

BRIEF REPORT

Excitatory and Inhibitory Brain Metabolites as Targets of Motor Cortex Transcranial Direct Current Stimulation Therapy and Predictors of Its Efficacy in Fibromyalgia

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Objective. Transcranial direct current stimulation (tDCS) has been shown to improve pain symptoms in fibromyalgia (FM), a central pain syndrome whose underlying mechanisms are not well understood. This study was undertaken to explore the neurochemical action of tDCS in the brain of patients with FM, using proton magnetic resonance spectroscopy (¹H-MRS).

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Methods. Twelve patients with FM underwent sham tDCS over the left motor cortex (anode placement) and contralateral supraorbital cortex (cathode placement) for 5 consecutive days, followed by a 7-day washout period and then active tDCS for 5 consecutive days. Clinical pain assessment and ¹H-MRS testing were performed at baseline, the week following the sham tDCS trial, and the week following the active tDCS trial.

Results. Clinical pain scores decreased significantly between the baseline and active tDCS time points ($P = 0.04$). Levels of glutamate + glutamine (Glx) in the anterior cingulate were significantly lower at the post-active tDCS assessment compared with the post-sham tDCS assessment ($P = 0.013$), and the decrease in Glx levels in the thalami between these time points approached significance ($P = 0.056$). From baseline to the post-sham tDCS assessment, levels of *N*-acetylaspartate (NAA) in the posterior insula increased significantly ($P = 0.015$). There was a trend toward increased levels of γ -aminobutyric acid (GABA) in the anterior insula after active tDCS, compared with baseline ($P = 0.064$). Baseline anterior cingulate Glx levels correlated significantly with changes in pain score, both for the time period from baseline to sham tDCS ($\beta_1 = 1.31$, $P < 0.001$) and for the time period from baseline to active tDCS ($\beta_1 = 1.87$, $P < 0.001$).

Conclusion. The present findings suggest that GABA, Glx, and NAA play an important role in the pathophysiology of FM and its modulation by tDCS.

Chronic pain impacts ~100 million people in the US, with annual costs exceeding 635 billion dollars (1). Fibromyalgia (FM) is considered the prototypical central pain syndrome, and emerging data suggest that patients with FM have central nervous system alterations (2–4). Despite the availability of multiple treatments for this condition, many FM patients still report significant

unresolved pain and disability. A significant limitation to evaluating potential interventions for chronic pain syndromes, including FM, is the lack of an objective marker of pain. There has been significant interest in using neuroimaging methods, such as proton magnetic resonance spectroscopy ($^1\text{H-MRS}$), to develop an objective test of pain. $^1\text{H-MRS}$ can be used to measure levels of brain metabolites, including γ -aminobutyric acid (GABA) (the brain's major inhibitory neurotransmitter), Glx (a combined marker of glutamine and glutamate, the latter being the brain's major excitatory neurotransmitter), and *N*-acetylaspartate (NAA) (thought to be a measure of neuronal integrity). Our group has reported increased levels of Glx in the posterior insula, which is responsible for the graded sensory processing of pain (5,6), in patients with FM. We have also reported decreased levels of GABA in the anterior insula, which is important in the emotional processing and affective aspects of pain (6,7), in FM patients. Lower NAA levels within the hippocampus in the setting of FM have also been described (8).

One potential treatment for FM is transcranial direct current stimulation (tDCS), a brain-stimulating procedure that uses noninvasive weak direct current applied to the scalp. In FM as well as other pain conditions, tDCS has been shown to modulate experimental and clinical pain. Specifically, it has been found to improve pain symptoms in FM (9,10). Anodal stimulation from tDCS has been demonstrated to increase cortical excitability, which is postulated to mitigate pain symptoms through indirect effects on pain processing regions in the brain (9). However, the mechanisms underlying the efficacy of tDCS in chronic pain are not well understood, and trials assessing its effects on chronic pain have yielded inconsistent results (11). Our objective in the present study was to explore the underlying neurochemical action of tDCS in the brain in FM, using $^1\text{H-MRS}$.

