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Reductions in GABA following a tDCS-language intervention for primary progressive aphasia

Ashley D. Harris [a,b,*], Zeyi Wang [c], Bronte Ficek [d], Kim Webster [d,e], Richard AE. Edden [f,g], Kyrana Tsapkini [d,h]

[a] Department of Radiology, University of Calgary, Calgary, Alberta, Canada
[b] Hotchkiss Brain Institute and Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Alberta, Canada
[c] Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[d] Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA
[e] Department of Otolaryngology Head & Neck Surgery, Johns Hopkins School of Medicine, Baltimore, MD, USA
[f] Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins School of Medicine, Baltimore, MD, USA
[g] F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA
[h] Department of Cognitive Science, Johns Hopkins University, Baltimore, MD, USA

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ABSTRACT

Transcranial direct current stimulation (tDCS) has shown efficacy in augmenting the effects of language therapy in primary progressive aphasia (PPA). The mechanism of action of tDCS is not understood, but preliminary work in healthy adults suggests it modulates γ-aminobutyric acid (GABA) levels to create an environment optimal for learning. It is unknown if this proposed mechanism translates to aging or neurodegenerative conditions. This study tested the hypothesis that tDCS reduces GABA at the stimulated tissue in PPA. We applied GABA-edited magnetic resonance spectroscopy to quantify GABA levels before and after a sham-controlled tDCS intervention with language therapy in PPA. All participants showed improvements but those receiving active tDCS showed significantly greater language improvements compared to sham both immediately after the intervention and at 2-month follow-up. GABA levels in the targeted tissue decreased from baseline after the intervention and remained decreased 2 months after the intervention. This work supports the hypothesis that tDCS modulates GABAergic inhibition to augment learning and is clinically useful for PPA combined with language therapy.

1. Introduction

Primary progressive aphasia (PPA) is a neurodegenerative condition, which primarily affects language (Gorno-Tempini, et al., 2011). Symptoms include difficulties with word finding, word usage, comprehension, and sentence construction. The primary treatment tool is language therapy, which includes oral and written word generation cued by pictures, word association, writing words, and spelling practice, which has shown some therapeutic effects, although improvements are not sustained (Tippett et al., 2015).

Transcranial direct current stimulation (tDCS) passes a weak current between two electrodes and appears to modulate cortical excitability, increasing excitability at the anode and decreasing excitability at the cathode (Krause et al., 2013; Lefaucheur, 2016). We have previously shown in a small cohort of patients that 15 sessions of anodal tDCS over the left inferior frontal gyrus (IFG) improved spelling in PPA (Tsapkini et al., 2014), whereas Cotelli's group showed that 10 sessions of anodal tDCS over the dorsolateral prefrontal cortex improved oral naming (Cotelli et al., 2014). Subsequent studies using similar designs have confirmed the augmentative role of anodal tDCS over the left IFG (Gervits et al., 2016; McConathey et al., 2017) and over the left inferior parietal gyrus (Roncero et al., 2017). These studies indicate that tDCS may (1) provide greater therapeutic gains than language therapy alone (sham condition) and (2) sustain therapeutic benefits longer than language therapy alone. Despite the growing interest in using tDCS clinically, the mechanism by which tDCS effects are induced has not been adequately explored.

Mechanistic studies in young, healthy, control adults suggest that tDCS increases glutamate and/or decreases γ-aminobutyric acid (GABA) at the anode (Clark et al., 2011; Kim et al., 2014; Stagg, 2014; Stagg et al., 2009; Stagg and Nitsche, 2011). These changes in

* Corresponding author at: University of Calgary, Alberta Children's Hospital, Office B4-512, 28 Oki Drive NW, Calgary, AB T3B 6A8, Canada. Tel.: +1 4039552771; fax: +1 4039552440.
E-mail address: ashley.harris2@ucalgary.ca (A.D. Harris).

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glutamate and GABA and the associated changes in inhibition and excitation are proposed to provide an optimal neural environment for plasticity and learning (Krause et al., 2013). Reducing inhibitory tone would result in lower inhibition to synaptic signal transfer and therefore facilitate transmission of signal to the subsequent neuron(s). An increase in excitatory tone will have a similar effect. This conditional neuronal firing would result in neural learning for the behavioral task in Hebbian terms. Previous studies have provided evidence of a reduction in GABA after one session of tDCS (Stagg et al., 2009, 2011), whereas others have shown a glutamatergic increase (glutamate or glutamate measured with glutamine [Glx]) (Clark et al., 2011; Hunter et al., 2015; Stagg et al., 2009). These studies have served as foundational evidence of a GABA- and glutamate-mediated tDCS mechanism in healthy adult controls. For tDCS effects to have a significant clinical value, interventions likely need to be performed for multiple days, weeks, or even months. (Reis et al., 2009) showed that 5 consecutive sessions of tDCS appear to result in longer and more robust behavioral effects than a single session. It is important to study repeated tDCS sessions because behavioral effects of one tDCS application that last for only a few hours after stimulation have little clinical utility. Furthermore, understanding tDCS mechanisms in clinical populations is necessary to target and optimize therapy. To date, metabolite changes after tDCS applications for language therapy in clinical populations have not been investigated; thus, there is a lack of direction for effectively applying and improving tDCS therapy in language rehabilitation in any clinical population.

