexpensive form of noninvasive brain stimulation that involves the administration of a weak direct current to the scalp using sponge electrodes. tDCS has been shown to influence excitability of cortical areas directly beneath the electrodes as well as

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distant areas connected to the primary stimulated area[26] and its effects can last several hours.[39] Evidence suggests that tDCS of the primary motor cortex modulates pain through direct cortical effects on ventral lateral and anterior thalamic nuclei, as well as downstream effects on the medial thalamus, anterior cingulate, and upper brainstem.[17,48] Preliminary clinical trials have suggested that anodal tDCS of the primary motor cortex may be effective in treating chronic pain in conditions such as spinal cord injury,[12] fibromyalgia,[13,32] and chronic pelvic pain.[11]

In contrast to tDCS, diffuse noxious inhibitory controls (DNIC) refers to endogenous analgesic pathways involved in bottom-up modulation of the neural activity underlying pain. Recently termed conditioned pain modulation,[59] DNIC decreases sensitivity to a noxious stimulus (the "test-stimulus") after introduction of a heterotopic conditioning stimulus (the "conditioning-stimulus"), often summarized as "pain inhibits pain."[29] When pain signals ascend through the spinal cord from the periphery, supraspinal structures responsible for DNIC (such as the subnucleus reticularis dorsalis of the caudal medulla) mediate descending inhibition of lamina I neurons in the spinal dorsal horn.[28,55,56] Patients with hypersensitivity to pain have been observed to have impaired DNIC modulation,[24,27,30,54,58] and preoperative DNIC efficiency has been shown to be predictive of chronic postthoracotomy pain.[60] It has been theorized that certain pain therapies such as acupuncture may exert their effects, in part, through activation of DNIC.[6,35,40]

In the present study we aimed to assess whether top-down motor cortex modulation using anodal tDCS combined with bottom-up induction of DNIC using a cold-water immersion paradigm can result in synergistic effects on pain perception. This experiment follows from a growing literature suggesting that tDCS as a modulatory technique is more effective when combined with other therapeutic modalities. For instance, when coupled with transcutaneous electrical nerve stimulation (TENS) and visual illusion therapy, tDCS has been observed to be more effective at treating chronic pain.[3,47] Similarly, in the context of motor rehabilitation following stroke, outcomes are enhanced when tDCS is coupled with constraint-induced movement therapy.[57] This experiment also follows from research suggesting that DNIC is under cortical influence.[18] In order to gain additional mechanistic insights, we used magnetic resonance spectroscopy to determine whether particular brain metabolites correlate with the effects of these pain modulatory interventions. We predicted that decreased baseline glutamate levels would correlate with changes in pain thresholds following stimulation, given recent transcranial magnetic stimulation literature suggesting such an association.[14]

Methods

Study Design

We conducted a randomized, double-blinded, sham-controlled trial to evaluate the influences of tDCS and DNIC on pain thresholds in healthy subjects. The study was conducted in accordance with a protocol approved by the institutional review board of Spaulding Rehabilitation Hospital, Harvard Medical School. All subjects gave written, informed consent.

Subjects

Subjects 18 to 64 years old were recruited from the greater Boston area using flyers and online listings. Exclusion criteria included history of neurological or psychiatric disorders, history of substance abuse in the previous 6 months, regular use of medications, pregnancy, symptoms of chronic pain in the previous 6 months (also assessed by visual analog scale [VAS] for pain), and presence of contraindications to magnetic resonance imaging (MRI) or tDCS (eg, implanted brain medical devices). For sample size calculation, we decided to be conservative in our estimate. With a sample size of 15 subjects per group (cross-over study) and assuming a power of 80% and alpha of 5%, we would detect differences between groups of .45 given 2-tailed t-tests. This effect size is significantly smaller than in our previous study[4] and was thus adequate for testing our hypothesis. Fifteen subjects (mean age 36.7 $\pm$ 11.0 years, 9 females) were included in this study. Subjects received either active tDCS or sham tDCS in a randomized order during their first session. Both the rater and subjects were kept blinded to the stimulation conditions.