PATIENTS AND METHODS

Trial design. This longitudinal trial had 3 phases: 1) a baseline period to measure pain levels and perform $^1\text{H-MRS}$, 2) 5 consecutive days of sham tDCS, followed by pain assessment and $^1\text{H-MRS}$, and 3) 5 consecutive days of active tDCS, followed by pain assessment and $^1\text{H-MRS}$. The second and third phases were separated by a 7-day washout period. Randomization was not performed given the presence of significant carryover effects with active tDCS (12) and the small sample size.

Patients. Thirteen female subjects (mean \pm SD age 47.6 ± 10.6 years [range 27–64]) were recruited for this study. Twelve of the subjects completed the entire protocol; 1

dropped out after the baseline pain assessment/ $^1\text{H-MRS}$. The first 2 of the 12 subjects did not have GABA data collected at the baseline time point due to protocol change, but did have Glx and NAA data collected. All subjects met the American College of Rheumatology 1990 criteria for FM (13) with a symptom duration of at least 1 year, had reported continued presence of pain on $>50\%$ of days, and were willing to refrain from introducing any new medications or treatments for control of FM symptoms during the study. All subjects were right-handed and had a body mass index of ≤ 36 kg/m². We excluded FM patients who had a history of coexisting autoimmune or chronic inflammatory disease that causes pain (e.g., rheumatoid arthritis, systemic lupus erythematosus, or inflammatory bowel disease), who had a history of substance abuse or were currently taking opiates, or who had a history of severe psychiatric illness (e.g., current major depression and schizophrenia). FM patients who were pregnant, breastfeeding, or participating in other therapeutic clinical trials were also excluded. The study was approved by the University of Michigan Institutional Review Board. All subjects provided written informed consent.

Clinical assessments. In clinical assessments, the patients were asked to refer to the “average” symptoms experienced/perceived during 3 time periods: the week prior to the initial $^1\text{H-MRS}$, the period between first-trial initiation (sham tDCS) and second $^1\text{H-MRS}$, and the period between second-trial initiation (active tDCS) and final $^1\text{H-MRS}$. Pain intensity was measured using a 10-point visual analog scale (VAS), with 0 representing no pain and 10 representing the worst possible pain. Affective state was assessed using the positive and negative affect scores from the Positive and Negative Affect Schedule (PANAS) (14). Pain discrimination and subjective pain experience were evaluated using the long-form McGill Pain Questionnaire (15) for the baseline visit and the short-form McGill Pain Questionnaire (16) for the post-sham tDCS and post-active tDCS visits.

Transcranial direct current stimulation. For both the sham and active tDCS sessions, the anode electrode was placed on the scalp overlying the left motor cortex and the cathode electrode was placed on the scalp overlying the right supraorbital cortex. During the active tDCS sessions, 2 mA of transcranial direct current stimulation was applied for 20 minutes. For the sham tDCS, the current was applied for only 30 seconds at the beginning and end of the session. A 30-second application of current is considered a method of sham stimulation, as sensations arising from tDCS treatment occur mostly at the beginning and end of application (17). Individual measurements determined the anatomic location for placement of the electrodes using the convention of electroencephalography 10/20 system. Placement of the electrodes was performed by the same operators (AFD and TDN) for all patients.