The present study addresses this paucity of mechanistic evidence. Specifically, under the hypothesis that GABA plays a mechanistic role in learning and is modulated with tDCS, we examined change in GABA after a tDCS intervention targeting the left frontal operculum (IFG) in combination with language therapy in a sham-controlled study. Secondary objectives included examining other metabolites (glutamate reported in combination with glutamine as Glx, creatine Cr, and N-acetyl aspartate [NAA]) and confirming the local specificity of this intervention.

2. Materials and methods

2.1. Patient recruitment

Patients diagnosed with PPA through consensus criteria (Gorno-Tempini, et al., 2011) (evaluated with neurological examination, cognitive and language testing, and imaging results) were recruited to participate in this study. Expert clinicians who based their diagnosis on the established criteria at specialized hospital or university centers confirmed patients' diagnoses. In all patients, the most robust symptom remained the progressive deterioration of language function(s) despite other preserved cognitive abilities. Additional inclusion criteria for study participation were at least a 12th grade education, right-handedness, and English as first language. Patients were excluded if they were not premorbidly proficient spellers, were in advanced stages of PPA or other dementia, were over 90 years of age, or had contraindications to magnetic resonance (MR) scanning such as severe claustrophobia. Participants with conditions such as previous stroke or any psychiatric or developmental disorder were also excluded. Patients were randomized to tDCS or sham therapy, to gain approximately equal groups matched for several demographic parameters (see Table 1 for demographic information). Twenty-two patients diagnosed with PPA were recruited. Of these patients, 6 were diagnosed with semantic variant PPA, 10 with nonfluent agrammatic variant PPA, and 6 with logopenic variant PPA. In this article, we did not stratify by variants that may have different pathology because there is no literature showing any effect of brain pathology on GABA or other metabolites. The FrontoTemporal Dementia Clinical Dementia Rate scale was used to assess overall disease severity, including language functions and other functions (memory, attention, independence) (Knopman et al., 2008).

2.2. Language therapy

We used an oral and written naming task with an adaptation of a spell-study-spell procedure described in previous studies (Beeson and Egnor, 2006; Rapp and Glucroft, 2009; Tsapkini et al., 2018). Briefly, each trial consisted of showing the patient a picture (presented on a computer). The patient named the object, first orally and then in writing. If the patient could not name the object, she/he was asked for three features of the object. If the patient made an error, she/he was told it was incorrect and given the chance to correctly name the object. If the patient orally named the object but wrote its name incorrectly, the clinician taught the correct spelling using a spell-study-spell procedure. Each letter was rehearsed individually and learning was reinforced with copying, as this repetition has been shown to have synergy with both oral and written naming (Beeson and Egnor, 2006). Scoring was quantified as a percentage of correct letter-to-sound correspondences in all words based on the accuracy of each letter, where each correct letter was given 1 point (0.5 points for correct identity and 0.5 points for correct position), and points (and half-points) were subtracted for letters deleted, added, substituted, or moved (Goodman and Caramazza, 1985). Two trained scorers followed this rule-based system independently and inter-rater reliability was 95%.

Table 1
Patient demographics

Characteristic	Combined (n = 22)	tDCS (n = 11)	Sham (n = 11)	*p*-value
Sex (F = female, M = male)	11 F, 11 M	6 F, 5 M	5 F, 6 M	1.000
PPA variant (L = logopenic, N = nonfluent, S = semantic)	6 L, 10 N, 6 S	4 L, 4 N, 3 S	2 L, 6 N, 3 S	0.857
Age	66.9 (7.5)	64.1 (8.4)	69.6 (5.7)	0.090
Years after onset	5.0 (3.0)	5.6 (3.4)	4.5 (2.5)	0.392
Language severity (FTD-CDR)	1.9 (0.8)	1.9 (0.8)	1.9 (0.8)	1.000
Total with language severity 0.5	2	1	1	—
Total with language severity 1	4	2	2	—
Total with language severity 2	12	6	6	—
Total with language severity 3	4	2	2	—
Total severity (FTD-CDR)	7.4 (4.8)	6.4 (3.6)	8.3 (5.7)	0.386
Marital status: single	4	3	1	0.587

For age, years after onset, and severity, values shown are mean (standard deviation); *p*-values are from two-sample permutation tests for continuous outcomes and Fisher's exact test for categorical outcomes. Language severity is based on the language subset from the FTD-CDR scale. Total severity refers to all language and behavior assessments as per (Knopman et al., 2008). The 2 groups (tDCS vs. sham) were matched in all measures.
Key: FTD-CDR, frontotemporal dementia Clinical Dementia Rate; tDCS, transcranial direct current stimulation; PPA, primary progressive aphasia.