Experimental Design

Our study consisted of 3 visits. During Visit 1, subjects underwent magnetic resonance spectroscopy (MRS) to measure baseline concentrations of brain metabolites. Within 3 days of Visit 1, subjects were randomized by blocks of 4 subjects to receive active or sham tDCS, which was preceded and followed by sensory and cognitive assessments (Visit 2). At least 7 days after Visit 2, subjects underwent identical procedures as in Visit 2, with the sole difference being that stimulation conditions were switched (sham or active tDCS, respectively). See Fig 1 for a diagram of the experimental paradigm.

Magnetic Resonance Spectroscopy

MRS was performed to index the baseline levels of glutamate and other brain metabolites in pain-related regions of interest. We performed ^{1}H-MRS using a Philips Achieva 3.0T (Philips Healthcare, Best, Netherlands) running Release 2.6 software. The subjects were instructed to lie still for the approximately 30 minutes that data were collected. Single-voxel proton MR spectra were acquired and quantified with LCModel to determine metabolite concentration ratios. The MRS voxels ($2 \times 2 \times 2$ cm, 8 cm^3) were positioned on the coronal, sagittal, and axial images from the areas of thalamus, anterior cingulate cortex (Brodmann area 24), motor cortex, and occipital cortex.[2,42] The spectra were acquired using a point resolved spectroscopy (PRESS) sequence with a TR of 2 seconds, short TE of 35 ms, spectral width of 5,000 Hz, 2,048 time points, partial water suppression. Shimming was performed using manufacturer-supplied shimming procedures.

Analysis of the metabolite concentration was performed using LC-Model (Stephen Provencher Inc., Oakville,

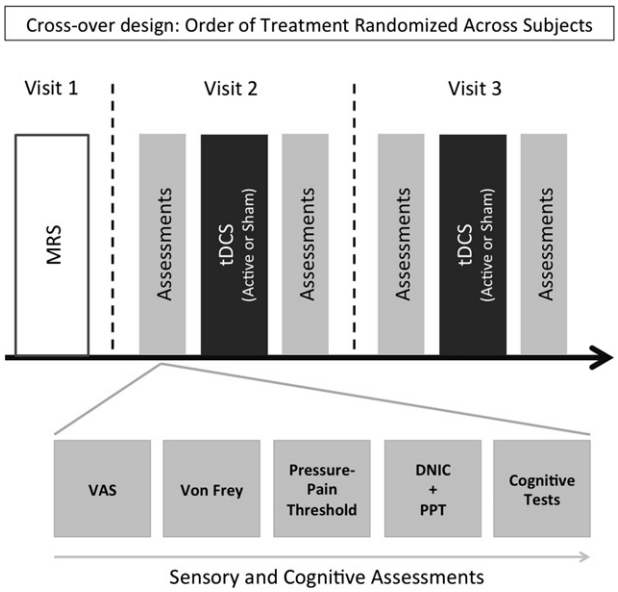

Figure 1. Experimental paradigm for evaluating the effects of motor cortex modulation and descending inhibitory systems on pain threshold. Our study consisted of 3 visits. During Visit 1, subjects underwent magnetic resonance spectroscopy (MRS) to measure baseline concentrations of brain metabolites. During Visit 2, subjects were randomized to receive active or sham anodal transcranial direct current stimulation (tDCS). tDCS was preceded and followed by a series of assessments, which included the visual analog scales (VAS) for anxiety, von Frey Hair sensory perception threshold test, pressure pain threshold (PPT) algometric measurements performed on the right thenar region, PPT following the first 30 seconds of the descending noxious inhibitory controls (DNIC) paradigm (cold-water immersion), and cognitive assessments. During Visit 3, subjects underwent identical procedures as in Visit 2, with the sole difference being the alternating of active and sham tDCS. This sequence allowed us to assess the effects of active tDCS, sham tDCS, DNIC, and combined conditions in all subjects (crossover design).