Proton magnetic resonance spectroscopy. A Philips Ingenia 3T system with a 15-channel receive head coil was used for imaging acquisition. Voxel placement was performed using a 3-dimensional magnetization-prepared rapid gradient-echo sequence with 0.9-mm³ isotropic voxel resolution. $^1\text{H-MRS}$ spectra were collected with 3.0 cm \times 2.0 cm \times 3.0 cm volumes from the right anterior insula, right posterior insula, and anterior cingulate and a 2.0 cm \times 4.0 cm \times 2.2 cm volume from bilateral thalami (data available from the corresponding au-

Table 1. Clinical scores and correlations between clinical scores and metabolite levels in various brain regions in the 12 patients with fibromyalgia*

	Baseline	Post-sham tDCS	Post-active tDCS
VAS pain score			
Mean \pm SD	5.1 \pm 2.3	4.1 \pm 2.1	3.3 \pm 2.8 [†]
Correlation (r)			
– With ACC GABA level	–0.74 [‡]	NS	NS
– With post. ins. NAA level	NS	NS	–0.60 [§]
– With thalamus GABA level	–0.75 [§]	NS	NS
– With thalamus NAA level	NS	–0.75 [‡]	NS
MPQ score (long form), mean \pm SEM	22.9 \pm 14.9	–	–
MPQ score (short form), mean \pm SEM	–	18.7 \pm 12.5	19.3 \pm 15.3
PANAS+ score			
Mean \pm SD	19.8 \pm 6.0	17.8 \pm 5.5	16.1 \pm 6.4
Correlation (r)			
– With ant. ins. Glx level	–0.87 [‡]	NS	NS
– With ant. ins. NAA level	–0.68 [§]	NS	NS
– With ant. ins. GABA level	NS	NS	0.71 [§]
– With post. ins. Glx level	–0.78 [‡]	NS	NS
– With post. ins. NAA level	NS	NS	0.61 [§]
– With thalamus GABA level	NS	–0.78 [§]	NS
PANAS– score	14.3 \pm 3.4	15.4 \pm 5.5	12.7 \pm 3.6 [¶]
– With post. ins. GABA level	NS	NS	–0.65 [§]
– With post. ins. NAA level	NS	NS	–0.63 [§]
– With thalamus GABA level	NS	–0.79 [‡]	NS

* tDCS = transcranial direct current stimulation; VAS = visual analog scale; ACC = anterior cingulate; GABA = γ -aminobutyric acid; NS = not significant; post. ins. = posterior insula; NAA = *N*-acetylaspartate; MPQ = McGill Pain Questionnaire; PANAS = Positive and Negative Affect Schedule; ant. ins. = anterior insula; Glx = combined glutamate + glutamine.

[†] $P = 0.04$ versus baseline.

[‡] $P \leq 0.01$.

[§] $P \leq 0.05$.

[¶] $P = 0.02$ versus baseline.

thor upon request). These regions were chosen a priori given their importance in pain perception processing, based on our prior work which has demonstrated significant alterations of GABA and/or Glx in the right insula and anterior cingulate of FM patients, and because they have also been shown, using other advanced neuroimaging techniques, to be altered under conditions of chronic pain (3,5,7,18).

Single-voxel point-resolved spectroscopy (PRESS) (repetition time [TR]/echo time [TE] 2,000/35 msec) was performed using VAPOR (variable pulse power and optimized relaxation) water suppression with 32 averages to measure Glx and NAA levels. A Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) experiment, which edits out the overlapping creatine peak at 3.0 parts per million (19), was performed to measure GABA levels. The MEGA-PRESS experiment was performed using the following parameters: TE 68 msec (TE₁ 15 msec, TE₂ 53 msec), TR 1.8 seconds, 256 transients of 2,000 data points, spectral width 2 kHz, frequency selective editing pulses (14 msec) applied at 1.9 ppm (on) and 7.46 ppm (off). Amplitude-modulated pulse GTST1203 (length 7 msec, bandwidth 1.2 kHz) was used for refocusing. Conventional PRESS spectroscopy data were analyzed with LCModel. MEGA-PRESS spectroscopy data were analyzed using MatLab in-house postprocessing software to fit Gaussian curves to the GABA and inverted NAA peaks. The absolute NAA concentration, as generated from LCModel analysis of the PRESS data, was multiplied by the GABA:NAA ratio as

generated from the MatLab analysis to determine the concentration of GABA (in arbitrary institutional units [AIU]). There was inadequate signal-to-noise ratio for the GABA spectra in the anterior cingulate and anterior insula in 1–2 subjects, and in the thalami in 2–3 subjects, at each of the trial phases.