2.3. Transcranial direct current stimulation

An investigational double-blind tDCS system was used (Model 1500, Soterix Transcranial Direct Current Stimulator Clinical Trials). The anode was placed over the left IFG, which corresponds to the F7 electrode in the electroencephalography, 10–20 system (Homan et al., 1987). In addition, we co-registered the IFG electrode placement to the pretreatment MRI scans using a fiducial marker. The reference electrode, the cathode, was placed on each participant's right cheek, a montage that has previously successfully delivered electrical current in the left motor and premotor areas (Buch et al., 2017; Homan et al., 1987; Hummel et al., 2005; Reis et al., 2009). The current modeling of this particular montage has shown to be delivering the current mostly in the intended area and has produced beneficial behavioral effects in our previous studies in PPA (Tsapkini et al., 2014, 2018). Saline-soaked electrodes (2 inches by 2 inches) delivered 2 mA of current (ramped up from 0 mA over the course of 30 seconds), for 20 minutes. A previously validated sham condition consisting of the 30 seconds of stimulation (the ramp phase) was applied to mimic skin sensations associated with active tDCS (Gandiga et al., 2006). Language therapy started at the beginning of stimulation and continued for a regular speech-language therapy session of 45–50 minutes, i.e., 25–30 minutes after the end of stimulation for both anodal tDCS and sham conditions for all therapeutic sessions. The participant, the therapist, and the technician who performed the language and cognitive evaluations were blind to the stimulation condition.

2.4. Magnetic resonance spectroscopy

All imaging and spectroscopy were performed at 3T (Philips Achieva). Before scanning, a fiducial marker was placed on the left temple to assist with landmarking. For voxel localization and subsequent tissue segmentation, a whole-brain MP-RAGE sequence was acquired (repetition time [TR]/echo time [TE] = 8/3.75 ms, 1 mm^3 isotropic voxels). Magnetic resonance spectroscopy (MRS) voxels ($3 \times 3 \times 3$ cm^3) were centered on the left IFG using the fiducial marker and anatomical landmarks of the lateral ventricles and the insula (see Fig. 1A). As a control region, the right sensorimotor cortex (SMC) was used, centering the voxel on the hand knob in the precentral gyrus. Voxels were placed to avoid the edge of the brain and the ventricles.

Owing to more abundant, overlapping peaks, a specialized MRS sequence is required to measure GABA, the most common being *J*-difference editing, such as Mescher-Garwood editing–point-resolved single voxel spectroscopy (MEGA-PRESS) (Harris et al., 2017). Data were acquired using a GABA-edited MEGA-PRESS acquisition (TR/TE = 2 s/68 ms, 14 ms editing pulses at 1.9 ppm and 7.46 ppm alternating every 2 averages across, 320 averages) to measure GABA and a standard PRESS acquisition (TR/TE = 2 s/32 ms, 48 averages) to measure glutamate (reported in combination with glutamine as Glx), NAA, choline (Cho), and creatine (Cr). For both acquisitions, 8 unsuppressed water scans were acquired for quantification.

GABA data were analyzed using the Gannet pipeline (Edden et al., 2014; Harris et al., 2015), including tissue correction to correct for voxel tissue content (which may be impacted by atrophy) as well as differences in GABA between white matter and gray matter, assuming that the level of GABA in gray matter is twice that in white matter (Harris et al., 2015). Short-echo PRESS data to quantify NAA, Cr, Cho, and Glx were analyzed using LCModel (Provencher, 1993) and subsequently correction accounting for cerebrospinal fluid-corrected using the same voxel fractions determined from the Gannet pipeline.

2.5. Statistical analysis

Changes in language scores were tested for time intervals "baseline to post-intervention" and "baseline to 2-month follow-up" with paired t-tests in the tDCS and sham groups. Similarly, metabolite changes for time intervals "baseline to post-intervention" and "baseline to 2-month follow-up" in the IFG and the SMC were tested with paired t-tests within the tDCS and sham groups separately. Dropouts on the 2-month follow-up were assumed to be completely at random. Welch two-sample t-tests with Satterthwaite degrees of freedom (DF) were applied for comparisons between the treatment groups because of the inequality of group variances or number of available observations (Ruxton, 2006).

