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Non-invasive brain stimulation for speech in Parkinson's disease: A randomized controlled trial

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ABSTRACT

Background: Hypokinetic dysarthria is a common but difficult-to-treat symptom of Parkinson's disease (PD).
Objectives: We evaluated the long-term effects of multiple-session repetitive transcranial magnetic stimulation on hypokinetic dysarthria in PD. Neural mechanisms of stimulation were assessed by functional MRI.
Methods: A randomized parallel-group sham stimulation-controlled design was used. Patients were randomly assigned to ten sessions (2 weeks) of real (1 Hz) or sham stimulation over the right superior temporal gyrus. Stimulation effects were evaluated at weeks 2, 6, and 10 after the baseline assessment. Articulation, prosody, and speech intelligibility were quantified by speech therapist using a validated tool (Phonetics score of the Dysarthric Profile). Activations of the speech network regions and intrinsic connectivity were assessed using 3T MRI. Linear mixed models and post-hoc tests were utilized for data analyses.
Results: Altogether 33 PD patients completed the study (20 in the real stimulation group and 13 in the sham stimulation group). Linear mixed models revealed significant effects of time ($F(3, 88.1) = 22.7$, $p < 0.001$) and time-by-group interactions: $F(3, 88.0) = 2.8$, $p = 0.040$) for the Phonetics score. Real as compared to sham stimulation led to activation increases in the orofacial sensorimotor cortex and caudate nucleus and to increased intrinsic connectivity of these regions with the stimulated area.
Conclusions: This is the first study to show the long-term treatment effects of non-invasive brain stimulation for hypokinetic dysarthria in PD. Neural mechanisms of the changes are discussed.

Introduction

Hypokinetic dysarthria (HD) occurs in 90% of Parkinson's disease (PD) patients [1]. It is a complex motor speech disorder characterized by manifestations such as monopitch and monoloudness, imprecise articulation, impaired speech rate and rhythm, and irregular pitch fluctuations [2]. HD in PD seems to be associated with abnormal activation and connectivity of subcortical and cortical brain areas engaged in dorsal language stream which maps acoustic speech signals to frontal lobe articulatory networks [3] [–] [6].

In healthy subjects, the right posterior superior temporal gyrus (STG) is associated with modulation of prosody and both left and right STG are involved in auditory feedback processing [7,8]. In PD patients, previous research showed that particularly the right STG plays an important role in modulation of motor aspects of speech production [3,9,10].

The effects of pharmacological or surgical interventions on HD are limited and variable. Currently, non-pharmacological non-

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invasive interventions seem to be a valid approach with the potential to treat HD in PD. One example is the Lee Silverman voice treatment (LSVT) program, which is being used in clinical practice in many countries; training is based on increasing voice loudness through auditory feedback control [11,12].

Another approach utilizes repetitive transcranial magnetic stimulation (rTMS), a non-invasive brain stimulation (NIBS) method that is based on the principle of electromagnetic induction and uses rapid changes of a magnetic field to modulate neuronal excitability. A meta-analysis demonstrated that rTMS may induce some effect on the reduction of PD motor symptoms [13]. Nevertheless, very few rTMS studies have focused on HD in PD patients and most of them applied rTMS over a primary orofacial area with inconsistent results [14–16]. Our previous study [17] demonstrated that low-frequency rTMS of the right posterior superior temporal gyrus (STG) (i.e. the auditory feedback area) may lead to significant improvement of articulation in PD. All the above-mentioned studies investigated only the short-term effects of single-session rTMS on HD.

We hypothesized that multiple (10 day) sessions of rTMS over the right posterior STG would induce immediate improvement of motor speech in PD that would last for several weeks. It has been shown that fMRI may serve as a valuable readout of rTMS-induced aftereffects [18–21]. We performed the first randomized sham-controlled fMRI-rTMS-behavioral longitudinal study to evaluate immediate and long-term effects of NIBS treatment for HD in PD. Our primary objective was to assess the effects of rTMS treatment by an experienced blinded speech therapist (MK) using a validated diagnostic tool [22]. We focused on speech features that were improved in our previous pilot study (i.e. articulation, prosody).

Our secondary objectives were to: 1. evaluate rTMS-induced changes in hemodynamic responses within the dorsal language pathway using a reading task fMRI, 2. explore NIBS-related changes in the intrinsic functional connectivity of the stimulated region with distinct seeds of the dorsal language pathway.