Ontario, Canada). Levels of N-acetylaspartate (NAA), N-acetylaspartateglutamate (NAAG), NAA+NAAG (total NAA), glutamate (Glu), glutamine (Gln), myoinositol (mI), creatinephosphocreatine (Cr), and choline were analyzed by fitting a linear combination of a basis set of metabolite model spectra to the data. Analyzing spectrum set from 3.8 ppm down to .2 ppm with no eddy-current correction and water scaling. The metabolite concentrations were expressed as mM and ratios relative to Cr peak. The metabolite concentrations and metabolite-to-creatine (Cr) ratios were determined in these 3 spectra for each subject.

Transcranial Direct Current Stimulation

Direct current (DC) was applied to the scalp using 2 sponge-enclosed rubber electrodes (35 cm^2) soaked in saline solution. The electrodes were attached by wires to a battery powered DC generator (Activa Dose, Salt lake City, UT) and held in place by rubber bands. During each stimulation session, the anode electrode was placed on the scalp above the left primary motor cortex (M1) and the cathode was placed over the right supra-orbital area. This seems to be an optimal montage for pain modulation studies according to a recent study.[32] M1 was localized using the electroencephalogram (EEG) 10/20 system used in previous studies[12] and correct localization was confirmed through MRI by placement of Vitamin E using similar procedures prior to MRS scans (Visit 1). The electrode sponges encompassed a large segment of the motor cortex, which included the upper limb and parts of the lower limb and face, similar to Fregni et al.[12] Such positioning has previously been shown to enhance M1 excitability.[38]

During active tDCS conditions, we applied 2 mA of anodal tDCS for 20 minutes according to current stimulation protocols.[37] To avoid visual sensations and other side effects, current level was slowly increased and decreased at the start and end of stimulation for 10 seconds, respectively. During sham tDCS conditions, the same montage was used, but current was only applied for the first 30 seconds out of the 20-minute session. Studies have shown that application of current for less than 3 minutes does not affect cortical excitability, and application of current for 30 seconds is a valid method of blinding.[5,16,38] Both subjects receiving active and sham stimulation experienced the current as an itching sensation beneath both electrodes at the beginning of stimulation that would typically wane over time. A single session of stimulation with 2 mA has been shown to be safe in healthy, nonpregnant adults, with only minor and short-lasting side effects.[22,44]

Assessments

During Visits 2 and 3, a blinded rater conducted all assessments immediately before and after the 20-minute stimulation sessions. The procedures were administered in the following order (details can be found below): 1) VAS for anxiety; 2) Beck Depression Inventory (before stimulation only); 3) von Frey Hair Assessment; 4) Pressure Pain Threshold (PPT) Assessment; 5) PPT assessment during cold water immersion (DNIC); 6) Trail-Making Tests A & B; 7) Stroop Test; and 8) Simple Reaction Test. Following stimulation, 2 questionnaires were immediately administered to assess the occurrence of side effects and success of blinding procedures, and these were then followed by repetition of the above assessments.

Sensory Assessments

Our main aim was to compare changes in pain perception induced by exogenous (tDCS) and endogenous (DNIC) modulation pathways. We therefore administered several assessments for evaluating changes in perception of pain.

von Frey Hair (VFH). Using von Frey monofilaments (sizes 1.65 to 6.65, corresponding to target forces in grams of .008 to 300 g), we assessed subject thresholds for perceiving mechanical pressure using 1 measurement with ascending intensities.[46] While the subject's eyes were closed or turned away, we applied increasingly thick monofilaments to the right thenar region until subjects reported perceiving the stimulus (perception threshold).

Pressure Pain Threshold (PPT). PPT recordings involved applying an increasing amount of blunt pressure using the 1-cm^2 hard-rubber end of an FDA-approved assessment device (Commander Algometer, JTECH Medical, Salt Lake City, UT). A series of discrete pressures were successively

applied to the right thenar region at an approximate rate of 2 lb/second until the subjects reported perceiving pain, at which point the device was removed and the PPT value was recorded. This procedure was repeated 3 times.