In addition, data were analyzed with nonparametric permutation testing because of the small sample size. Changes in the metabolite data from IFG and the SMC were tested with paired (one-sample) permutation tests within the tDCS and sham groups separately. The changes in language scores for "baseline to post-intervention" and "baseline to 2-month follow-up" were also tested using the same approach. Two-sample permutation tests were applied for comparisons between the treatment groups. Dropouts on the 2-month follow-up were assumed to be completely at random. The null distributions of all permutation tests were approximated via Monte Carlo resampling (Good, 2000); *p*-values were calculated with two-sided tests. For one-sample permutation tests, at each iteration step, the signs of changes were resigned by 1 or −1 with equal probabilities and the test statistic was computed as the mean of the resigned changes. For two-sample permutation tests, at each iteration step, the treatment group labels were randomly permuted and the test statistic was computed as the difference between the means of the 2 groups after label permutation. The total iteration number of all permutation tests was 10,000.

3. Results

3.1. Patient demographics and tDCS tolerability

A summary of patient demographics is shown in Table 1. All patients completed at least 10 language therapy and tDCS sessions, and most completed the full 15-session protocol (mean 13.5 sessions, standard deviation 1.7). Both the tDCS and sham groups were almost identical in the number of sessions; the variance was due to unavoidable events in this aging population (flu, colds, other medical appointments, etc.). No serious adverse events occurred, no patients reported any side effects past the stimulation period, and no one discontinued the study because of pain complaints. Patients tolerated tDCS well in general, except for tingling or itching sensations reported at the beginning of the session. This was reported in both tDCS and sham conditions, and patients could not differentiate between conditions. The mean pain ratings for tDCS was 2.51 (standard deviation 3.50, range 0–10), and for sham, it was 2.04 (standard deviation 2.08, range 0–10). All patients completed the full assessment at the end of the therapy intervention (time point "post"). Four patients did not have any language assessments and were not scanned at the 2-month follow-up, and 1 additional patient was not scanned at the 2-month follow-up. Table 2 summarizes the demographics of the patients in tDCS and sham groups at the 2-month follow-up time point, illustrating no apparent bias from the dropouts.

3.2. Language score changes

After treatment, all patients showed improvement with therapy on an individual and group level: tDCS group mean change in

