

# Effect of High-Definition Transcranial Direct Current Stimulation on Headache Severity and Central $\mu$ -Opioid Receptor Availability in Episodic Migraine

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**Objective:** The current understanding of utilizing HD-tDCS as a targeted approach to improve headache attacks and modulate endogenous opioid systems in episodic migraine is relatively limited. This study aimed to determine whether high-definition transcranial direct current stimulation (HD-tDCS) over the primary motor cortex (M1) can improve clinical outcomes and endogenous  $\mu$ -opioid receptor ( $\mu$ OR) availability for episodic migraineurs.

**Methods:** In a randomized, double-blind, and sham-controlled trial, 25 patients completed 10-daily 20-min M1 HD-tDCS, repeated Positron Emission Tomography (PET) scans with a selective agonist for  $\mu$ OR. Twelve age- and sex-matched healthy controls participated in the baseline PET/MRI scan without neuromodulation. The primary endpoints were moderate-to-severe (M/S) headache days and responder rate ( $\geq 50\%$  reduction on M/S headache days from baseline), and secondary endpoints included the presence of M/S headache intensity and the use of rescue medication over 1-month after treatment.

**Results:** In a one-month follow-up, at initial analysis, both the active and sham groups exhibited no significant differences in their primary outcomes (M/S headache days and responder rates). Similarly, secondary outcomes (M/S headache intensity and the usage of rescue medication) also revealed no significant differences between the two groups. However, subsequent analyses showed that active M1 HD-tDCS, compared to sham, resulted in a more beneficial response predominantly in higher-frequency individuals ( $>3$  attacks/month), as demonstrated by the interaction between treatment indicator and baseline frequency of migraine attacks on the primary outcomes. These favorable outcomes were also confirmed for the secondary endpoints in higher-frequency patients. Active treatment also resulted in increased  $\mu$ OR concentration compared to sham in the limbic and descending pain modulatory pathway. Our exploratory mediation analysis suggests that the observed clinical efficacy of HD-tDCS in patients with higher-frequency conditions might be potentially mediated through an increase in  $\mu$ OR availability.

**Conclusion:** The 10-daily M1 HD-tDCS can improve clinical outcomes in episodic migraineurs with a higher baseline frequency of migraine attacks ( $>3$  attacks/month). This improvement may be, in part, facilitated by the increase in the endogenous  $\mu$ OR availability.

**Clinical Trial Registration:** [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), identifier - NCT02964741.

**Keywords:** HD-tDCS, neuromodulation, migraine, headache, pain, mu-opioid receptor

## Introduction

Migraine is a highly disabling primary headache disorder featuring recurrent headache attacks associated with throbbing pain, vomiting, nausea, and photophobia/phonophobia,<sup>1,2</sup> with a 1-year prevalence of nearly 15% in the general population.<sup>3</sup> Although our understanding of the pathogenesis of migraine has progressed much, currently available therapeutics to reduce the severity or prevent attacks are still unsatisfactory to many migraine sufferers.<sup>4</sup> Moreover, inadequate management of pain

and overuse or misuse of medications such as opioids could exacerbate the disease process (eg, increased headache frequency), which deteriorates the quality of life of affected individuals.<sup>5</sup> In this context, non-invasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) have showed promise as a potential non-pharmacological treatment approaches for those who are ill-suited to existing treatment options.<sup>6</sup>

tDCS is designed to shift the excitability of the brain, either hyperpolarizing (inhibitory) or depolarizing (excitatory), by focalizing current flow using two types of electrodes consisting of an anode and cathode; thereby, tDCS modulates the neural network by promoting network synchronization and plasticity.<sup>7</sup> To this end, the primary motor cortex (M1) has been studied extensively as a target for alleviating pain symptoms.<sup>8</sup> Indeed, M1-tDCS applied to episodic migraineurs has produced promising results in reducing headache days and pain intensity in a safe and well-tolerated way.<sup>9,10</sup> However, we are aware that there are remaining challenges to be addressed to optimize outcomes.<sup>11,12</sup> One key challenge identified is to increase the number of sessions at a clinical setting to optimize and extend the therapeutic effect of neuromodulation on a patient's outcome. To address this challenge, we have been developing a new protocol that incorporates home-based HD-tDCS to increase the dose of treatment and patients' adherence to the treatment regimen.<sup>8</sup>

Accumulating evidence has shown that tDCS with novel high-definition (HD) montages can promote the focality of current distribution compared to conventional tDCS, which activates the brain far beyond the targeted region, for example, through widespread structural and functional neural connection.<sup>13,14</sup> Moreover, HD-tDCS outperformed the conventional tDCS in terms of inducing neuroplasticity which lasted longer<sup>15,16</sup> and was validated for its potential in managing chronic pain such as temporomandibular disorder<sup>17</sup> and fibromyalgia.<sup>18</sup> However, no studies have been conducted to establish the efficacy of M1 HD-tDCS in episodic migraineurs and, most importantly, the neuro-mechanism by which it relieves migraine symptoms.

It has been revealed that  $\mu$ OR concentration would be compromised as acute pain develops in a chronic form,<sup>19</sup> which can be observed by a higher release of  $\mu$ OR activating peptides (eg, beta-endorphin, enkephalin). These observations hold for various chronic pain disorders, including migraine,<sup>20</sup> fibromyalgia,<sup>21</sup> orofacial pain,<sup>22</sup> and neuropathic pain.<sup>23</sup> We showed that  $\mu$ OR availability was lower in patients with chronic migraine compared to episodic migraine in the right amygdala and left parahippocampal gyrus implicated in pain chronification<sup>24</sup> and hypersensitivity.<sup>25</sup> Moreover, reduced  $\mu$ OR availability was correlated with a lower pain threshold and headache severity,<sup>20</sup> indicating that  $\mu$ OR characteristics are possibly associated with ineffective pain processing, which might be the neural correlate of migraine severity.

Herein, we assessed whether M1-tDCS with a highly focused neuromodulation montage would reduce headache days and pain severity in episodic migraineurs. We further evaluated the hypothesis that the therapeutic effect of HD-tDCS would be mediated by restoring  $\mu$ OR availability in the limbic regions.

## Materials and Methods

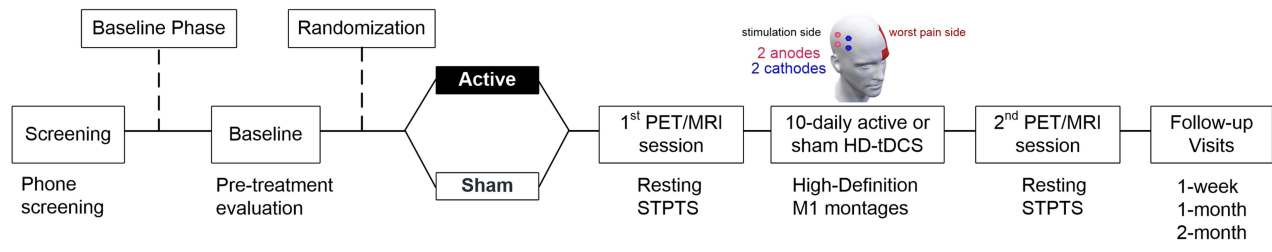
### Study Design

This was a single-center, randomized, double-blind, and sham-controlled study including five phases of evaluation following: baseline screening, pre-treatment Positron Emission Tomography (PET)/Magnetic Resonance Imaging (MRI) 10-daily M1 HD-tDCS either active or sham over 2 weeks, post-treatment PET/MRI, and 1-week, 1-, and 2-month follow-up assessments between February 2017 and April 2020 (Figure 1A). Of note, according to the guidelines of the International Headache Society for controlled trials of preventive treatment of migraine attacks in migraine,<sup>26,27</sup> our pre-specified outcomes in the original protocol were switched from a change in pain intensity to moderate-to-severe (M/S) headache days (primary) and headache intensity and rescue medication use (secondary). This switch in clinical outcomes was also in agreement with the National Institute of Health.