PPT During Cold Water Immersion. By measuring PPT during cold water immersion, we evaluated the degree to which pain perception is modulated by DNIC following presentation of an initial heterotopic noxious stimulus. In this paradigm, the cold-water immersion served as the conditioning-stimulus and blunt algometric pressure (PPT) served as the testing stimulus. Subjects immersed their left hands into cold water (10–12°C) for 1 minute total. During the last 30 seconds of cold-water immersion, the PPT procedure was administered to their right hands. In a few instances in which the subjects found 12°C too cold, the water temperature was raised to more tolerable, but still reportedly painfully cold, levels. The temperature was held constant across the experiment for each subject.

Confounding Variable Assessments

We assessed changes in anxiety and depression since these variables can be significant confounders for changes in the experience of pain.

Visual Analog Scale (VAS) for Anxiety. This consisted of subjects rating their present level of anxiety on a visual scale from 0 to 10 (where 10 = worst possible anxiety).

Beck Depression Inventory (BDI). This consisted of 21 multiple-choice questions, targeted at evaluating the presence and extent of depression in adults.

Cognitive Assessments

In order to assess potential positive and deleterious effects of 20 minutes of tDCS administration on cognitive function, we conducted several cognitive assessments: Trail-Making Tests A & B (provides a measure of working memory function and attention); Stroop Test (assesses selective attention and interference proclivity and provides a measure of executive function); and Simple Reaction Test (tests attention and response time with 30 brief trials performed using Superlab pro v2.0 software [Cedrus Corporation, San Pedro, CA]).

Side-Effects and Blinding Assessments

Safety Assessment. Immediately following administration of tDCS during both Visits 2 and 3, subjects were asked about the occurrence of any side effects such as tingling, headache, neck pain, scalp burns, scalp pain, skin redness, sleepiness, acute mood change and trouble concentrating.

Blinding Assessment. Following the safety assessment, subjects were asked to guess whether they believe they had just received active or real tDCS and rate how confident they were that their guesses were correct.

Statistical Analysis

Analyses were done using Stata® statistical software (version 9.1, College Station, Texas). We ran a Shapiro-Wilk test for normality. We then performed a repeated-measures analysis of variance (ANOVA) in which the dependent variable was change in pain threshold (lbs) as measured by PPT and the independent variables were conditions (active tDCS, sham tDCS, DNIC, both active tDCS and DNIC) and the random variable subject ID to control for within-subject variability. Post hoc comparisons using 2-tailed paired t-tests were then performed using correction for multiple comparisons when appropriate. Similar 2-tailed paired t-test analyses were used to compare changes across the active and sham tDCS conditions for von Frey perception, VAS anxiety, Trail-Making A & B, Stroop Test, and Simple Reaction Test. Finally, we computed Pearson correlation coefficients to assess the relationship between baseline concentrations of brain metabolites and changes in pain threshold outcomes during specific experimental conditions as well as age. These secondary analyses were considered exploratory and we did not apply Bonferroni adjustments (significance level set at $P = .05$).

Results

Fifteen subjects (mean age 36.7 ± 11.0 years, 9 females) were included in this analysis. Subjects tolerated the procedure well and there were no significant adverse effects. Additionally, adverse effects were not significantly different between active and sham tDCS sessions. See Table 1 for the side effects reported by subjects following administration of active and sham tDCS. We analyzed the data for possible order effects by comparing side effects in those receiving active tDCS during Visit 1 and those receiving sham tDCS during Visit 1. Although it seemed that there was an order effect for certain side effects, this order effect did not relate to the condition of stimulation. For instance, subjects seemed to report more tingling in the first session regardless of the condition (for those receiving active and then sham the frequency was reported to be 83% versus 50%, respectively, and for those receiving sham and then active this was reported to be 78% and 67%, respectively). For other side effects such as sleepiness there seemed to be no order effect (the same analysis showed 33% versus 33% and 44 versus 33%, respectively). Blinding assessments revealed that 5 subjects (33%) correctly guessed both when they received active and sham tDCS while the other 10 subjects did not, suggesting that guessing success was at the level of chance ($P = .2$, comparison between correct and incorrect guesses). Tests for normality revealed that our data is