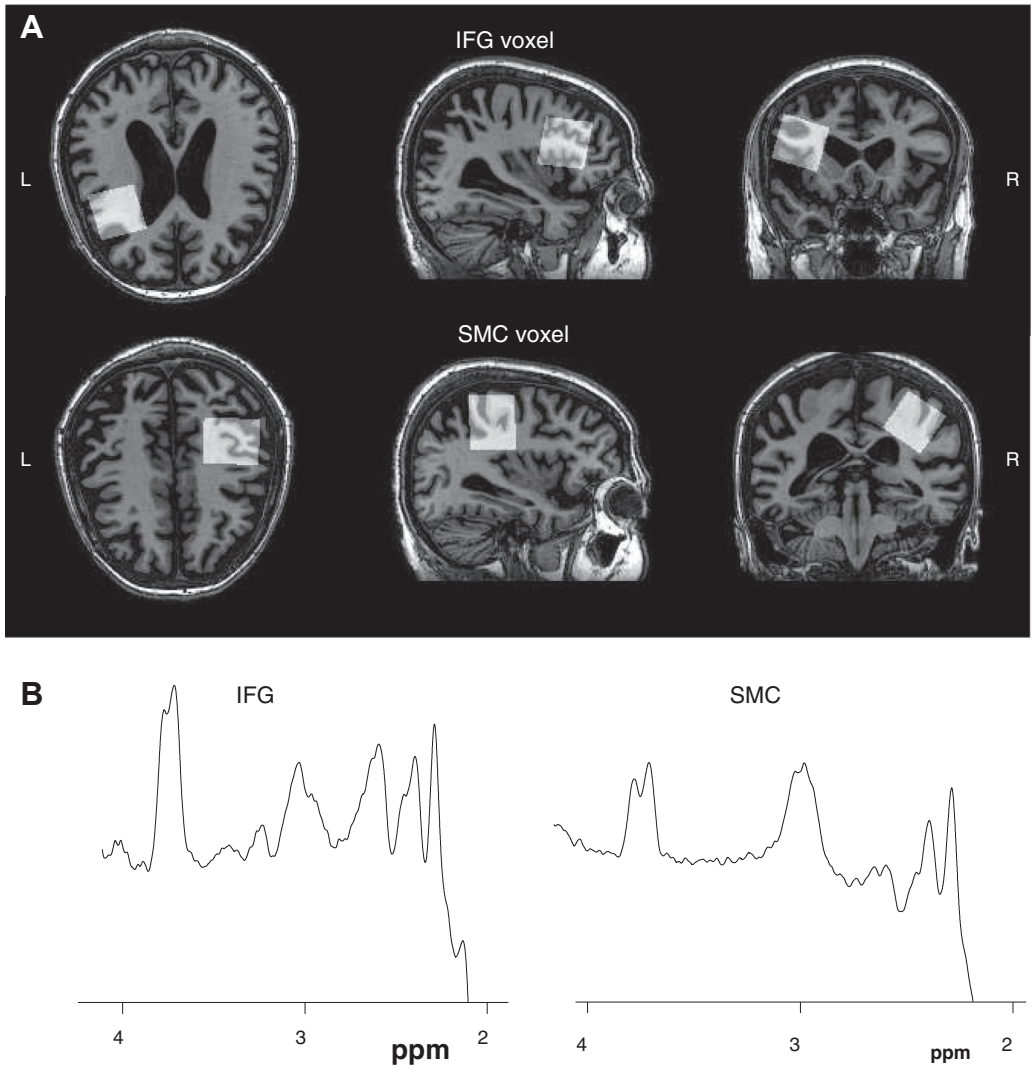

Fig. 1. Exemplar voxel placement (A) and GABA spectra (B) for the IFG voxel and the SMC voxel. Abbreviations: GABA, γ-aminobutyric acid; IFG, inferior frontal gyrus; SMC, sensorimotor cortex.

language scores = 42.62, DF = 10, T-statistic = 5.13, $p < 0.001$ and sham group mean change in language scores = 22.76, DF = 10, T-statistic = 5.02, $p < 0.001$ (see Table 3 and Fig. 2). Patients who received anodal tDCS showed greater improvements than the sham group (mean difference in scores: 19.86, DF = 15.47, T-statistic = 2.10, $p = 0.053$). The permutation testing showed consistent results; significant improvements were seen in both the tDCS ($p < 0.0001$) and sham groups ($p = 0.0021$), and the tDCS group showed significantly greater language improvements than the sham group ($p = 0.048$)

At the 2-month follow-up, both groups had significantly improved language scores compared with baseline. The tDCS group showed an increase in language scores of 40.94, DF = 6, T-statistics = 4.34, $p = 0.005$, and the sham group showed an increase in language scores of 13.29, DF = 10, T-statistic = 4.57, $p = 0.001$. The tDCS group showed significantly higher language scores than the sham group at the 2-month follow-up (difference in improved language scores = 27.65, DF = 7.15, T-statistic = 4.57, $p = 0.001$). Similarly, the nonparametric permutation testing showed significant language improvements at 2-month follow-up for tDCS ($p = 0.014$) and sham groups ($p = 0.0019$) and that tDCS showed significantly greater language improvements than sham ($p = 0.0019$).

3.3. Metabolite changes

Exemplar spectra are shown in Fig. 1B. GABA in the IFG significantly decreased from baseline in the tDCS group directly after the tDCS intervention (change = −0.50 i.u., DF = 10, T-statistic = −2.70, p-value = 0.022, Table 4) and decreased at a trend level at the 2-month follow-up time point (change = −0.33 i.u., DF = 6, T-statistic = −2.37, p-value = 0.056, see Fig. 3). GABA did not change in the IFG of the sham group after the intervention (estimated change = −0.07, DF = 10, T-statistic = −0.47, p-value = 0.65) or at 2-month follow-up (estimated change = −0.09, DF = 9, T-statistic = 0.65, p-value = 0.61). No changes were seen in the SMC voxel of either group at either time point (Fig. 3), and no changes were seen in any other metabolite (Glx, NAA, Cr, or Cho). For validation, our permutation testing showed generally consistent results; from baseline to post-intervention, there was a significant decrease in GABA in the tDCS group ($p = 0.021$) but not in the sham group. However, in the permutation testing, GABA was significantly decreased at 2-month follow-up ($p = 0.047$) compared with the trend-level decrease detected with the parametric statistics. No changes in GABA were detected in the SMC voxel with the permutation testing.

Table 2
Dropout report for the 2-mo follow-up visit

Characteristic	Combined (n = 22)	Complete cases (n = 17)	GABA dropouts (n = 5)	Language dropouts (n = 4)
Treatment group	11 tDCS, 11 sham	7 tDCS, 10 sham	4 tDCS, 1 sham	4 tDCS, 0 sham
Sex (F = female, M = male)	11 F, 11 M	7 F, 10 M	4 F, 1 M	3 F, 1 M
PPA variant (L = logopenic, N = nonfluent, S = semantic)	6 L, 10 N, 6 S	4 L, 8 N, 5 S	2 L, 2 N, 1 S	2 L, 2 N, 0 S
Age	66.9 (7.5)	66.6 (6.7)	67.6 (11.0)	65.8 (11.8)
Years after onset	5.0 (3.0)	5.0 (3.0)	5.0 (3.2)	4.9 (3.6)
Language severity (FTD-CDR)	1.9 (0.8)	1.8 (0.8)	2.0 (0.7)	2.0 (0.8)
Total with language severity 0.5	2	2	0	0
Total with language severity 1	4	3	1	1
Total with language severity 2	12	9	3	2
Total with language severity 3	4	3	1	1
Total severity (FTD-CDR)	7.4 (4.8)	7.3 (5.3)	7.6 (2.8)	7.6 (3.2)
Marital status: single	4	2	2	2