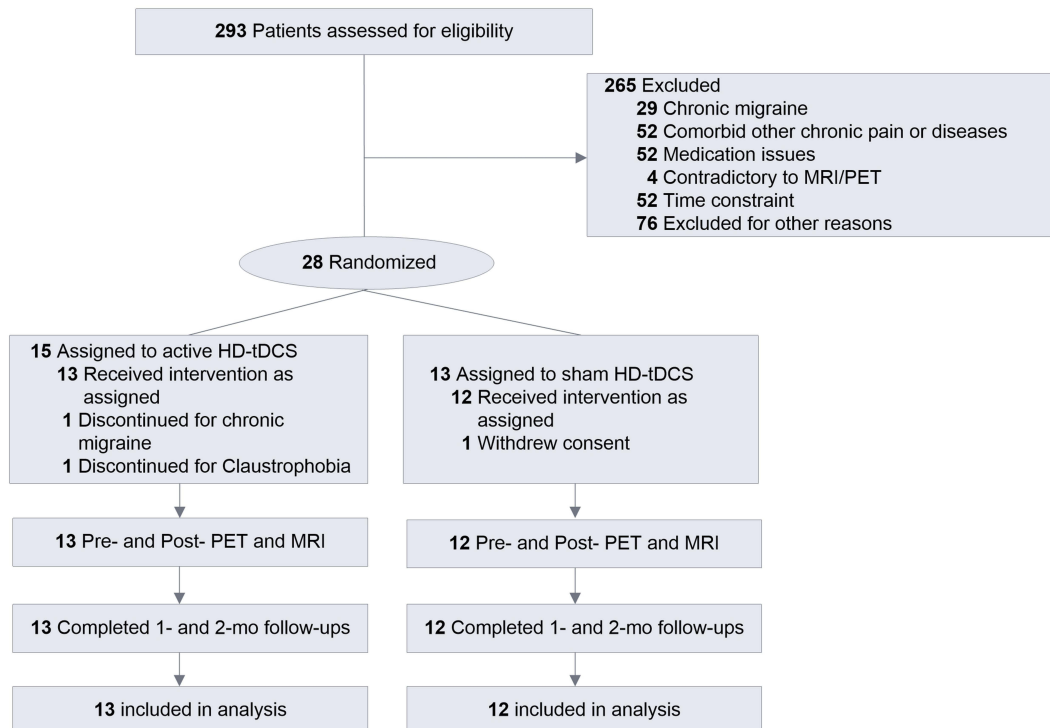
### Patient Recruitment

Study participants were recruited from the University of Michigan Hospital clinics or the local community via advertisements posted on bulletin boards. Episodic migraine with or without aura was defined by the International Classification of Headache Disorders (ICHD), 3rd edition (beta version) (ICHD-3.1/2),<sup>1</sup> experiencing 2 to 14 headache days per month and at least one year of history. The exclusion criteria for this study were 1) a history of other chronic

## A Trial design



## B CONSORT study flow



**Figure 1** Trial design and CONSORT study flow. **(A)** This study included five phases of evaluation: screening; baseline PET/MRI session; 10 daily M1 HD-tDCS, either active or sham, over two weeks; follow-up PET/MRI session; and follow-up assessments at 1-week, 1-month, and 2-month after the end of the treatment. **(B)** CONSORT flow diagram includes screening, allocation to treatment, follow-ups, and analyses. Adapted from Schulz KF, Altman DG, Moher D, CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials. *PLoS Med.* 2010;7(3):e1000251. Copyright: © 2010 Schulz et al. Creative Commons Attribution License.<sup>28</sup>  
**Abbreviations:** HD-tDCS, high-definition transcranial direct current; MRI, magnetic resonance imaging; PET, positron emission tomography.

pain disorders and other types of migraine such as hemiplegic migraine or cluster headache, 2) medication overuse or opiate intake for over 6 months, 3) hormonal contraceptives, 4) a history of concurrent psychiatric disorders or neurological disorders, 5) ongoing and unresolved disability litigation, 6) preventive medication and use of an investigational drug or device within 30 days of study entry, 7) pregnant or planning to become pregnant, or 8) contraindication to MRI such as implanted pacemaker or claustrophobia. The patients agreed to take abortive rescue medicine for managing headaches but not for preventive purposes.

## Randomization and HD-tDCS Intervention

Among 293 patients assessed for eligibility, we enrolled 28 patients and randomized them into active or sham treatment in a 1:1 ratio using the Taves covariate adaptive randomization method balancing sex and age.<sup>29</sup> We reported the study flow under the Consolidated Standards of Reporting Trials (CONSORT) guideline (Figure 1B).<sup>28</sup> A sample size of twenty-five who completed the study would have 80% power to detect an effect size of 1.2 SD with  $\alpha = 0.05$  two-sided

Type I error. Twelve age- and sex-matched healthy control participants consisting of 1 male and 11 females (mean age, 32.4, SD 15.3 years) were also included for the baseline PET/MRI scan without neuromodulation. The principal investigator, research staff, and patients were blinded to the treatment allocation during and until the end of the data analyses, except for the research staff responsible for operating HD-tDCS.

We used M1 HD-tDCS stimulator (Soterix Medical Inc., NY, USA) to promote spatial focality of stimulation, thereby increasing current intensity on the targeted region.<sup>14</sup> A  $2 \times 2$  ring configuration with 12-mm diameter disk electrodes, developed in-house, consisting of two anodes and two cathodes, was placed posterior to the anterior direction across the face/head homuncular M1 region contralateral to the worst headache side. In the case of bilateral pain, we placed electrodes on the left M1. Explicit 10–10 system locations for anodes were C3 and C5, and cathodes were FC3 and FC5. An active M1 HD-tDCS with 2 mA was applied for 20 minutes/day over 2 weeks. The same montages were used for a sham session; however, the current was applied for only 30 seconds at the beginning and end of the session, delivering a tingling sensation to the scalp,<sup>30</sup> believed to be reliable for a sham experiment.

## PET/MRI Acquisition

We acquired the pre-treatment and post-treatment PET with [<sup>11</sup>C]CFN data several days before and after M1 HD-tDCS treatment (4.7 and 6.2 days on average, respectively). [<sup>11</sup>C]CFN was synthesized as previously described.<sup>31</sup> Each PET session consisted of a 40-min early resting state after [<sup>11</sup>C]CFN injection followed by a 50-min late phase, including the Sustained Thermal Pain Threshold Stress (STPTS) challenge. The PET/MRIs were performed outside attacks (interictal period). Detailed procedures for PET and T1-weighted MRI acquisition, pre-processing, and analyses are described in [Supplementary Data](#). Of the 25 patients who participated in active or sham HD-tDCS treatment, 13 and 11 patients for each active and sham group were included in the PET analyses due to a technical failure during pre-treatment PET scanning for one sham patient.