Methods

Participants

We recruited 39 patients with clinically established PD [23] with mild to moderate HD, based on the assessment of a speech therapist. None of the subjects had a history or presence of hallucinations, psychosis, depression, or dementia. All participants were on a stable dopaminergic medication at least 4 weeks prior to baseline assessment and during the whole study. The patients were tested in the ON medication state without dyskinesias; none of them underwent speech therapy during the study. All participants were right-handed, and they reported Czech as their first language. All patients signed an informed consent form that was approved by the local ethics committee. This trial was registered in clinicaltrials.gov (NCT04203615).

Study design

We used a parallel group single-blinded randomized sham-stimulation-controlled design. Considering the medium effect size (Cohen's d = 0.681) from our previous single-session rTMS study [17], when setting the type I error probability level at $\alpha = 0.05$, and the power of the test at $\beta = 0.8$, we calculated the effect size to be 19 subjects in the active arm. Of note, the sample size was calculated based on immediate aftereffects of one rTMS session while in the current study we used a multiple stimulation session protocol which may induce more pronounced effects than a single session of rTMS [24].

Participants were randomly assigned in a 1:1 ratio to real or sham rTMS group. A computerized simple randomization procedure was implemented by an independent investigator. All of the researchers, except for the investigator who applied the rTMS, were blinded to the treatment condition.

Participants in the real rTMS group and the sham group underwent a baseline assessment (T0); 10 stimulation sessions (in a 2-week period between T0 and T1); a follow-up assessment right after rTMS treatment (T1) and follow-up assessments 6 weeks (T2) and 10 weeks (T3) after the baseline assessment (see Fig. 1). Each assessment lasted 1.5 h and consisted of MRI scanning, speech recording, and speech evaluation by a speech therapist. These methods are described in detail below. At baseline, all participants were also examined with a complex cognitive battery, for details, see Supplementary Materials.

rTMS treatment

All subjects underwent ten rTMS (DuoMAG™ XT-100, Deymed Diagnostic, Czech Republic) sessions for two weeks at Central European Institute (CEITEC), Masaryk University. Each stimulation session lasted about 40 min. An air-cooled figure-eight-shaped coil was placed over the right posterior STG (MNI coordinate X = 40, Y = −38, Z = 14) [25]. Frameless stereotaxy was used for coil placement navigation. Both real rTMS (1 Hz, 100% intensity of the resting motor threshold, 1800 pulses per session) and sham rTMS were utilized over the STG. The sham coil was placed in the same manner as a real coil and produced similar clicking sounds, however without any magnetic field induction or electrical scalp stimulation. The parameters of our stimulation protocol were based on our previous research [17].

Speech assessment

The perceptual rating of speech performance was made by a speech therapist who was blinded to the study protocol. The speech evaluation was performed at each visit (see Fig. 1). The validated diagnostic tool The 3F Test – Dysarthric Profile (3FT) was used for this purpose [22], see Table S2 in Supplementary Materials for the 3FT details. This test consists of three subtests: Faciokinesis, Phonorespiration, and Phonetics. The overall score of the 3F Test is the sum of 45 items with a maximum score of 90 (normal speech), and a minimum score of 0. Each item is assessed on an ordinal scale 0–2. In our main analysis, we focused on the Phonetics subtest score, because this subtest evaluates speech features that were improved in our previous pilot study [17], i.e. articulation, prosody, and speech intelligibility.

Speech task in MR scanner

Participants performed a reading task inside the MR scanner; the task is described in detail elsewhere [25]. In brief, the task consists of reading short emotionally neutral sentences aloud and

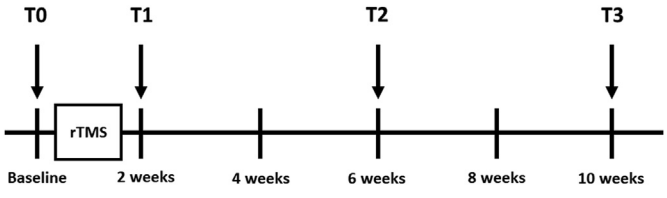

Fig. 1. Longitudinal study design.

viewing a string of "Xs" (i.e. a baseline condition). Altogether there were 48 sentence reading trials and 24 baseline trials, which alternated pseudo-randomly. The duration of all stimuli was 5 s, a black screen was displayed for 11 s in between stimuli. The whole fMRI reading task lasted about 15 min.