Table 1. Side Effects of tDCS Administration

	Active tDCS	*Sham tDCS*
Tingling	11 (73)	10 (67)
Skin redness	7 (47)	8 (53)
Sleepiness	5 (33)	6 (40)
Itching	2 (13)	1 (7)
Scalp pain	1 (7)	2 (13)
Scalp burning	1 (7)	0 (0)
Headache	1 (7)	1 (7)
Pins and needles	1 (7)	0 (0)
Neck pain	0 (0)	0 (0)
Trouble concentrating	0 (0)	0 (0)
Acute mood change	0 (0)	0 (0)

Abbreviation: tDCS, transcranial direct current stimulation.
NOTE. Number of subjects and percentage (in parentheses) reporting the side effect.

normally distributed. Indeed for our main outcome (PPT), results showed a W score of .97 ($P = .18$).

Pressure-Pain Threshold

To analyze whether active tDCS, DNIC, or combined tDCS and DNIC were associated with decreased sensitivity to pain, we performed a repeated-measures ANOVA in which the dependent variable was change in pain threshold (lbs) and the independent variables were condition (active tDCS, sham tDCS, DNIC, both active tDCS and DNIC) and the random variable subject ID (to control for within-subject variability). This analysis revealed a significant difference in outcomes between conditions ($F_{(3,42)} = 8.12$; $P < .001$).

We then conducted post hoc testing to compare the effects of motor cortex modulation using tDCS and DNIC in modulating pain thresholds. Analyses using paired t-tests for the pain threshold measurements revealed significant increases in pain threshold following active tDCS compared with sham conditions ($P < .05$), following DNIC compared with sham conditions ($P < .005$), and following combined active tDCS and DNIC compared with sham ($P < .005$) (Fig 2). There was no significant difference in pain threshold changes following active tDCS alone compared with DNIC alone ($P = .35$), suggesting that the modulatory effects of these 2 techniques are comparable. The effects of combined active tDCS and DNIC were significantly greater than DNIC alone ($P < .01$). While the combined tDCS and DNIC was greater than active tDCS alone, this difference did not reach significant levels ($P = .19$).

One interesting finding was that subjects who responded to tDCS alone had similar response increases when receiving combined tDCS and DNIC. In fact, using a Pearson's correlation, we observed that increases in pain threshold following active tDCS were positively correlated with increases in threshold following both tDCS and DNIC (r =.54, $P < .05$); therefore both interventions likely share the same predictors of response.

Von Frey Hair Test

We analyzed results from the von Frey Hair test using ANOVA as well as 2 tailed paired t-tests. The subjects' change in threshold for perceiving the stimulus ($Threshold_{after}$–$Threshold_{before}$) increased significantly when receiving active tDCS compared with sham tDCS ($F_{(1,14)} = 5.88$, $P < .05$; .29 ± .43 filament units versus .00 ± .37 filament units, $P < .05$).

Reported and Behavioral Measures

No significant difference in subject scores on the Beck Depression Inventory were observed when comparing scores before receiving active and sham stimulation (1.20 ± 2.14 versus 1.27 ± 2.49, $P = .86$). No significant differences were observed when comparing changes in reported anxiety following active versus sham stimulation, as measured by the VAS. Average reported anxiety before stimulation (both active and sham) was significantly higher than after stimulation (1.13 ± 1.48 VAS units before versus .83 ± 1.14 VAS units after, $P < .05$). No significant differences in changes in performance on the cognitive tests following active and sham tDCS were observed.