## Clinical Outcomes

The two primary endpoints were the number of moderate-to-severe (M/S) headache days and the proportion of participants with  $\geq 50\%$  reduction in the M/S headache days 1-month following the end of the two-week tDCS session. The M/S headache days were defined as days with moderate or severe pain intensity with a minimum value of 4 based on a 0–10 numeric rating scale (NRS) and at least 4 hours of untreated headache. However, a minimum time was not specified if patients had rescue medication (migraine-specific, non-steroidal anti-inflammatory drug, or non-aspirin pain reliever). The secondary endpoints were the headache intensity that classified none-to-mild pain (NRS: 0–3) or M/S pain (NRS: 4–10) and any rescue medication intake over a 1-month post-intervention (yes/no). At each visit, headache frequency, intensity, and medication usage were assessed by patients' recall and recorded using PainTrek technology (University of Michigan, MI), a 3D-pain tracking mobile application. Following data collection over a one-month period, we computed the average headache intensity within that timeframe. As an additional exploratory outcome, we also recorded the maximum pain intensity and location (eg, body subregion, side) using the PainTrek. We utilized the PainTrek-technology to enable patients to record not only their pain intensity but also the specific area and location of their headaches, allowing for a comprehensive analysis of the spatial aspects of their pain and enhancing the overall understanding of their migraine episodes.<sup>32</sup>

## Statistical Analysis

Demographic and clinical characteristics between diagnostic and treatment groups were compared using an independent sample *t*-test and chi-square test for continuous variables and categorical variables, respectively. We used Poisson regression, suitable for count type measures, on the number of days with M/S headache over 1-month post-intervention as the outcome variable and treatment indicator (active vs sham) as a predictor. We performed logistic regression for the other primary outcome – a responder rate (achieving 50% or more reduction in M/S headache days compared to baseline), and for secondary outcome measures, including headache intensity (none-to-mild or M/S) and rescue medication use (yes/no) over 1-month post-intervention as the outcome variable and treatment indicator as a predictor. We sought to assess if the intervention effect varied by baseline migraine severity as measured by the

migraine attack frequency. Therefore, we included the monthly migraine attacks at baseline (during the last 30 days) and their interaction with treatment in the model for each primary and secondary outcome. A significant interaction term would indicate a differential treatment effect based on the baseline frequency of migraine attacks. We displayed predicted values and 95% confidence interval (CI) of clinical outcomes by treatment group and pre-treatment frequency of migraine attacks. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC), and statistical significance was set as  $p < 0.05$  (2-sided).

## PET Data Analysis

Linear mixed-model analysis was applied to measure longitudinal changes of  $BP_{ND}$  during the resting state and during the STPTS challenge phase including treatment group (active vs sham), time, and a group-by-time interaction term as factors of interest (fixed effect) and subject as a random effect while adding age as a covariate of no interest using Statistical parametric Mapping (SPM) 12 (Wellcome Department of Cognitive Neurology, University College London, England; <http://www.fil.ion.ucl.ac.uk/spm/>) implanted in MATLAB (version R2016b, MathWorks, Inc). Only voxels with values over 0.1 ( $BP_{ND}$ ) were included in the analysis. We utilized the small-volume correction (SVC) approach to focus on our a priori hypothesized regions for both basal resting and STPTS phase including the left and right amygdala, parahippocampal gyrus, temporal pole, hypothalamus, thalamus, and rostral anterior cingulate cortex (rACC) linked to opioidergic pain modulatory function, migraine pathophysiology, and tDCS effects as identified in previous studies.<sup>33–36</sup> We used the Harvard-Oxford cortical and subcortical structural atlas to define a priori mask regions. For the hypothalamus, we used Neurosynth meta-analytic maps (<http://neurosynth.org>) with the term “hypothalamus” (Figure S1). The significance was set to voxel-level  $p < 0.005$  (uncorrected), combined with a cluster-level family-wise error (FWE)-corrected  $p < 0.05$ . Additionally, we assessed the ventrolateral periaqueductal gray (vlPAG) as a region of interest due to its small size and tested changes of vlPAG  $\mu$ OR  $BP_{ND}$  only during the STPTS challenge phase, considering its essential role in  $\mu$ OR-related anti-nociception.<sup>37–39</sup> The coordinates and size for right and left vlPAG were MNI (Montreal Neurological Institute)  $xyz = 4, -30, -8$  and  $xyz = -4, -30, -8$  surrounded by 3-mm radius sphere, respectively.<sup>38,39</sup>

Acknowledging that M1 HD-tDCS efficacy on clinical outcomes was varied by baseline frequency of migraine attack, we assessed if regional changes in  $\mu$ OR  $BP_{ND}$  (showing significant interaction between time and treatment group) would differ between active and sham treatment in each stratified group into higher ( $>3$  attacks/month) and lower ( $\leq 3$  attacks/month) using the Mann–Whitney  $U$ -test. Bonferroni correction was applied to correct multiple comparison ( $\alpha = 0.006$ ; 0.05/8 comparisons).

Moreover, we conducted an exploratory mediation analysis to examine and corroborate, in higher-frequency patients, whether the treatment effect (predictor) on M/S headache days (outcome) was mediated through the voxel-wise changes in  $\mu$ OR  $BP_{ND}$  (mediator) within our a priori masks including temporal pole, hippocampus, parahippocampal gyrus, hypothalamus, and amygdala. As shown in Figure S2, path a denotes the association between predictor and mediator, and path b the association between mediator and outcome controlling for predictor. Path c represents the overall relationship between predictor and outcome, whereas path c' is the direct effect controlling for the mediator. The mediating effect ( $a*b$ ) was tested based on the significance of  $c-c'$  determined with a bootstrap resampling ( $N = 10,000$  iterations) using the Mediation Toolbox for Matlab (<https://github.com/canlab/MediationToolbox>).<sup>40,41</sup>

## Patient-Reported Side Effects

We used a questionnaire administered every tDCS session to assess side effects, including headache, neck pain, scalp pain, scalp burns, tingling, skin redness, sleepiness, trouble concentrating, acute mood change, and others. Symptom severity was classified as absent, mild, moderate, and severe. In each side effect, we calculated the percentage of patients by dividing the number of patients who reported complaints at least once out of ten sessions by the total number of patients in each treatment group.

## Results

### Baseline Characteristics

Among those screened for eligibility ( $n = 293$ ), 28 episodic migraineurs were enrolled, and a total of 13 active and 12 sham participants completed 10 daily HD-tDCS and follow-ups. The two treatment groups were similar in terms of baseline demographic and migraine characteristics, including M/S headache days, pain intensity, the proportion of patients taking rescue medication, frequency, chronicity, and presence of an aura (Table 1).

### MI HD-tDCS Effect on Primary Outcomes

M1 HD-tDCS was well tolerated without any serious adverse effects in either group, consistent with the known low risk of tDCS.<sup>42,43</sup> The M/S headache days over 1-month following HD-tDCS treatment were not different between the active and sham-treated groups; 2.9 days (95% confidence interval [CI], 2.1 to 3.9) and 3.3 days (95% CI 2.4 to 4.4), respectively ( $p = 0.64$ ).