MRI sequences and processing

Subjects were scanned with a 3T Siemens Prisma MR scanner (Siemens, Erlangen, Germany). High-resolution anatomical T1-weighted images were acquired at baseline assessment for the Brainsight neuronavigation system (TR = 2300 ms, TE = 2.33 ms, FA = 8°, FOV = 224 mm, slice thickness 1 mm, 240 sagittal slices, matrix size 224 × 224).

Reading task fMRI and resting-state fMRI scans were acquired at each visit. EPI BOLD sequences during the speech task (47 transversal slices, slice thickness = 3 mm, TR = 12000 ms, scan acquisition time = 2750 ms, length of pause = 9250 ms, TE = 35 ms, FA = 90°, FOV = 204 mm, matrix size 68 × 68, 73 vol), multi-echo EPI resting-state sequences (60 transversal slices, slice thickness = 2.5 mm, TR = 800 ms, TE = {15, 33, 52} ms, FA = 26°, FOV = 200 mm, matrix size 68 × 80, 735 vol), diffusion-weighted images (TR = 8700 ms, TE = 97 ms, FOV = 228 mm, isovoxel 2 mm) were acquired. MRI scanning protocol lasted up to 40 min. DTI data will be analyzed and published elsewhere.

Preprocessing and data analyses were performed in SPM12 running under Matlab 2014a. The preprocessing of the functional data consisted of realignment and unwarping, normalization into standard anatomical space (MNI), and spatial smoothing with 5 mm FWHM. The level of motion was assessed visually during scanning sessions by trained technicians. In addition, the resting-state extent of motion data was controlled in terms of framewise displacement. The subjects with at least 15% of scans with FD > 0.75 mm were excluded. Moreover, the six-motion parameter time series (obtained from the realign procedure in SPM), the framewise displacement time series, and the signals from white matter and cerebrospinal fluid were regressed out of the data in subsequent analysis.

We were specifically interested in task-induced BOLD signal changes in the regions of interest (ROIs), i.e. brain regions engaged in the dorsal language pathway and implicated in pathophysiology of HD in PD [3–6], including the left orofacial sensorimotor cortex (OFSM1) (-58 -4 22) [25], supplementary motor area (SMA) (8 8 62) [3], left inferior frontal gyrus (IFG) (−44 23 15) [26], left caudate nucleus (CN) (-14 -1 17) [27], left anterior insula cortex (AIC) (−32 16 2) [27], left supramarginal gyrus (SMG) (-42-52 37) [26], the right posterior superior temporal gyrus (STG) (40 -38 14) [9] (see Fig. 2). The ROIs were centered on the nearest local maxima (peak voxel values) of the mean group activations within the selected brain regions (reading sentences in contrast to baseline condition) at the baseline examination, calculated using a one-sample *t*-test. Representative activations within the spheres with a radius of 6 mm covering the ROIs were calculated as the mean values of fMRI contrast between the reading task and the baseline condition.

Because of the well-known placebo effects of rTMS, we additionally evaluated rTMS-induced activations within the ventral striatum (VS) which is engaged in the mesolimbic pathway and seems to be involved in placebo effect in PD [28]. The masks of parcellated striatum into subregions were based on a probabilistic atlas of sub-striatal regions (extracted from the FSL package http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases/striatumconn) [29]. We used the relative changes of the BOLD signal increases to obtain approximately normal distribution of residuals.

Resting-state analysis

We explored whether intrinsic functional connectivity was changed due to the rTMS treatment between the stimulated region (STG) and the ROIs with significant rTMS-induced differences in activation. We extracted the time series of the BOLD signal within these ROIs and computed representative mean signals. Resting-state functional connectivity was calculated as Pearson's correlation coefficient between the representative time series and then converted to z values using Fisher's r-to-z transformation.