Dropouts *only* occurred at the 2-mo follow-up. Four patients did not attend the appointment, and 1 patient had the language assessment but was not scanned. For age, years after onset, severity, and total treatment sessions, values shown are mean (standard deviation). Language severity is based on the language subset from the FTD-CDR scale. As mentioned previously, total severity refers to the sum of boxes, including language and behavior as per (Knopman et al., 2008).
Key: FTD-CDR, frontotemporal dementia Clinical Dementia Rate; tDCS, transcranial direct current stimulation; PPA, primary progressive aphasia; GABA, γ-aminobutyric acid.

4. Discussion

The IFG has previously been shown to be a hub for oral and written naming and spelling as it is an area recruited in numerous language studies in recent reviews and meta-analyses (Price, 2010; Purcell et al., 2011; Roux et al., 2014). Furthermore, the left IFG has been shown to be involved in semantic selection of the appropriate word (Thompson-Schill et al., 1999) and active retrieval (Owen et al., 1996), thus is crucial for earlier and later stages of word production. Targeting the IFG with anodal tDCS has been shown to be an effective way to improve the results of a language intervention for PPA (Cotelli et al., 2014; Tsapkini et al., 2014).

The present study tested the hypothesis that a reduction in the inhibitory neurometabolite GABA is modulated by anodal tDCS in PPA. Patients with PPA who participated in a language intervention (written naming/spelling) were randomized to anodal tDCS or sham-tDCS targeting the left IFG for ~15 sessions. GABA-edited MRS and conventional MRS were performed in 2 locations in the brain—the left IFG as the tDCS target and the right SMC as a control location to detect regional changes in GABA and glutamate after a language and tDCS intervention. After the intervention, all patients (both anodal and sham-tDCS groups) showed improved language scores. However, we show that repeated, consecutive tDCS applications resulted in larger improvements in language outcomes than sham and there was a decrease in GABA in the tissue targeted by the tDCS anode. Furthermore, the additional behavioral improvements and GABA reduction were sustained for 2 months and were significantly greater than sham effects. To our knowledge, this is the first time GABA reductions after tDCS have been demonstrated long (weeks) after the intervention. None of the other metabolites examined (Glx, NAA, Cr, and Cho) showed a significant change after stimulation, indicating a GABA-specific mechanism. Moreover, GABA concentrations did not change in the sham condition or at the control area, the right SMC. These results indicate the localized effects of tDCS on the metabolite GABA. To our knowledge, this is also the first study examining GABA reduction as a mechanism of tDCS in a neurodegenerative condition with consecutive tDCS sessions.

Table 3
Reports of means, SDs, and number of observations for language scores on different time points within different treatment groups

Time points	Treatment group	Mean (SD) of language scores	Number of observations
Base	tDCS	51.16 (28.48)	11
Post	tDCS	93.78 (9.87)	11
2-mo follow-up	tDCS	83.97 (15.87)	7
Base	Sham	50.18 (27.52)	11
Post	Sham	72.94 (26.03)	11
2-mo follow-up	Sham	63.47 (27.08)	11

Key: tDCS, transcranial direct current stimulation; SD, standard deviation.

These behavioral results are consistent with previous behavioral studies in neurodegenerative diseases, including ours (Cotelli et al., 2014; Tippett et al., 2015; Tsapkini et al., 2014, 2018); patients who received anodal tDCS showed significantly greater improvements than those who received sham treatments. Little is known about the brain mechanisms of tDCS. Previous studies, mainly from Stagg's group, have shown GABA decreases at the stimulation site after 1 application of anodal tDCS over the primary motor cortex (M1) (Bachtiar et al., 2015; Stagg et al., 2014). There is evidence that resting-state connectivity is also altered after tDCS (Bachtiar et al., 2015; Ficek et al., 2018), although it is not clear whether alterations in functional connectivity cause improvements in task performance or if task improvements result in changes in functional connectivity.