In addition, a responder rate ( $\geq 50\%$  reduction of M/S headache days compared to baseline) was greater for active (76.9%, 95% CI 55.1 to 99.2) than the sham group (50.0%, 95% CI 22.6 to 76.5) but not significant ( $p = 0.15$ ). However, M1 HD-tDCS efficacy on M/S headache days over 1-month after treatment varied between the two groups by baseline frequency of migraine attacks ( $\beta = -0.35$ , 95% CI  $-0.53$  to  $-0.18$ ,  $p < 0.001$  for interaction) (Figure 2A).

### MI HD-tDCS Effect on Secondary Outcomes

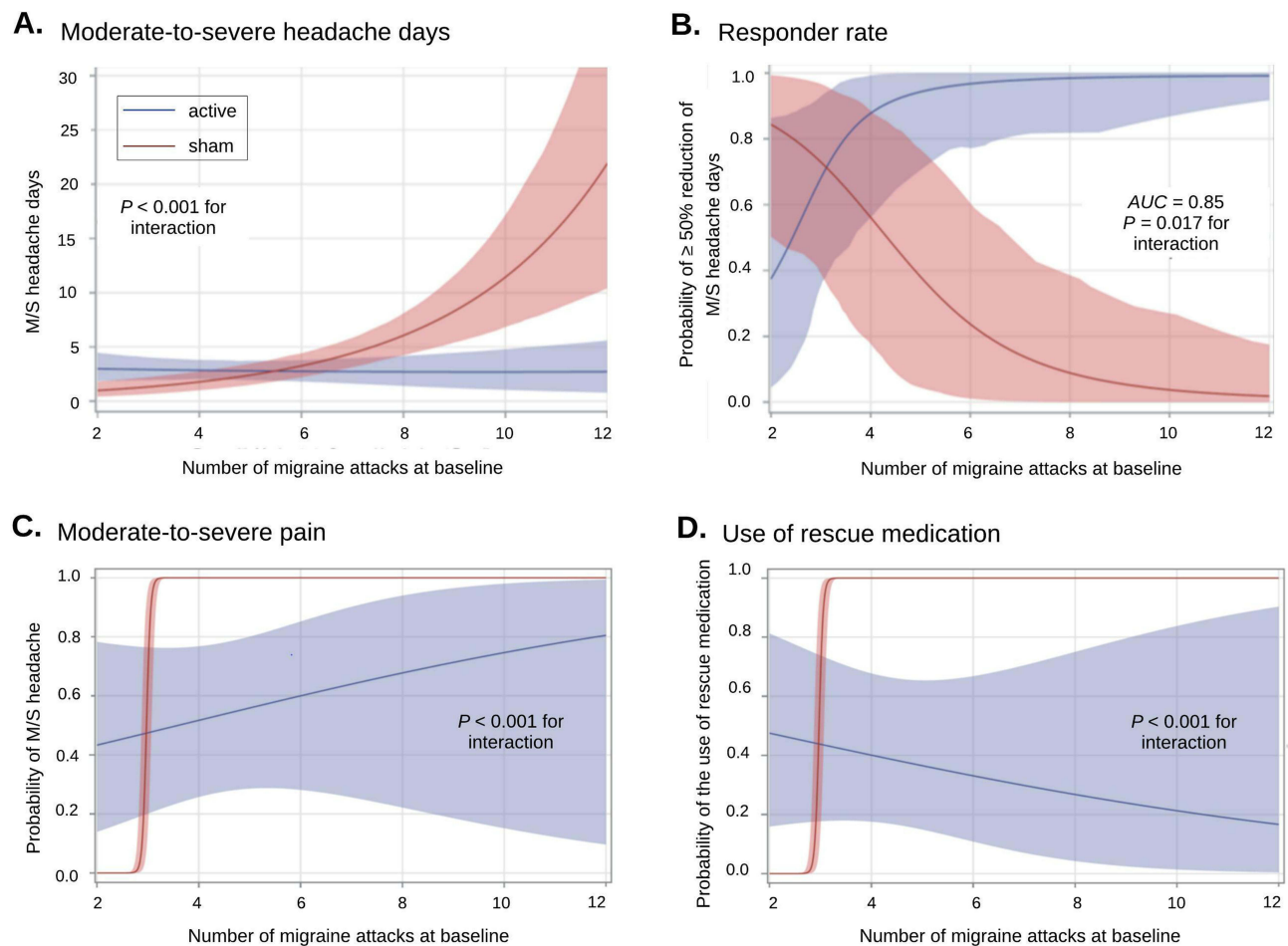
The secondary outcome assessed the presence of M/S headache pain and the use of rescue medication over 1-month follow-up. Our findings indicate that the occurrence of M/S headache for the active group was 53.8% (95% CI, 28.2 to 77.6) and the sham group was 66.7% (95% CI 37.6 to 86.9). The difference between the groups, however, was not statistically significant ( $p = 0.52$ ). Similarly, the use of rescue medication was 38.5% (95% CI 17.0 to 65.6) in the active group and 66.7% (95% CI 37.6 to 86.9) in the sham group. Again, the difference between the groups was not statistically significant ( $p = 0.16$ ).

**Table 1** Baseline Characteristics of Episodic Migraineurs<sup>a</sup>

Variables	Active HD-tDCS (n = 13)	Sham HD-tDCS (n = 12)	Comparisons t or $\chi^2$ (P value)
Age, years	30.1 (13.8)	30.3 (8.8)	-0.03 (0.97)
Gender (male/female)	2/11	1/11	0.30 (0.59)
Body Mass Index <sup>b</sup>	25.0 (4.6)	24.1 (3.5)	0.55 (0.59)
Beck Depression Inventory	3.2 (3.1)	3.1 (2.9)	0.12 (0.90)
Age at migraine onset, y	18.7 (11.1)	16.2 (9.6)	0.61 (0.55)
Years of migraine, yrs	11.4 (7.5)	14.1 (9.8)	-0.78 (0.45)
Presence of aura, N (%)	6 (46.2)	5 (41.7)	0.05 (0.82)
Number of migraine attacks per month	4.6 (2.8)	4.8 (2.8)	-0.19 (0.85)
Moderate-to-severe headache days per month <sup>c</sup>	5.9 (3.4)	5.2 (2.7)	0.55 (0.59)
Rescue medication use, N (%)	11 (84.6)	12 (100)	2.01 (0.16)
MIDAS score <sup>d</sup>	24.5 (15.7)	22.3 (15.1)	0.35 (0.73)
Expectancy for treatment <sup>e</sup>	5.6 (1.8)	6.3 (0.8)	-1.13 (0.27)

**Notes:** Continuous variables are reported as mean (standard deviation, SD). Categorical variables are reported as counts. <sup>a</sup>Unpaired t-test was used for continuous variables and Chi-square test for categorical variables. <sup>b</sup>The body mass index is the weight in kilograms divided by the square of the height in meters. <sup>c</sup>Moderate-to-severe headache were defined by  $\geq 4$  on a 0–10 numeric rating scale. <sup>d</sup>MIDAS score ranges from 0 to 27 with 0–5 indicating little or no disability, 6–10 indicating mild disability, 11–20 indicating moderate disability, and  $\geq 21$  indicating severe disability related to migraine. <sup>e</sup>Expectancy for treatment was assessed on a 0–10 visual analog scale.