Statistical analysis

We used linear mixed models (LMM) adjusted for multiple comparisons via Bonferroni correction, to evaluate the effects of rTMS on perceptual ratings of speech performance, tasks-induced brain activation within our ROIs, and resting-state connectivity between our seeds of interest. Fixed factors in LMM were treatment group (real vs. sham), time (T0, T1, T2, T3), and the time-by-treatment group interaction. A Spearman correlation analysis was used to assess associations between the rTMS-induced behavioral and fMRI changes with time. Post-hoc pairwise comparisons of estimated marginal means were performed between each time point, adjusting for six comparisons using the Bonferroni correction. Age, gender, and levodopa equivalent dose (LED) were used as covariates in all LMMs. These statistical procedures were performed with IBM SPSS Version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Participants

Twenty individuals were randomly assigned to the real rTMS group and 19 participants were assigned to the sham group. The real and sham stimulations were well tolerated and did not induce any side effects. During the study, 6 participants did not complete the 10 stimulation sessions. The most common reasons were unexpected health problems, and withdrawal of the consent with the study protocol. Our final research sample consisted of 20 patients in the real rTMS group and 13 patients in the sham group. The real rTMS group and sham group did not significantly differ in demographic and clinical data. For details, see Table 1. Since the drop-out was predominantly within the sham group, we additionally checked for demographic and clinical data of those six drop-outs. The three groups did not statistically or numerically differ in their demographic and clinical data (see Supplementary Materials for box plots and results).

Speech assessment

Based on assessment of speech therapist most patients had mild hypokinetic dysarthria.

For the total 3FT score, the LMM revealed statistically significant effect of time ($F(3, 81.3) = 36.1$, $p < 0.001$), while a time-by-treatment group interaction was not significant ($F(3, 81.3) = 2.0$, $p = 0.119$), for relative changes of 3FT total scores see Fig. S2 in Supplementary Materials.

For the 3FT Phonetics score, i.e. our score of interest [8], the LMM showed a significant effect of time ($F(3, 88.1) = 22.7$, $p < 0.001$) and a significant time-by-treatment group interaction ($F(3, 88.0) = 2.8$, $p = 0.040$). Additional LMM analyses were performed separately for the real rTMS group and the sham group. These models revealed that the effect of time on Phonetics scores was significant for both the real rTMS group ($F(3, 50.9) = 12.5$, $p < 0.001$) and the sham rTMS group ($F(3, 37.4) = 5.6$, $p = 0.003$).

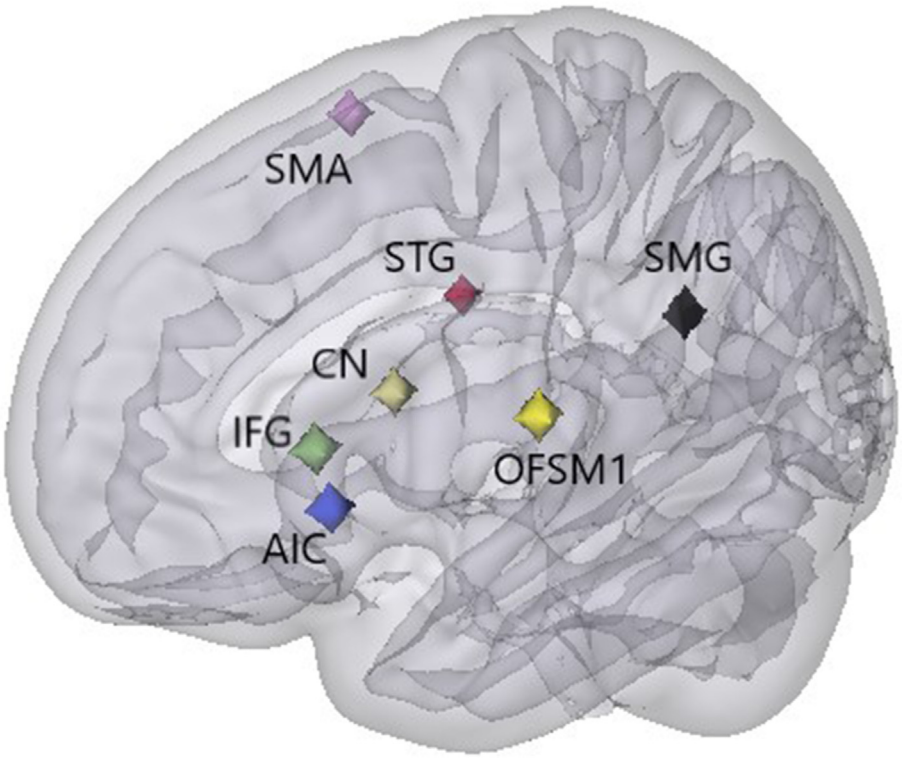

Fig. 2. Regions of interest in the dorsal language pathway.
AIC- anterior insular cortex, **CN**- caudate nucleus, **IFG**-inferior frontal gyrus, **OFSM1**-primary orofacial sensorimotor cortex, **SMA**-supplementary motor area, **SMG**-supramarginal gyrus, **STG**-superior temporal gyrus.