Brain Metabolites

We conducted post hoc, exploratory correlational analyses between baseline concentrations of various metabolites in the brain with experimental conditions. Increase in pain threshold (indicating decrease in pain perception sensitivity) following active tDCS was positively associated with total NAA concentration in the anterior cingulate cortex (r = .58, $P < .05$; mean baseline concentration = 14.5 mM), mI concentration in the anterior cingulate cortex and occipital cortex (r = .66, $P < .01$; r = .52, $P < .05$, respectively) and negatively correlated with Gln concentration and Gln/Cr in the thalamus (r = −.60, $P < .05$; r = −.61, $P < .05$, respectively). Increase in pain threshold following sham tDCS was negatively associated with Gln concentration in the motor cortex, anterior cingulate cortex, and occipital cortex (r = −.53, $P < .05$; r = −.59, $P < .05$; r = −.54, $P < .05$, respectively) and Gln/Cr in the motor cortex (r = −.67, $P < .01$). Pain threshold during administration of DNIC prior to active stimulation was negatively correlated with Glu and Glu/Cr in the occipital cortex (r = −.54, $P < .0$; r = −.59, $P < .05$, respectively). Pain threshold following active tDCS was positively correlated with mI in the anterior cingulate cortex (r = .58, $P < .05$) and negatively correlated with Glu/Cr in the occipital cortex (r = −.53, $P < .05$).

Discussion

Increase in Pain Thresholds

In the present study we aimed to assess whether top-down motor cortex modulation using anodal tDCS combined with bottom-up induction of DNIC using a cold-water immersion paradigm can result in synergistic effects on pain perception. We found that when administered alone, both tDCS and cold-water-induced DNIC can significantly increase the thresholds at which subjects perceive pain. Interestingly, these increases were not significantly different in magnitude. Furthermore, when combined, these top-down and bottom-up modulatory techniques appear to have an additive effect in increasing pain threshold. The combined effect increased pain threshold significantly greater than DNIC alone. These findings are consistent with previous studies that found increased efficacy of tDCS in treating pain and other conditions when combined with other therapies such as TENS, visual illusion therapy, and constraint-induced movement therapy.[3,47,57]

tDCS and DNIC are theorized to influence pain perception through different neural pathways. It is thought that stimulation of the primary motor cortex modulates pain through direct effects on ventral lateral and anterior thalamic nuclei, as well as downstream effects on the medial thalamus, anterior cingulate, and upper brainstem.[12,17,26,48] In contrast, DNIC is believed to exert its effects via supraspinal structures such as the subnucleus reticularis dorsalis of the caudal medulla when pain

signals ascend through the spinal cord from the periphery.[21,28,55,56] Some have suggested that neural mechanisms underlying DNIC may also include corticoamygdaloid regulation of endogenous opioid release to modulate pain perception and involvement of primary somatosensory cortex and periaqueducatal gray to modulate nociceptive motor reflexes.[43] Given these understandings, there are several possible ways of understanding the mechanisms underlying our findings. It is possible that the observed additive effect results from combined modulation of 2 different sources of neural activity underlying pain. It is also possible, contrary to previous understandings, that tDCS and DNIC in fact influence similar neural pathways, having an additive effect when the combination essentially increases the modulatory dose administered to these neural pathways. A third mechanism might involve 1 modulatory technique synergistically potentiating the other. For instance, excitatory anodal tDCS of the motor cortex might facilitate activity in DNIC-related neural networks and therefore enhance their bottom-up effects, or vice versa. Whereas DNIC is dependent on afferent neural activity to modulate neural activity, tDCS influences cortical activity through modulation of resting membrane potentials, potentially allowing for such a complementary relationship. Of note, neurons of the subnucleus reticularis dorsalis receive massive corticofugal projections and are modulated by the cingulate cortex,[10,61] potentially explaining how cortical stimulation could directly modulate DNIC.