**Abbreviations:** HD-tDCS, high-definition transcranial direct current stimulation; MIDAS, Migraine Disability Assessment.



**Figure 2** Primary and secondary outcomes after M1 HD-tDCS treatment. **(A)** The baseline monthly attack frequency was related to clinical outcomes. Results are presented as a predicted moderate-to-severe (M/S) headache frequency and **(B)** likelihood of  $\geq 50\%$  reduction of M/S headache days from baseline over 1-month after HD-tDCS with a 95% confidence interval (CI) by baseline monthly attack frequency. **(C)** Secondary outcomes of headache pain intensity (M/S or non-to-mild) and **(D)** the use of rescue medication over 1-month after HD-tDCS were presented with 95% CI by baseline monthly attack frequency.

## Subgroup Analysis

Following a significant interaction term between baseline frequency and M/S headache days at 1-month follow-up, we stratified the patients into higher ( $n = 14$ ; 8 for active and 6 for sham) and lower frequency groups ( $n = 11$ ; 5 for active and 6 for sham). Among higher-frequency patients ( $>3$  attacks/month), the active group had fewer M/S headache days (2.6, 95% CI 1.8 to 4.2) compared with sham group (5.5, 95% CI 3.9 to 7.7;  $p = 0.012$ ), whereas this therapeutic effect was opposite in direction in the lower-frequency patients ( $\leq 3$  attacks) (active: 3.4, 95% CI 2.1 to 5.5; sham: 1.2, 95% CI 0.6 to 2.4;  $p = 0.017$ ). Logistic regression analysis revealed that the treatment effect on responder rate was changed by baseline frequency of migraine attacks ( $\beta = 2.37$ , 95% CI 0.74–4.62,  $p = 0.017$  for interaction) (Figure 2B), such that higher-frequency patients of the active group showed a better response rate than the peers of the sham group (active: 87.5%, 95% CI 46.3 to 98.3; sham: 6.7%, 95% CI 2.3 to 63.1;  $p < 0.018$ ), whereas this effect was not observed in lower-frequency patients ( $p = 0.36$ ).

As to the secondary outcomes, the effect of treatment on the probability of having an M/S headache and the use of rescue medication over 1-month post-intervention differed by monthly migraine attack frequency ( $p < 0.001$  for interaction) (Figure 2C and D). We then determined the predicted probabilities of secondary endpoints in each frequency group. We found that the active treatment reduced the likelihood of having M/S headache pain compared with the sham group (62.5% vs 100%;  $p < 0.001$ ), only for higher-frequency patients. Similarly, the active treatment for higher-frequency patients reduced the likelihood of abortive medication intake compared to the sham counterpart (37.5% vs

100%;  $p < 0.001$ ). However, we did not observe treatment effects of HD-tDCS neither on the headache intensity (active vs sham, 40.0% vs 33.3%;  $p = 0.82$ ) nor on the abortive medication intake (40.0% vs 33.3%;  $p = 0.82$ ) in lower-frequency patients.

## Additional Secondary Outcomes Specific to the Side of the MI HD-tDCS

To investigate whether the HD-tDCS specifically modulates pain across ipsilateral or contralateral side regions to the stimulation, we additionally compared the maximum pain intensity subdivided into 4 categories (0, none; 1, mild; 2, moderate; 3, severe). Briefly, active HD-tDCS, compared with the sham control, lowered the probability of having M/S headache pain contralateral to the stimulation for 1-month after treatment as the migraine attack frequency at baseline increased ( $p < 0.001$  for interaction) (Figure S3A). However, this treatment effect was not observed on the ipsilateral side of the stimulation ( $p = 0.35$  for interaction) (Figure S3B). Similarly, higher-frequency patients treated with active HD-tDCS were also less likely to have M/S intensity pain on the contralateral side of HD-tDCS compared to those with sham HD-tDCS (active vs sham, 75.0% vs 100%;  $p < 0.001$ ). This effect was absent in the lower-frequency patients (40.0% vs 33.3%,  $p = 0.82$ ). In contrast, we did not find any beneficial effect in the ipsilateral side to HD-tDCS, on either higher-frequency group (50.0% vs 66.7%,  $p = 0.54$ ) or lower-frequency group (40.0% vs 16.7%,  $p = 0.40$ ).

## MI HD-tDCS Effect on $\mu$ OR Availability

Notably, there was an interaction effect between time and treatment on voxel-wise  $\mu$ OR BP<sub>ND</sub> in a priori hypothesized regions, including the right amygdala (peak MNI xyz = 28, 4, -28;  $k = 14$ ) and left temporal pole (xyz = -28, 8, -46;  $k = 50$ ) during the resting state and right hypothalamus (xyz = 2.4, -18;  $k = 8$ ) and left parahippocampal gyrus (xyz = -18, -2, -32,  $k = 54$ ) during the late phase, driven mainly by increased  $\mu$ OR BP<sub>ND</sub> in the active group or decrease in the sham group (Figure 3A). Further, the  $\mu$ OR BP<sub>ND</sub> increases were primarily observed in higher-frequency patients treated with active HD-tDCS compared to sham counterparts specifically in the amygdala (during resting) and the parahippocampal gyrus (during STPTS challenge), with a significant change found in the parahippocampal gyrus after a Bonferroni correction (Figure 3B).

As to the ROI analysis of vIPAG, we found a similar pattern, such that active treatment increased right vIPAG  $\mu$ OR BP<sub>ND</sub> compared to sham during STPTS challenge (Figure 4A). Furthermore, we observed a marked increase only in patients with higher-frequency condition, although the significance was marginal (Figure 4B). When we re-analyzed the interaction effects between treatment group and time while only including higher-frequency patients with the same statistical threshold, we additionally found increased  $\mu$ OR BP<sub>ND</sub> in the right rostral anterior cingulate cortex (rACC) after active treatment compared to sham (Figure S4).