The effect sizes, computed from the differences between T0 and T3, were large for both the real rTMS group (*Cohen's d* = 1.391) and the sham rTMS group (*Cohen's d* = 0.996), while the difference between the effects of both stimulations was medium (T3-T0, Hedges' g = 0.589). In the real rTMS group, post-hoc pairwise comparisons showed that Phonetics scores significantly improved immediately after 10 sessions of rTMS (T0 vs T1, mean diff. = −2.5, p < 0.001), and further improved at the 10-week follow-up visit (T1 vs T3, mean diff. = −1.8, p = 0.029). In the sham group, post-hoc pairwise comparisons revealed that Phonetics scores significantly improved immediately after 10 sessions of rTMS (T0 vs T1, mean diff. = −1.6, p = 0.038); however, it remained stable during the T2 and T3 assessments (T1 vs T3, mean diff. = −0.6, p = 0.9).

We found similar differences between the real and sham groups when we used relative changes to baseline in the LMM. For relative changes of Phonetics subtest scores, the LMM showed a significant effect of group (F(1, 30.3) = 4.2, p = 0.048). In the real rTMS group, the relative change of Phonetics scores was higher than in the sham group (real vs sham mean diff. = 10.28, p = 0.048); see Fig. 3. The post-hoc comparison revealed that the significant difference between the real and sham groups was at the T2 follow-up visit (mean diff. = 12.5, p = 0.026) and at the T3 follow-up visit (mean diff. = 12.5, p = 0.031) (see Fig. 3).

The LMMs for Faciokinesis and Phonorespiration subtest scores did not reveal any significant time-by-treatment group interactions (data not shown).

fMRI results

Regarding reading task-induced activations in the ROIs, LMM revealed significant time-by-group interactions for OFSM1 (F(3, 34.4) = 3.2, p = 0.032) and CN (F(3, 40.2) = 3.3, p = 0.029), see

Table 1
Demographic and clinical variables.

	PD real rTMS group	PD sham rTMS group	Mann-Whitney
Gender Female/Male	6/14	4/9	p > 0.05
Age (years)	Mdn 71.7 (IQR 62.2–75.5)	Mdn 71.5 (IQR 60.9–78.0)	p > 0.05
Duration of PD (years)	Mdn 4.0 (IQR 2.0–10.5)	Mdn 3.0 (IQR 1.0–8.2)	p > 0.05
LED	Mdn 990.0 (IQR 610.0–1416.2)	Mdn 750.0 (IQR 500.0–970.0)	p > 0.05
UPDRS III	Mdn 14.0 (IQR 11.0–20.0)	Mdn 13.5 (IQR 8.7–17.0)	p > 0.05
3F Test	Mdn 64.0 (IQR 54.0–71.0)	Mdn 74.0 (IQR 62.0–77.5)	p > 0.05
Faciokinesis	Mdn 21.0 (IQR 17.5–25.0)	Mdn 20.0 (IQR 19.0–26.0)	p > 0.05
Phonorespiration	Mdn 22.0 (IQR 20.0–25.5)	Mdn 24.0 (IQR 20.7–25.5)	p > 0.05
Phonetics	Mdn 21.0 (IQR 17.0–24.0)	Mdn 22.5 (IQR 19.5–28.0)	p > 0.05

Mdn- Median, IQR-Interquartile range.