This experiment follows from a growing literature suggesting that tDCS as a modulatory technique is more effective when combined with other therapeutic modalities. For instance, when coupled with TENS and visual illusion therapy, tDCS has been observed to be more effective at treating chronic pain.[3,47] Similarly, in the context of motor rehabilitation following stroke, outcomes are enhanced when tDCS is coupled with constraint-induced movement therapy.[57] While the present study involves healthy subjects, our results suggest that future investigations ought to explore whether the combination of tDCS and DNIC-related pain modulation might be effective in alleviating chronic pain in patients with conditions such as fibromyalgia. Patients with fibromyalgia have been shown to have dysfunctional endogenous pain inhibition (DNIC)[24] and this deficiency has been linked to diminished activation of the rostral anterior cingulate cortex (rACC) and associated brainstem regions, which both play important roles in the central pain regulatory system.[23] It is possible that tDCS, which has been shown to influence activity in the anterior cingulate cortex,[26] could therefore enhance deficient endogenous pain modulatory activity in these patients by indirectly activating medial cortical regions such as the rACC.

Insights From MRS to Measure Brain Metabolites

In order to gain additional mechanistic insights, we used magnetic resonance spectroscopy to determine whether particular brain metabolites, such as glutamate, correlate with the effects of these pain modulatory pathways. We found that increase in pain threshold following active tDCS was positively associated with baseline total NAA and that pain thresholds negatively correlated with glutamine and glutamate levels, with varying consistency across regions of the brain.

These findings are in alignment with previous understandings of the role of these metabolites in brain function and related experimental findings. Glutamate is a major excitatory neurotransmitter that can be modulated by noninvasive brain stimulation methods.[31] NAA has been viewed as a marker of neural function.[33,45,51] Previous MRS findings suggest that pain levels negatively correlate with glutamate and NAA concentrations in areas of the brain involved in processing both the somatosensory and affective aspects of pain. With respect to somatosensory processing areas, 1 study showed a negative correlation between glutamate levels in the left insular region and subjective pain intensity resulting from provoked experimental dental pain.[20] Another study comparing brain metabolites in subjects with chronic pain following spinal cord injury and healthy subjects found that patients with chronic pain had lower concentrations of NAA in the thalamus.[41] Finally, our recent MRS study showed that improvement in chronic visceral pain corresponded to increases in glutamate and NAA levels across treatment of the secondary somatosensory cortex (II) and that decreased baseline levels of glutamate predicted likelihood of pain improvement following noninvasive brain stimulation (repetitive transcranial magnetic stimulation).[14] With respect to brain regions involved in affective processing of pain, patients with chronic pain have been found to have lower concentrations of NAA in the prefrontal cortex and anterior cingulate cortex. In fact, most of these patients needed psychological intervention according to this study.[15] Along these lines, patients with depression and chronic pain also show decreased NAA levels in the dorsolateral prefrontal cortex.[19]

Our combined findings advance our understanding of this area of investigation by demonstrating that brain metabolite levels of healthy subjects can also be utilized to predict response to a potential analgesic intervention. Interestingly, we showed that lower levels of glutamine in the thalamus predict higher pain threshold increases following tDCS and that higher levels of baseline NAA in the anterior cingulate cortex (suggesting a lower pain profile) predict higher pain threshold increases following tDCS. In this context, tDCS may enhance the biochemical brain profile associated with a reduced level of pain processing.

Limitations

There are some limitations to our study that must be addressed. As we did not find any significant difference between the effects of tDCS or DNIC alone, it is important to recognize that manipulation of dosage could greatly change pain responses. tDCS, for instance could potentially be administered with varying levels of current density and duration, and DNIC could have been