## Increased $\mu$ OR Availability as a Potential Mediator of HD-tDCS Efficacy

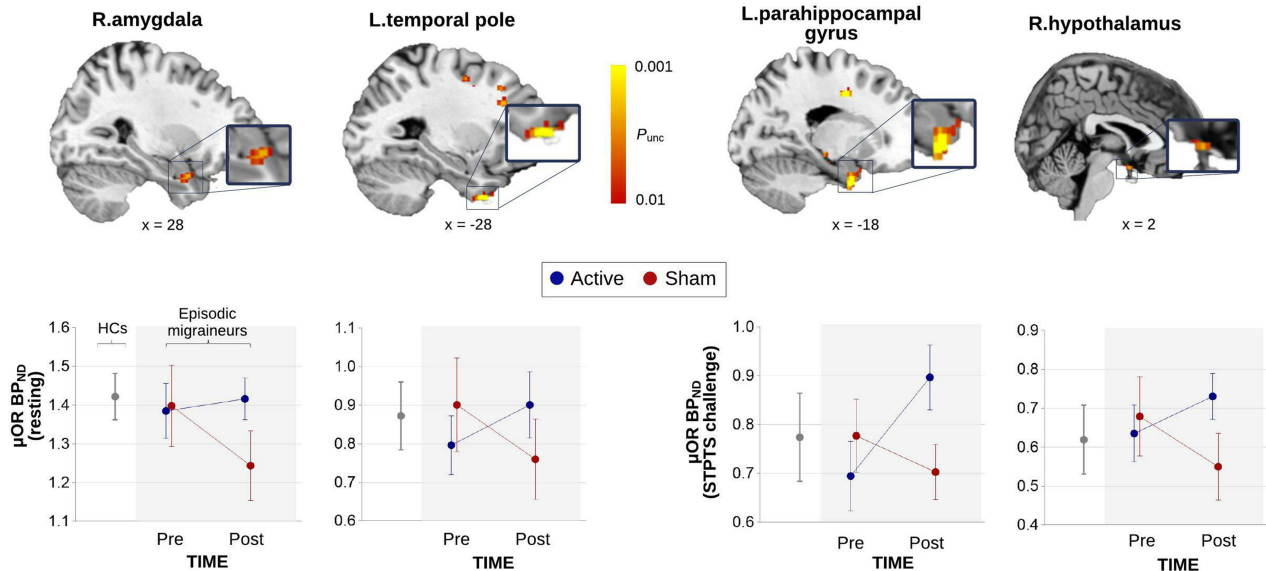
We conducted the mediation analysis to identify voxels of mediators within the amygdala and temporal pole (resting-state) and hypothalamus and parahippocampal gyrus (STPTS phase) with a treatment indicator as the predictor and M/S headache days over the 1-month follow-up as the outcome in higher-frequency patients. The result revealed that a portion of the left parahippocampal gyrus mediated the relationship between treatment and M/S headache days over the 1-month follow-up (Figure S2). Specifically, the active HD-tDCS over M1, compared to sham, increased the  $\mu$ OR BP<sub>ND</sub> in the anterior portion of the left parahippocampal gyrus (path a: peak voxel xyz coordinates: -22, 0, -36;  $Z = 3.83$ ,  $p = 0.0001$ ), which predicted a fewer M/S headache days (path b:  $Z = -4.03$ ;  $p = 0.0001$ ; path a\*b:  $Z = -4.67$ ;  $p < 0.0001$ ), controlling for treatment indicator ( $q < 0.05$  false discovery rate-corrected).

## Patient-Reported Side Effects

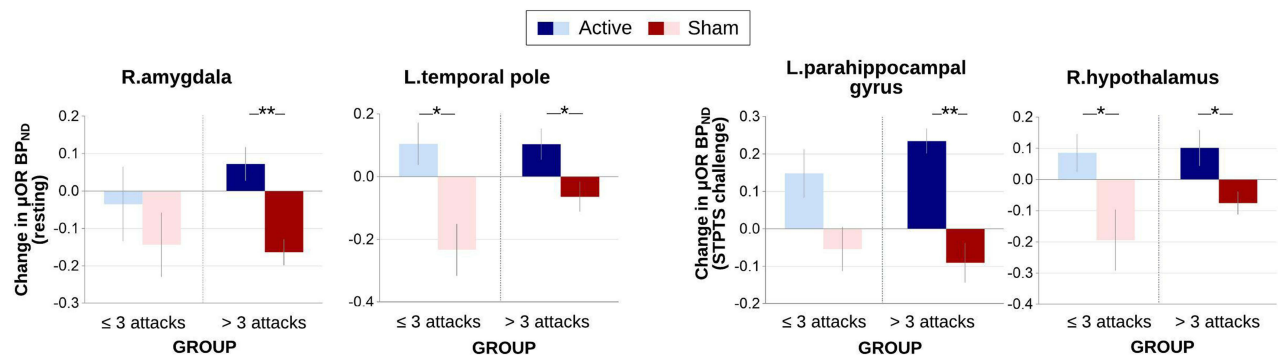
As to side effects reported by participants after each HD-tDCS session, the tingling sensation, primarily mild, was the most frequently reported; 53.8% for the active and 91.7% for the sham group (Figure 5). We confirmed discomfort on the head and neck from the treatment soothed shortly after each session. In addition, the patients acknowledged that they could withdraw their participation at any point during the study. There were no treatment-related serious adverse events. No patients decided to stop treatment due to adverse events.



### A. Changes in $\mu$ OR BP<sub>ND</sub> after M1 HD-tDCS



### B. Changes in $\mu$ OR BP<sub>ND</sub> according to baseline frequency of migraine attack

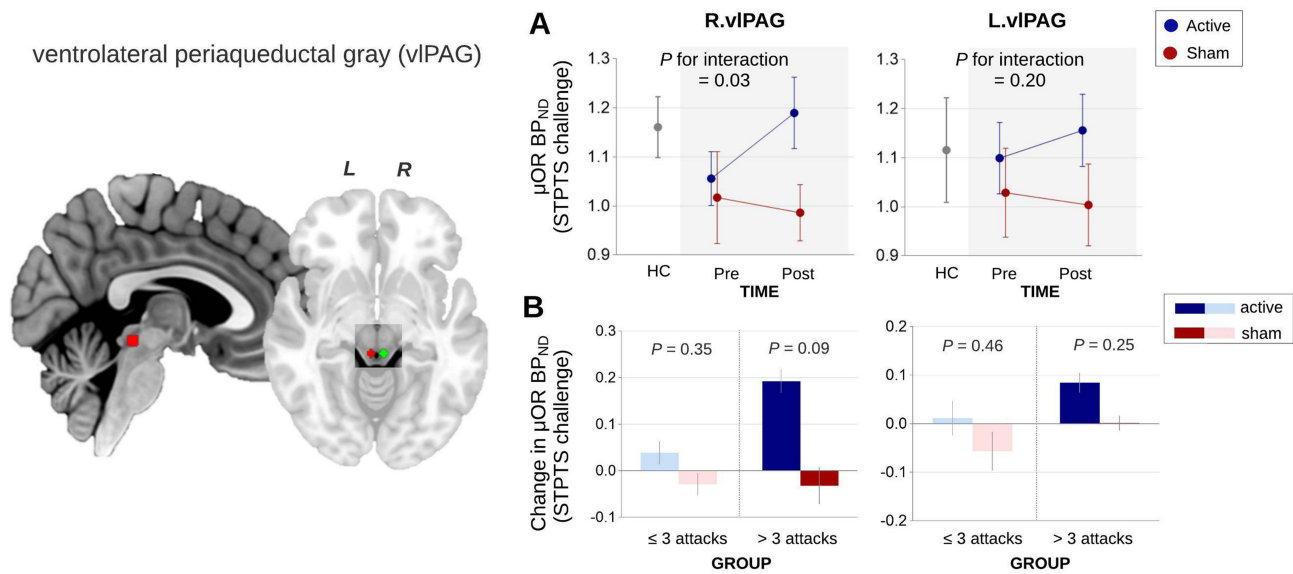


**Figure 3** Changes in  $\mu$ OR BP<sub>ND</sub> after HD-tDCS treatment. **(A)** Interaction effects between treatment groups and time (pre- vs post-treatment) on  $\mu$ OR BP<sub>ND</sub> during early resting-state (5–40 mins after radiotracer injection) in the right amygdala and left temporal pole and right hypothalamus and left parahippocampal gyrus during late Sustained Thermal Pain Threshold Stimulus (STPTS) challenge phase (45–90 mins after radiotracer injection). The inclusion of healthy participants was to provide a point of reference to show any deviations observed in the patient group as well as to visualize the directionality of treatment effect whether post-treatment effect was in the direction to healthy control group. The left is ipsilateral to the stimulation side. Images were displayed at  $P_{uncorrected} < 0.01$  for visualization purposes. **(B)**  $\mu$ OR BP<sub>ND</sub> changes from the above 4 regions were primarily observed among the higher-frequency migraineurs (> 3 attacks/month) treated with active HD-tDCS. The number of higher-frequency patients is 8 for active and 6 for sham; lower-frequency patients is 5 for active and 5 for sham, respectively. The between-group difference in each frequency range was tested by Mann–Whitney *U*-Test. After applying Bonferroni correction for multiple comparisons with an adjusted threshold of  $\alpha = 0.006$  (0.05/8), the parahippocampal gyrus remained only significant for the comparison between active vs sham in higher-frequency patients. The error bar indicates a standard error of the mean. \*\*\* $P < 0.005$ , \* $P < 0.05$ .