Statistical analysis. Stata version 11 was used for statistical analysis. The significance of changes in brain metabolite levels in the different brain regions between baseline and sham tDCS, between baseline and active tDCS, and between sham tDCS and active tDCS was determined by paired 2-tailed *t*-test. We also correlated the scores from the VAS scale and the McGill and PANAS questionnaires at each of the time points with the respective brain metabolite levels from the different voxel locations. Linear regression analysis was performed to assess correlations between brain metabolite values at baseline and changes in the pain score from baseline to sham tDCS and from baseline to active tDCS. *P* values less than 0.05 were considered significant.

RESULTS

We observed substantial longitudinal changes in clinical pain scores, with a significant decrease in the VAS pain score from baseline to active tDCS ($P = 0.04$) (Table 1). There was a trend toward a decrease in the

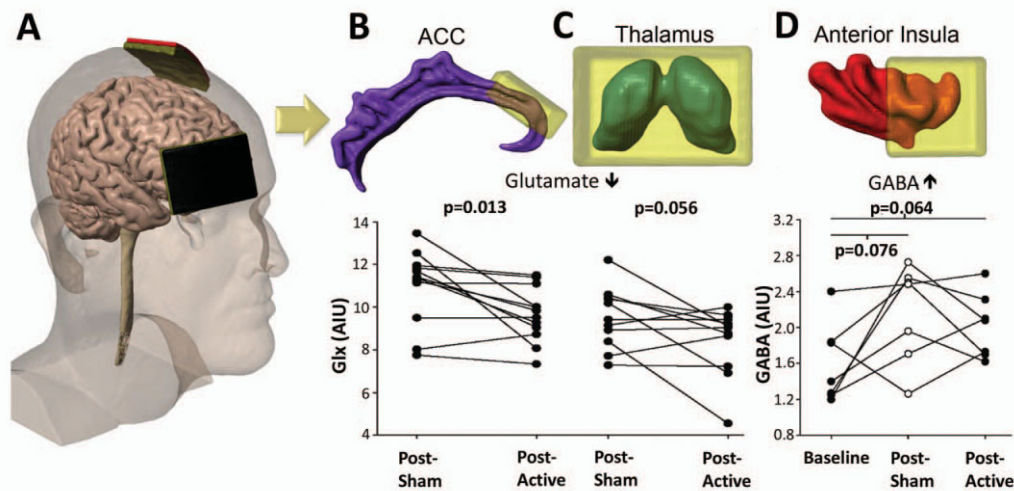


Figure 1. Transcranial direct current stimulation (tDCS) electrode placement and proton magnetic resonance spectroscopy results. **A**, tDCS montage, showing the anode placed over the primary motor cortex (M1) and the cathode over the contralateral supraorbital cortex. **B** and **C**, Significantly decreased levels of Glx (glutamate + glutamine) in the anterior cingulate (ACC) following active tDCS treatment (**B**), with a trend toward a significant decrease in levels of Glx in the thalamus (**C**). **D**, Trend toward a significant increase in levels of γ -aminobutyric acid (GABA) in the anterior insula after sham and active tDCS treatments. Each symbol represents an individual fibromyalgia patient. AIU = arbitrary institutional units.

VAS score from baseline to sham tDCS ($P = 0.10$). Between sham tDCS and active tDCS the VAS pain score did not change significantly ($P = 0.16$). The PANAS score decreased significantly from baseline to active tDCS ($P = 0.02$). There were no additional significant changes in the PANAS score for the different time point comparisons. Between sham tDCS and active tDCS there was a significant decrease in Glx levels in the anterior cingulate ($P = 0.013$) and a trend toward a decrease in Glx levels in the thalamus ($P = 0.056$) (Figure 1). From baseline to active tDCS there was a trend toward an increase in GABA levels in the anterior insula ($P = 0.064$). In addition, NAA levels in the posterior insula increased significantly from baseline (mean \pm SD 7.68 ± 0.45 AIU) to sham tDCS (8.24 ± 0.58 AIU) ($P = 0.015$).