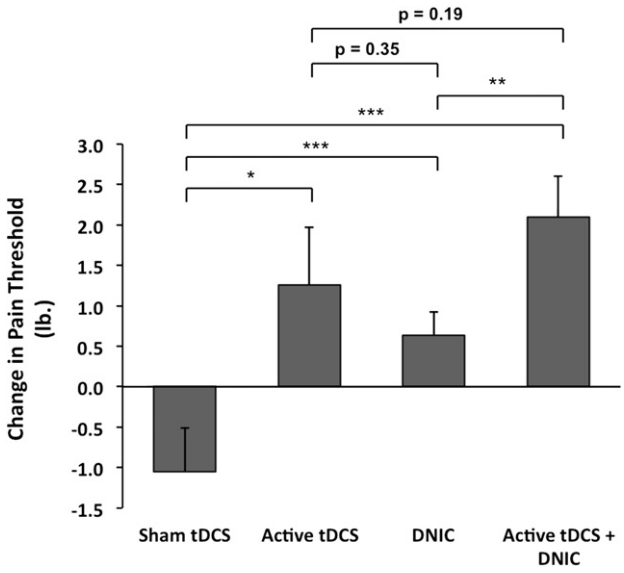

Figure 2. Motor cortex modulation and descending inhibitory systems increase pain thresholds. We compared subjects' baseline right hand algometric pain thresholds to their thresholds following administration of active and sham anodal transcranial direct current stimulation (tDCS) of the left motor cortex, during immersion of the left hand in cold water (descending noxious inhibitory controls, DNIC), and during cold-water immersion following active tDCS. Significant increases in pain thresholds were observed following active tDCS, DNIC, and combined conditions compared with sham conditions. No significant difference was observed in change in pain threshold following active tDCS alone compared with DNIC alone. While combined active tDCS and DNIC led to an increase in pain threshold that was greater than that observed following either method alone, this only reached significant levels when compared with DNIC conditions. *$P < .05$; **$P < .01$; ***$P < .005$ (paired t-test, 2-tailed).

induced with varying water temperatures and durations of immersion.[50] As the mechanisms underlying these forms of modulation continue be explored, the dosage of each should be modified to explore optimal combinations and parameters for increasing pain thresholds. Furthermore, the present paper examines the effects of anodal tDCS on pain thresholds, specifically following from a large literature demonstrating anodal stimulation to be effective in chronic pain populations.[12,13,34,47,53] Other parameters of tDCS, such as cathodal tDCS (of M1), also seem to exert significant effects in modulating experimental pain.[1,7,49] Future studies thus ought to examine the effect of cathodal tDCS on pain as well.

Studies suggest that stimulation of different areas of the cerebral cortex, such as primary motor cortex and dorsolateral prefrontal cortex, may influence pain through different mechanisms.[8,36] It is important that future studies examine the interaction between DNIC and other cortical areas as well. Furthermore, the influence of attentional factors in modulating DNIC's effect on pain when combined with the tDCS ought to be explored.[9]

Of note, our current design includes DNIC administration during the assessments both before and after tDCS administration. Our study assumes that there is no DNIC carryover effects when assessing thresholds immediately following tDCS administration. This assumption is supported by the brief length of the cold water immersion (1 minute) and the fact that we had at least 15 minutes of cognitive assessments and 20 minutes of active/sham tDCS stimulation before the post-tDCS assessments took place. In contrast, when assessing combined tDCS and DNIC, we assumed that there was a carryover effect from tDCS after DNIC administration since studies suggest brief sessions of tDCS (up to 13 minutes) can have effects lasting up to 90 minutes[39] and the pain threshold assessments were performed within minutes after tDCS and DNIC administration.

With respect to our MRS findings, it is also important to note that given our small sample size, our findings should be interpreted with caution. There was no power to correct for multiple comparisons for our post hoc exploratory analyses, a limitation of which the reader should be aware due to increased false positive rate. In this experiment we chose not to run postmodulation MRS studies since subjects only underwent single modulation sessions. Future studies, however, ought to include several sessions over a period of days in patients with chronic pain that are both preceded and followed by MRS in order to assess changes in brain metabolites across the experiment.

Conclusions

Our results suggest that noninvasive neuromodulation can increase the threshold at which humans perceive pain and that this effect can be enhanced when combined with bottom-up techniques to induce endogenous analgesic DNIC pathways. Our study further demonstrates that baseline levels of brain metabolites can have some predictive value for the effects of these modulatory pathways. Future studies ought to examine the effects of alternative parameters for tDCS and DNIC induction. Finally, these results suggest that future studies explore whether outcomes of other pain therapies can be enhanced through combination with noninvasive brain stimulation.

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