Several studies have shown that GABA decreases during protocols designed to induce cortical plasticity and there is increasing evidence that it is necessary for GABA to decrease for long-term potentiation (LTP)–induced plasticity to occur (Stagg et al., 2011). Building on this premise, modulating GABA levels with noninvasive neurostimulation during training may provide a mechanism to improve learning and/or therapeutic effects of behavioral interventions. tDCS has been shown to induce long-term language and motor improvements after several consecutive applications, possibly through the mechanism of LTP (Fritsch et al., 2010; Monte-Silva et al., 2013). The present study additionally showed that the decrease in GABA immediately after treatment was sustained for 2 months. This may indicate that changes in GABA persist longer than anticipated through these LTP-based mechanisms, or it may indicate that in this population, learning persists beyond the intervention. As patients are motivated to maintain their language improvements after this intense language therapy intervention, it is may be that these patients actively engage in activities to maintain their language skills. This may result in the brain maintaining a more plastic state with lower GABA for ongoing learning. These activities may be overt, such as practicing and repeating learning exercises from the language intervention or through less formalized events such as actively engaging in challenging conversations to have the same effect of maintaining language skills.

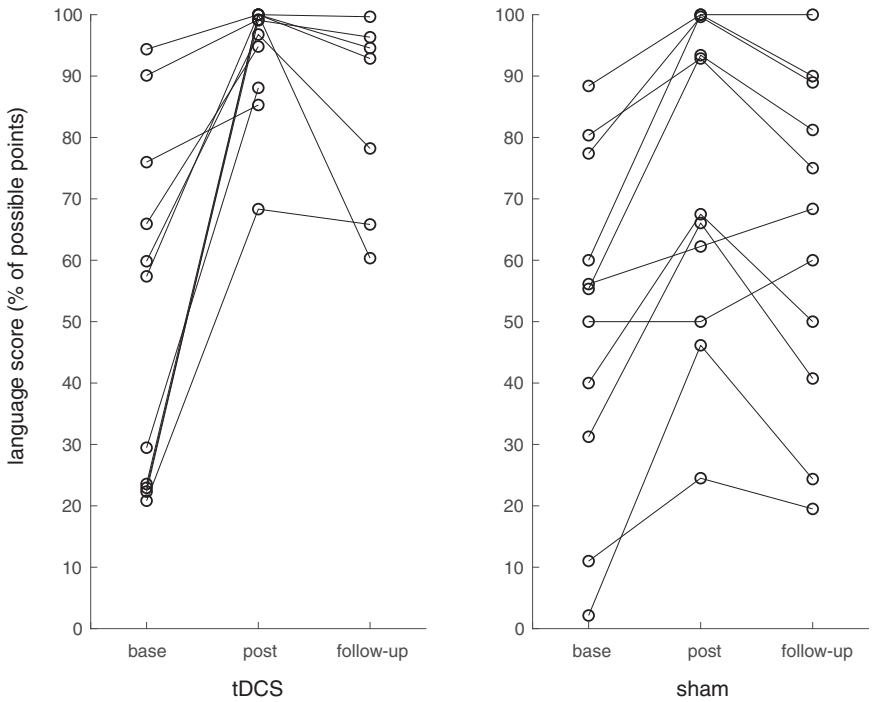

Fig. 2. Language scores at pre-intervention (base), after the tDCS protocol (post), and at 2-month follow-up (follow-up). Scores are presented as a percentage of the possible points for each patient. Abbreviation: tDCS, transcranial direct current stimulation.

Studies in young and healthy control populations have shown increased glutamate or GLX local to anodal tDCS (Clark et al., 2011; Kim et al., 2014; Stagg et al., 2009), which may be related to increases in network connectivity (Hunter et al., 2015). However, in aging or clinical populations, such as PPA, these observations may not be seen. In a very recent study of elderly control subjects (Antonenko et al., 2017), only changes in GABA were observed and no changes in GLX were seen. This may indicate that the cortical response to tDCS may change with age (or the ability to detect metabolite changes is impacted with age). This indicates that further studies are warranted to better delineate the mechanisms of tDCS across the life span, as it appears incorrect to assume that results from young adult populations will translate (Antonenko et al., 2017). Age may also modulate the impact of tDCS on learning as reported through changes in task performance. In a study comparing young and elderly populations receiving tDCS during a language-learning task, the elderly population showed significant learning after anodal tDCS compared with sham, whereas the young population did not show the same effects (Fiori et al., 2017).

Table 4
Reports of means, SDs, and number of observations for GABA in the IFG and the SMC areas on different time points within different treatment groups

Time points	Treatment group	Mean (SD) of IFG GABA	Mean (SD) of SMC GABA	Number of observations
Base	tDCS	2.03 (0.57)	2.09 (0.29)	11
Post	tDCS	1.52 (0.52)	2.24 (0.29)	11
2-mo follow-up	tDCS	1.61 (0.46)	2.19 (0.45)	7
Base	Sham	1.40 (0.37)	1.93 (0.41)	11
Post	Sham	1.33 (0.59)	2.23 (0.38)	11
2-mo follow-up	Sham	1.48 (0.48)	2.05 (0.50)	10

Key: GABA, γ-aminobutyric acid; IFG, inferior frontal gyrus; tDCS, transcranial direct current stimulation; SD, standard deviation; SMC, sensorimotor cortex.