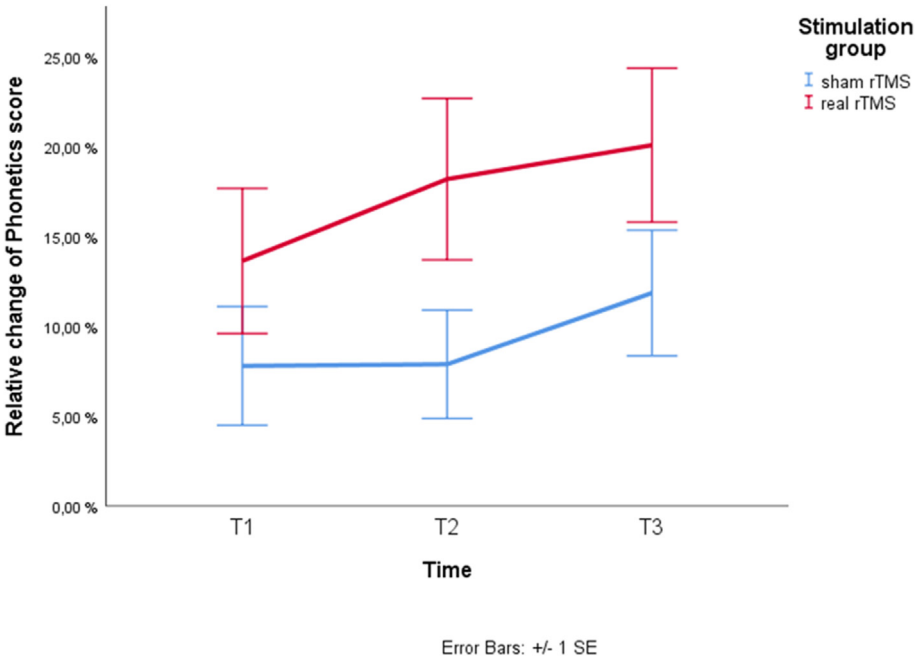

Fig. 3. Relative change of Phonetics scores.

Fig. 4a and b. A time-by-group interaction was observed also for AIC although it did not reach statistical significance ($F(3, 43.8) = 2.6$, $p = 0.06$).

Speech task-induced hemodynamic responses in the above-mentioned regions seemed to increase across T0-T1-T2 in the real rTMS group; in the sham group, the post-hoc pairwise comparisons revealed a significant BOLD signal decrease at T2 as compared to T0, particularly in the OFSM1 (mean diff. = 69.2, $p = 0.009$). The post-hoc comparison between the real and sham groups at the T2 visit showed a significant difference both for the OFSM1: mean diff. = 66, $p = 0.004$, and for the CN: mean diff. = 36.5, $p = 0.011$).

For the relative change of the BOLD signal increases in the ventral striatum, LMM showed a significant effect of stimulation group ($F(1, 28.7) = 5.2$, $p = 0.029$). In the sham group, the relative change of the hemodynamic response was higher than that in the real rTMS group (real vs sham, mean diff. = −117.4, $p = 0.029$). The direct comparison between the real and sham groups revealed a significant difference at T1, i.e. immediately after 10 stimulation