There were a number of moderate-to-strong correlations between brain metabolite levels and the clinical rating scales. Higher levels of GABA and NAA and lower levels of Glx from the prescribed brain region voxels were associated with better clinical pain and affect scores (Table 1). Linear regression analysis revealed that Glx levels in the anterior cingulate at baseline correlated significantly with changes in the VAS pain score between baseline and sham tDCS ($\beta_1 = 1.31$, $P < 0.001$) and between baseline and active tDCS ($\beta_1 = 1.87$, $P < 0.001$) (Figure 2), with higher levels of Glx in

the anterior cingulate at baseline associated with greater reductions in clinical pain following both the sham and active treatment phases.

DISCUSSION

In the present longitudinal trial we observed that the anterior cingulate was the region most affected by tDCS treatment in FM patients, with a significant decrease in Glx levels. Furthermore, we found that patients with higher levels of Glx in the anterior cingulate at baseline tended to show a greater degree of improvement in clinical pain after both the sham and the tDCS treatments. The anterior cingulate is a key component in the brain's pain modulating regions and is also involved in the emotional processing of pain (6). Its structure and function have been found to be altered in chronic pain states; specifically, focal atrophy and reduced connectivity in the rostral anterior cingulate have been described (12,18). Furthermore, the rostral anterior cingulate has been shown to be an important region for descending inhibition of pain (12). Our findings suggest that "normalization" of Glx in the anterior cingulate may have an important role in the mechanism of action of tDCS for the treatment of fibromyalgia. In addition, the strength of the regression coefficient and high level of significance between Glx levels in the anterior cingulate and

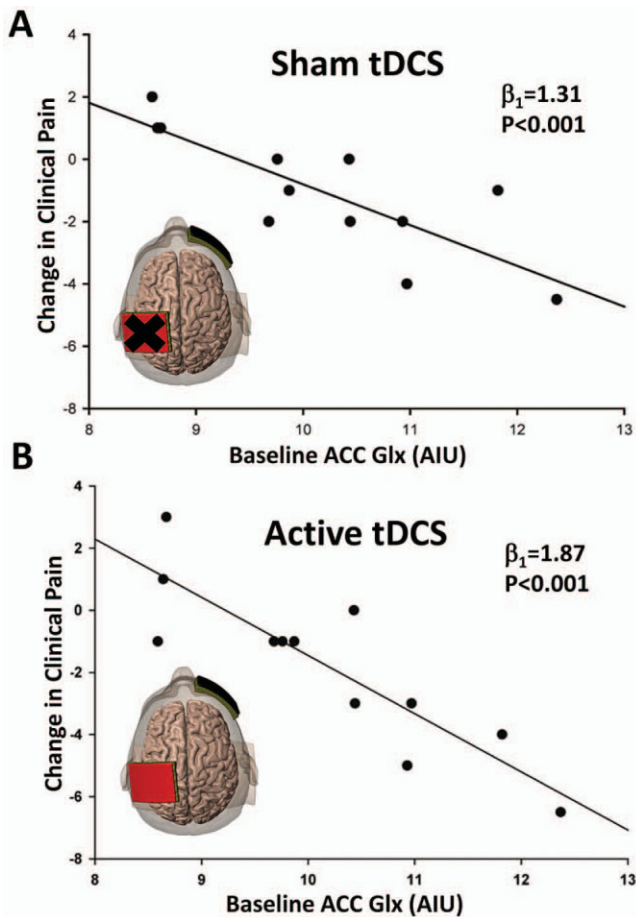


Figure 2. Pretreatment levels of Glx in the anterior cingulate are predictive of subsequent changes in clinical pain in fibromyalgia patients receiving sham or active tDCS. Scatterplots show baseline levels of Glx versus posttreatment changes in clinical pain (rated on a visual analog scale) following sham tDCS (A) and active tDCS (B). Patients with higher levels of Glx in the anterior cingulate at baseline had greater reductions in clinical pain following both sham and active tDCS. See Figure 1 for definitions.