Another effect of age that we need to consider, because it may influence effectiveness of tDCS, is the loss of gray matter volume itself. This drives the majority of age-related decline in GABA (Gao et al., 2013; Porges et al., 2017; Puri et al., 2015). In healthy populations, decreases in gray matter drive observed decreases in GABA concentration; the concentration of GABA in gray matter itself (i.e., the gray matter remaining) is not decreasing (Porges et al., 2017). It is unknown if this is also true in PPA. In a recent study, baseline gray matter density was correlated with behavioral improvements after 15 days of language therapy and tDCS of the dorsolateral prefrontal cortex (Cotelli et al., 2016). Similarly, tissue volumes of specific areas of the language network and the hippocampus predict therapy scores immediately after therapy and 2 months after therapy (Tsapkini, 2017). In the present study, we explicitly correct for the tissue composition of the voxel, but age may interact with tDCS effects in ways we do not completely understand.

4.1. Limitations of the present study

A potential limitation of our study is that initial GABA levels were higher in the tDCS group, despite the fact that the participants were randomized and the groups did not differ in language and dementia severity or in initial naming and spelling performance. Although this appears to be a random occurrence, it may be that GABA levels can only decrease during learning if they are initially high enough, i.e., if GABA levels are too low, they cannot further decrease. If this is the case, it is possible that had the GABA levels in the sham group been higher at baseline, they may have also shown decreases; this is, however, highly speculative.

Second, there were 4 dropouts in our anodal tDCS group (Table 4). These dropouts were due to unrelated issues (e.g., falls, cancer). Our demographic data analysis showed no bias in dropout,

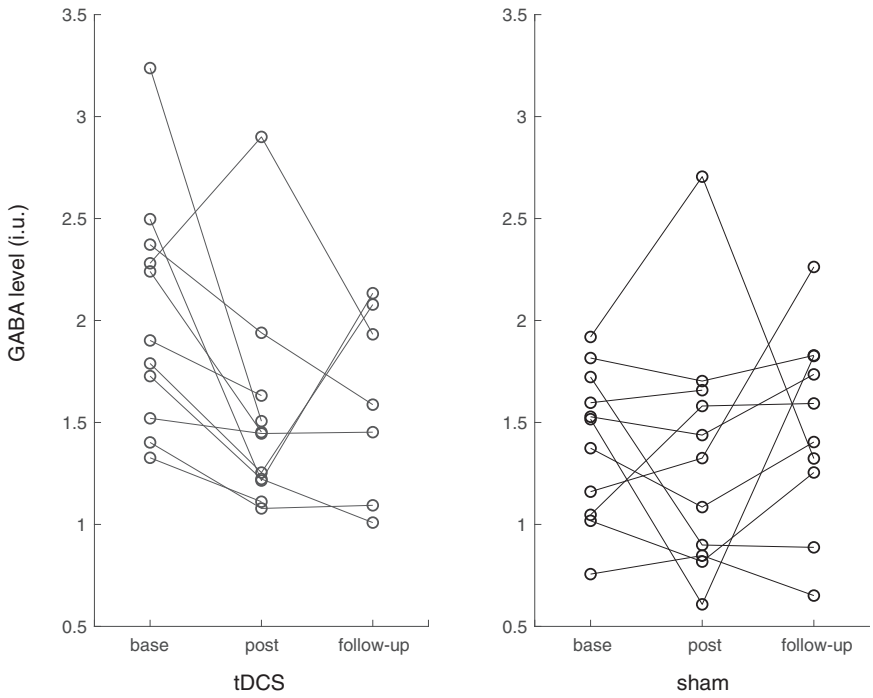

Fig. 3. GABA levels in the IFG voxel for each patient at pre-intervention (base), after the tDCS protocol (post), and at 2-month follow-up (follow-up). Abbreviations: GABA, γ-aminobutyric acid; IFG, inferior frontal gyrus; tDCS, transcranial direct current stimulation.

so to the best of our ability we have shown these dropouts were random and did not impact the results.

Finally, we did not find any relationship between behavioral measures and metabolite levels. Future studies with larger sample sizes are needed to evaluate whether the tDCS effects seen in behavioral measures correlate with measures of GABA concentrations at the stimulated site.

Disclosure

The authors have no conflicts of interest relevant to the subject matter of this manuscript.

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