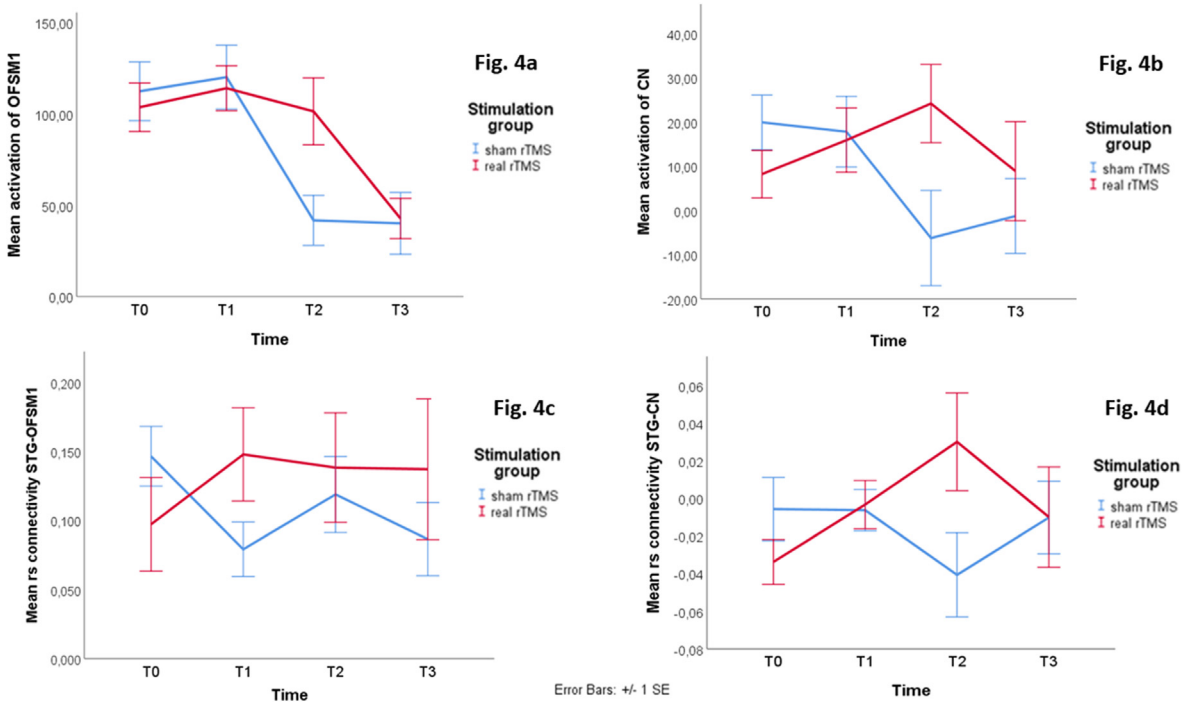

Fig. 4. Mean activation of OFSM1 and CN (Fig. 4a,b) and their resting-state connectivity with STG (Fig. 4c,d).

sessions (mean diff. = −151.7, p = 0.008) (see Table S4 in Supplementary Materials).

Regarding the resting-state functional connection of the stimulated region (STG) to our seeds of interest (OFSM1, CN), the LMM revealed a significant time-by-group interaction for the STG-OFSM1 connection ($F(3, 21.1) = 3.1$, $p = 0.045$) and a non-significant time-by-group interaction for the STG-CN connection ($F(3, 25.4) = 2.8$, $p = 0.059$), see Fig. 4c and d. Connectivity seemed to increase in the real rTMS group and it seemed to decrease in the sham group (see Fig. 4c and d). Moreover, a significant positive correlation was detected between the temporal evolution of the Phonetics score and the STG-OFSM1 functional connectivity changes in the real stimulation group ($R = 0.449$, $p = 0.013$).

Finally, an effect of stimulation group was insignificant for the STG-VS relative change of the resting-state connectivity ($F(1, 36.2) = 1.3$, $p = 0.261$).

Discussion

Behavioral results

Our longitudinal randomized controlled study demonstrated for the first time significant effects, lasting at least eight weeks, of multiple sessions of real rTMS as compared to sham rTMS on phonetic aspects of HD in PD. The 3FT Phonetics score evaluates the precision of articulation, spontaneous speech, reading, a diadochokinetic ("pa-ta-ka") task, as well as quality of speech intonation, speech rhythmicity, and, most importantly, speech intelligibility.

Interestingly, the post-hoc analyses did not reveal significant differences between the real and sham groups immediately after the NIBS treatment completion (at the T1 follow-up visit), and there was significant improvement immediately after the rTMS treatment completion as compared to the baseline assessment in both groups (T1-T0). While this result could have been related to placebo effects of rTMS (see the discussion below), further significant improvements at follow-up visits occurred only in the real rTMS group, but not in the sham group. The delayed long-term effects of the active multiple session rTMS treatment may relate to the rTMS-induced long-term potentiation effects and/or elevation of the brain-derived neurotrophic factor (BDNF) that may contribute to changes in synaptic plasticity [30,31] and lead to long-lasting plasticity changes [32,33]. It has been shown that the process of plastic reorganization after rTMS may take time to develop and cause more pronounced aftereffects 4–15 weeks later [34–36]. This accords well with changes in our real stimulation group that gradually built up during the 8 weeks after the rTMS treatment completion. Of note, the initial behavioral effect in the sham group at T1 persisted throughout the 8-week post-treatment evaluation. This may seem long, however, similar long-term placebo effects of rTMS have been documented, for example in rTMS studies focused on PD patients [37] or patients with schizophrenia [38].