clinical pain improvement suggest that $^1\text{H-MRS}$ may prove to be a clinically useful predictor of tDCS efficacy in FM. We speculate that the higher regression coefficient for the correlation of baseline Glx levels with the change in pain score after active tDCS ($\beta_1 = 1.87$) relative to the change in pain score after sham tDCS ($\beta_1 = 1.31$) may reflect additive placebo response to the tDCS treatment.

The present findings complement our results in a study of pregabalin treatment, which demonstrated that baseline Glx levels were associated with pain response, albeit in a different brain region (3). Increases in Glx and NAA levels following tDCS stimulation have been

observed in the parietal cortex underneath the anode (20), suggesting that tDCS locally increases glutamatergic activity and neuronal metabolism.

There was a trend toward decreased levels of Glx in the thalamus following tDCS treatment. The thalamus, which is a critical pain relay and processing center, has exhibited alterations in blood flow, white matter structure, and connectivity in FM (21). We also observed a trend toward increased levels of GABA in the anterior insula following tDCS treatment, complementing our earlier findings of reduced levels of GABA in FM patients as compared to healthy controls (7). The pain processing regions of the brain also demonstrated significant correlations between GABA and Glx levels and clinical pain scores: patients with higher levels of GABA and lower levels of Glx had lower levels of pain intensity (as measured by VAS) and negative affect (as measured by PANAS-) and higher levels of positive affect (as measured by PANAS+). These findings are consistent with the notion that in FM there is an excitatory:inhibitory ratio imbalance in the brain, resulting in up-regulation of pain response/experience, as we have previously proposed (5,7). Similar results were demonstrated with regard to NAA levels in the anterior insula, posterior insula, and thalamus: patients with lower pain scores, lower levels of negative affect, and higher levels of positive affect tended to have higher levels of NAA. Our findings, in addition to the increase in NAA levels in the posterior insula that we observed following sham tDCS treatment, suggest that neuronal integrity, as measured by NAA, is important in chronic pain.

Limitations of the present study include the relatively small number of subjects and lack of randomization. The large number of statistical comparisons raises the possibility of Type I error. Furthermore, we were unable to demonstrate a significant improvement in clinical pain scores between the sham tDCS and active tDCS time points, which may be due in part to placebo response and the small sample size. We suggest that given the exploratory nature of this study, subsequent efforts be undertaken to confirm our results. In addition, the GABA editing acquisition includes contributions from macromolecules. GABA editing requires relatively large voxel sizes and long time acquisitions, limiting the number of potential brain regions to interrogate. For future pain studies, it would be interesting to measure local $^1\text{H-MRS}$ changes underneath the anode.

In conclusion, our findings suggest that GABA, Glx, and NAA play important roles in the pathophysiology of chronic pain and its modulation by tDCS. There were significant alterations in the levels of metabolites

for various pain centers in the brain following both the sham and active tDCS phases of the present trial. Furthermore, baseline Glx levels in the anterior cingulate predicted response to treatment. These findings encourage further work to pursue targeted therapy with tDCS and other noninvasive brain stimulation modalities in FM and other chronic pain conditions.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Foerster had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Clauw, Zubieta, Harris, DaSilva.

Acquisition of data. Foerster, Nascimento, DeBoer, Bender, Rice, DaSilva.

Analysis and interpretation of data. Foerster, Nascimento, DeBoer, Rice, Truong, Bikson, Zubieta, Harris, DaSilva.

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