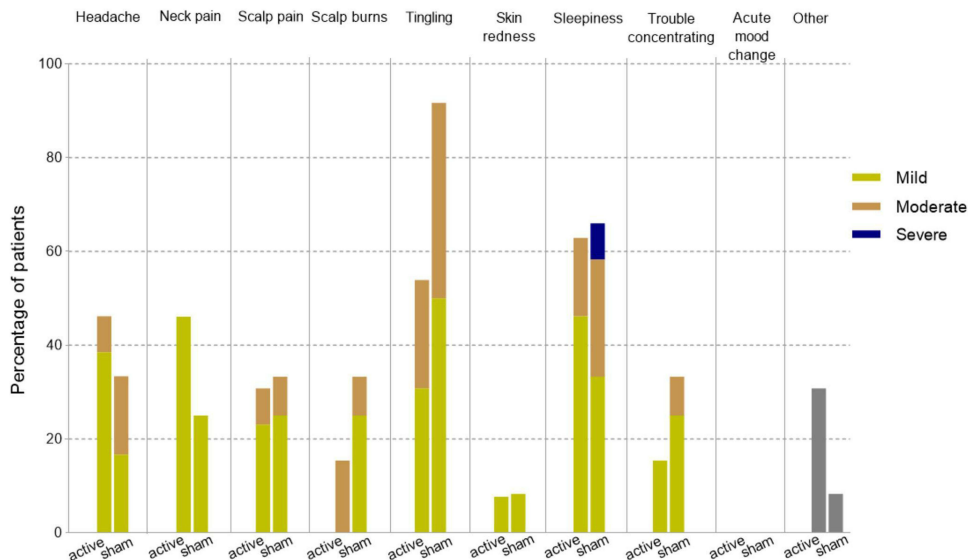
## Discussion

### Overall Observations from the Study Participants

The primary outcomes, specifically the number of moderate-to-severe (M/S) headache days and 50% responder rate, and the secondary outcomes (M/S headache intensity and use of rescue medication) were not significantly different between the active and sham groups following the 2-week session of HD-tDCS treatment. However, in subsequent analyses, we discovered that the efficacy of M1 HD-tDCS on M/S headache days over 1-month period after treatment varied between the two groups depending on the baseline frequency of migraine attacks. Specifically, the active HD-tDCS was more effective than the sham in both the primary and secondary outcomes in those with higher baseline frequency (>3 attacks/month). These favorable outcomes in high-frequency patients were potentially mediated by an increase in the concentration of  $\mu$ OR in the brain regions involved in pain modulation,<sup>44</sup> stress response,<sup>45</sup> and possible origin of a migraine attack.<sup>46</sup>



**Figure 4** Changes of  $\mu$ OR BP<sub>ND</sub> in the ventrolateral periaqueductal gray. **(A)** We observed increased  $\mu$ OR BP<sub>ND</sub> of the right vIPAG in the active group compared to the sham group. **(B)** Higher-frequency patients (> 3 attacks/month) treated with active HD-tDCS showed an increase in  $\mu$ OR availability compared with their sham counterparts. The number of higher-frequency patients is 8 for active and 6 for sham; lower-frequency patients is 5 for active and 5 for sham, respectively. The error bar indicates a standard error of the mean. **Abbreviation:** HC, healthy control.



**Figure 5** Patient-reported HD-tDCS side effects. Patients reported their symptoms after each HD-tDCS treatment. For each category, the number of patients who reported complaints at least once out of 10 sessions was divided by the total number of patients in each group. In the other symptom category, participants reported some symptoms, including dizziness, nausea, scalp tenderness, and mild itching. We confirmed that the side effects and discomfort related to HD-tDCS were fully resolved on that day. All patients showed their willingness to continue the remaining sessions.

## Efficacy of tDCS in Comparisons with Previous Data

Although still debatable as to tDCS efficacy in migraine,<sup>12,47</sup> combined efforts over the past years have demonstrated that enhancing the focality of the stimulation target<sup>15</sup> or increasing the number of treatment sessions<sup>9</sup> can overcome prior mixed results. We first applied M1 HD-tDCS with molecular neuroimaging arms to episodic migraine, and this neuromodulation technique was well tolerated in patients, with few adverse events aside from tingling and sleepiness. The impact of M1 HD-tDCS seemed to take effect shortly after treatment since we found reduced M/S headache days over 1-month after the end of treatment. However, the therapeutic benefit for active M1 HD-tDCS compared to sham was only seen in patients with higher

frequency migraine attacks (>3 attacks/month), while no significant differences were observed in patients with lower frequency ( $\leq 3$  attacks/month) before treatment. These trends were also noted in the secondary outcomes, including pain intensity and abortive medication, such that higher-frequency patients treated with active HD-tDCS were less likely to exhibit M/S pain or use of rescue medication over 1-month post-treatment compared with their sham counterparts. The present result partly aligns with previous investigations showing excitatory M1 stimulation reduced headache intensity and attack frequency.<sup>10</sup> However, it is worth noting that the effectiveness of excitatory conventional M1 tDCS in reducing headache intensity and attack frequency remains still inconsistent. Hence, further research is still needed to better understand the precise mechanisms through which tDCS exerts its effects on episodic migraines.

## Evidence of Contralateral Pain Management Induced by HD-tDCS

On a further note, maximum pain intensity (pain edge) measured by PainTrek over a 1-month follow-up according to the baseline attack frequency was only observed on the contralateral side of the stimulation but not on the ipsilateral one, suggesting that focused M1 HD-tDCS modulates migraine pain in a highly controlled way as demonstrated in our previous M1 HD-tDCS study involving patients with temporomandibular disorder.<sup>17,32</sup> It is well known that the primary sensorimotor cortices (S1/M1) mainly control pain and movement in/from the opposite body side. Thus, this study extends the idea that bilateral excitatory-focused M1 stimulation could be more effective in treating chronic pain disorders, given that many chronic pain patients suffer from bilateral pain symptoms.<sup>8</sup>