Neural underpinnings of rTMS and their relation to rTMS- induced behavioral changes

Neural mechanisms of motor speech impairment of PD are not yet fully understood [2]. Accurate speech production depends on auditory-motor integration, which is mediated by posterior (phonological) and anterior (articulatory) components of the dorsal language pathway [6]. The STG, stimulated in this study based on our pilot one-session rTMS study results [17], is involved in phonological processing and auditory feedback [39]. In PD patients, abnormal activation and connectivity of this region seems to play an important role in motor speech impairment [3,4,9]. In this study we provided further evidence for the importance of this region in the control of articulation and prosodic aspects of overt speech production in PD. By manipulating the excitability of this region with rTMS, we observed changes in remote areas functionally connected with this region. Our results show that significant changes occurred not directly in the stimulated region (STG) but in specific regions engaged in the articulatory network, particularly in the OFSM1 and CN. Moreover, we demonstrated that intrinsic connection of the STG with both abovementioned regions increased due to stimulation in the active as compared to the sham stimulation group, particularly with the OFSM1. The temporal evolution of the described fMRI changes followed (and correlated with) the temporal pattern of behavioral changes; in other words, the intrinsic connectivity changes induced by rTMS were linked to our behavioral outcomes.

Previous works demonstrated that the effects of rTMS could be remote and spread from the directly stimulated brain area to other functionally connected regions within the distinct brain networks [40,41]. Our fMRI findings accord well with this hypothesis of stimulation-induced propagation of changes within the functionally connected regions. Here we report rTMS-induced neural changes in remote regions of the articulatory network that were functionally connected with the stimulated STG area, namely the OFSM1 and CN. Importantly, the increased connection between the STG and OFSM1 was linked to alleviation of HD symptoms as assessed by the Phonetics score. Both OFSM1 and CN play an important role in overt speech production. The left OFSM1 is engaged in the execution of speech movements [27,42,43]. Activation of this region was correlated with several aspects of speech of PD patients: a negative correlation was found for speech initiation time [25,44], a positive correlation was found for speech loudness and prosody [25]. Positive effects on speech loudness and quality of voice were observed after high frequency rTMS was applied directly over the left OFSM1 [16]. However, in that previous crossover study directly comparing the effects of one stimulation session of rTMS over the OFSM1 and STG in PD, the effects of STG stimulation surpassed those of OFSM1 stimulation [17]. The CN plays an important role in speech motor planning [27] and decreases in connections between the CN and prefrontal cortices were observed in PD patients both on and off dopaminergic medication during speech production [4]. Successful LSVT led to increases in CN activation [45], and levodopa induced changes of functional connectivity of the CN which were linked to speech intonation variability [10].

Finally, as reported above, similar immediate behavioral aftereffects were observed in both the real and sham stimulation groups which could have been related to placebo effects of rTMS [46]. Previous studies [47,48] described sham rTMS-induced dopamine release, particularly in the VS in PD patients, that led to the immediate improvement of some motor symptoms. We showed that sham rTMS led to increased activations of the VS that were more pronounced than activations in the real stimulation group. However, the rTMS-induced STG-VS connectivity changes did not significantly differ between both groups. Therefore, the fMRI results of VS and their potential relation to placebo effects of rTMS have to be interpreted with caution.

Study limitations

A study limitation is the fact that most of the participants had only mild dysarthria and therefore the rTMS effects cannot be generalized to the whole PD population. The sham and real stimulation groups were unbalanced and dropout was predominantly within the sham group. However, linear mixed model is quite robust for analyses of unbalanced groups [49,50] and we showed

that the patients who prematurely withdrew the study were not outliers in terms of their baseline demographic and clinical data.

Conclusion

In conclusion, we showed for the first time in a randomized controlled trial that multiple sessions of low-frequency rTMS over the right STG may have long-lasting clinically relevant effects on motor speech impairment due to PD. Results of fMRI analysis revealed rTMS-induced increases of activation in remote areas of the dorsal language stream. We provided further evidence that rTMS effects may propagate to brain areas distant from the stimulation site, probably via modulation of its intrinsic connection with regions of the same functional network. This study underlies the importance of STG manipulation for the treatment of HD in PD and provides the first evidence of the therapeutic potential of rTMS for this otherwise difficult-to-treat syndrome of PD. In future research, more intensified and individualized rTMS protocols might be used. To achieve the best treatment effects, rTMS might also be combined with other methods such as the LSVT.

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Declaration of competing interest

None.

CRediT authorship contribution statement

Lubos Brabenec: Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft. **Patricia Klobusiakova:** Formal analysis, Data curation, Writing – original draft. **Patrik Simko:** Investigation, Data curation, Writing – original draft. **Milena Kostalova:** Investigation, Writing – review & editing. **Jiri Mekyska:** Conceptualization, Methodology, Writing – review & editing. **Irena Rektorova:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

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