## Clinical Implication of $\mu$ OR Function in Migraine

Migraine attacks typically appear in adolescents or young adults (eg, 17.5 yr on average in the current study).<sup>3</sup> Thus, migraineurs' brains likely have a high  $\mu$ -opioid peptide drive to manage long-term attacks, pain, and stress, which might translate into  $\mu$ OR downregulation or internalization.<sup>19</sup> This phenomenon can significantly impact the effectiveness of exogenous opioid in pain relief during severe migraine attacks. As opioids primarily bind to the  $\mu$ ORs, and any alterations or decreased availability of these receptors may result in limited efficacy of opioids in managing migraine pain. Here, our repetitive active M1 HD-tDCS enhances baseline  $\mu$ OR concentration, compared to the sham. The observed changes occurred in the limbic and paralimbic regions; specifically, we found sustained or increased  $\mu$ OR BP<sub>ND</sub> in the right amygdala and left temporal pole during the resting state in the active group, whereas the sham group showed only decreased patterns. Likewise, we found an increased  $\mu$ OR BP<sub>ND</sub> in the parahippocampal gyrus and hypothalamus during the thermal pain threshold challenge. Notably, aligned with the clinical findings, higher-frequency patients treated with active M1 HD-tDCS displayed a marked increase in  $\mu$ OR BP<sub>ND</sub>. This was particularly evident when compared to the sham counterpart, especially in areas such as the right amygdala and left parahippocampal gyrus, which is responsible for emotional pain processing<sup>48</sup> and stress response to aversive stimuli.<sup>49</sup>

These results align with the studies supporting the involvement of limbic systems in pain relief<sup>50</sup> and even a migraine attack.<sup>51,52</sup> Indeed, recent research showed that the hypothalamus plays a pivotal role in generating migraine attacks supposedly through inter-relationship with limbic regions.<sup>51</sup>

## Changes of $\mu$ OR Availability as a Potential Mechanism of Brain Stimulation

It is also known that the  $\mu$ -opioid system plays an essential role in the regulation of hedonic homeostasis, such as promoting stress responses.<sup>53</sup> Moreover,  $\mu$ OR acts as an interface between emotional and physical stress regulation.<sup>54</sup> Thus, it is reasonable to assume that an increased  $\mu$ OR concentration might enable migraineurs to cope better with internal/external stressors, which might protect migraineurs from having another attack and related pain, given that stress is one of the most probable trigger factors in migraine symptoms.<sup>55</sup> The exploratory mediation analysis supported the hypothesis mentioned above that the  $\mu$ OR BP<sub>ND</sub> changes in the parahippocampal region potentially facilitated the therapeutic efficacy of the M1 HD-tDCS on M/S headache days. The possibility that tDCS could potentially elevate  $\mu$ OR availability within the regions crucial for pain perception and modulation gives it a distinct advantage over prescribed opioids. Traditional opioid medications for pain relief are notorious for their significant risk of adverse side effects, including tolerance, addiction, and deleterious physical and psychological consequences. In contrast, tDCS appears to enhance our natural pain-modulating systems, potentially offering a therapeutic benefit while avoiding the risks linked

with opioid usage. However, given the small sample size in our study, we caution against drawing definitive conclusions from our exploratory mediation analysis. Instead, these findings should be viewed as initial observations, requiring further investigation in larger-scale studies.

Previous preclinical and clinical studies have demonstrated that M1 stimulation facilitates descending pain modulatory pathways in concert with the opioid receptor system.<sup>56</sup> In an invasive M1 stimulation combined with longitudinal PET with non-selective opioid receptor agonist [<sup>11</sup>C]diprenorphine applied to neuropathic pain, there was significant pain reduction associated with an endogenous opioid release in the anterior middle cingulate cortex and PAG.<sup>57</sup> In our previous single M1 tDCS study with a specific  $\mu$ OR radiotracer [<sup>11</sup>C]CFN PET, we observed stimulation-induced  $\mu$ -opioid peptide release in pain modulatory regions in chronic pain and healthy participants.<sup>58,59</sup> Conversely, opioid antagonist naloxone, exhibiting the greatest affinity to  $\mu$ OR, abolished the analgesic effect of M1 stimulation.<sup>60</sup> These findings collectively suggest that the therapeutic effects of tDCS might rely on the  $\mu$ OR function.

Notably, M1 HD-tDCS treatments conducted over a 2-week period increased  $\mu$ OR BP<sub>ND</sub> in the right vIPAG, a region involved in descending pain modulation, during STPTS challenge, compared to sham, with a more marked increase in the higher-frequency migraineurs. Thus, these findings align with previous reports that suggest an impact of M1 stimulation on the opioid system, albeit with a slight deviation. Specifically, we observed an increase in  $\mu$ OR availability, also suggesting less release of  $\mu$ OR-activating peptides. We speculate that this increase might reflect to enhanced efficiency of the  $\mu$ OR system, which potentially facilitates better pain modulation or stress responses.

## Limitations

Regarding other factors potentially contributing to the treatment effect, it can be argued that non-therapeutic factors, such as regression to the mean, could be associated with clinical improvement.<sup>61</sup> Also, treatment expectancy is a known factor contributing to pain relief, possibly by activating endogenous opioid circuits; however, using a sham control group reduces the likelihood that placebo-related mechanisms underlie the observed effects of M1 HD-tDCS. While a 10-10 method is a widely adopted method for tDCS target placement especially for the motor cortex, we acknowledge the potential limitation of this method. To address this limitation, future studies could consider incorporating additional techniques, such as neuroimaging or neuro-navigation, to improve the precision and reliability of electrode placement.

Additionally, most of our subjects were female; therefore, the current results may not be entirely generalizable to male migraineurs. Moreover, although we used M/S headache days as a primary outcome and clinical correlation, patients were monitored in our frequent in-person lab sessions, not at home, in the first 1-week and 1- and 2-month follow-ups using the PainTrek. At this time, we acknowledge that our findings are primarily applicable to the specific population studied here; larger studies are warranted to extend these findings to a broader population of migraine with potentially varying characteristics and more treatment days to extend the current findings as well as maximize the HD-tDCS efficacy on migraine symptoms.

## Conclusion

Altogether, our results highlighted that M1 HD-tDCS could be beneficial in reducing M/S headache days, pain intensity, and the use of rescue medication in high-frequency patients with episodic migraine along with increased  $\mu$ OR availability in the brain in a manner proportional to the improvement in migraine severity, representing a potential therapeutic mechanism of M1 HD-tDCS actions on clinical outcomes. Lastly, our results highlight that the indirect modulation of  $\mu$ ORs by M1 HD-tDCS – as opposed to direct by externally administered opioid medications – may be an effective non-pharmacological treatment option to explore in high-frequent migraine patients. However, a study with a larger sample size is needed to confirm the robustness of our findings.

## Data Sharing Statement

The data supporting this study's findings are available from the corresponding author (adasilva@umich.edu) upon reasonable request.

## Ethics Approval and Informed Consent

The study protocol was approved by the University of Michigan Institutional Review Boards (HUM00107286) and registered at ClinicalTrials.gov (NCT02964741). We conducted the study under the Declaration of Helsinki. All study participants provided written informed consent.

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## Disclosure

The contents described within this study, PainTrek and H.O.P.E. M1 HD-tDCS, have been developed at the University of Michigan and disclosed to the University of Michigan Office of Technology Transfer. All intellectual property rights, including but not limited to patents/patent applications, trademarks, and copyright of software, algorithms, reports, displays, and visualizations, are owned by the Regents of the University of Michigan. Dr. DaSilva is the main inventor of the mobile technology now called PainTrek (previously GeoPain, MoxyTech), which is owned by the University of Michigan. Other authors declare no conflict of interest in this work. Present address for Manyoel Lim: Food Convergence Research Division, Korea Food Research Institute, Republic of Korea.

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