Clinical Neurophysiology 130 (2019) 1292–1298

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

The influence of one session of low frequency rTMS on pre-supplementary motor area metabolites in late stage Parkinson's disease

Anja Flamez [a,*], Wietse Wiels [a], Peter Van Schuerbeek [b], Johan De Mey [b], Jacques De Keyser [a,c], Chris Baeken [d,e,f]

[a] Department of Neurology, UZ Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium
[b] Department of Radiology, UZ Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium
[c] Department of Neurology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 Groningen, the Netherlands
[d] Department of Psychiatry, UZ Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium
[e] Ghent University, Department of Psychiatry and Medical Psychology, C. Heymanslaan 10, 9000 Ghent, Belgium
[f] Ghent Experimental Psychiatry (GHEP) Lab, Ghent University, Ghent, Belgium

ARTICLE INFO

Article history:
Accepted 27 April 2019
Available online 31 May 2019

Keywords:
Parkinson's disease
Dyskinesias
rTMS
Spectroscopy

HIGHLIGHTS

- One rTMS session in Parkinson's disease (PD) does not affect neuronal integrity.
- One rTMS session influences membrane turnover, inversely correlated with disease duration.
- The presence of dyskinesias did not influence spectroscopy outcomes.

ABSTRACT

Objective: To study the effect of Low Frequency repetitive Transcranial Magnetic Stimulation (LF rTMS) on brain metabolites in late stage Parkinson's disease (PD) patients (disease duration at least 4 years and Hoehn and Yahr (1969) score at least 2 in OFF). Several neuroimaging data support a role for pre-Supplementary Motor Area (pre-SMA) involvement in the pathogenesis of Parkinson's disease. Proton magnetic resonance spectroscopy (^{1}H-MRS) measures in vivo metabolites, but results in PD brain remain conflicting and little is known of the effect of LF rTMS thereupon.
Methods: We investigate the neurochemical profile of the right pre-SMA in 17 late stage PD patients (11 male and 6 female, mean age of 71 years) before and after one session of sham controlled 1 Hz rTMS (1000 pulses, 16 minutes), focusing on the tNAA/tCr and tCho/tCr ratios.
Results: The tNAA/tCr ratio was unaffected by one session of LF rTMS. We did observe a significant effect of real LF rTMS on the tCho/tCr ratio, inversely correlated with disease duration, and not related to the presence of dyskinesias. As expected, one session of LF rTMS did not affect clinical outcome.
Conclusions: LF rTMS at the right pre-SMA in late stage Parkinson's disease patients does not alter tNAA/tCr, but influences tCho/tCr ratio, in particular in patients with shorter disease duration.
Significance: Pre-SMA LF rTMS seems to influence membrane turnover, more importantly in patients with shorter disease duration. Larger LF rTMS treatment studies applying multiple sessions are needed.

1. Introduction

Therapeutic management of late stage Parkinson's disease (PD) patients remains a huge challenge. Several non-invasive stimulation techniques (LF and HF rTMS, deep rTMS, theta burst stimulation) have been investigated for the modulation of cortical excitability in Parkinson's disease and in the treatment of its motor and nonmotor symptoms (Brys et al., 2016; Cohen et al., 2018; Lefaucheur et al., 2004; Zamir et al., 2012). In healthy control subjects, motor cortex excitability has been documented to be decreased by LF rTMS when applied directly over the motor cortex (Chen et al., 1997). Since hyperactivity of the primary and

* Corresponding author at: Department of Neurology, UZ Brussel, Laarbeeklaan 101, 1090 Jette, Belgium.
E-mail address: anja.flamez@uzbrussel.be (A. Flamez).

https://doi.org/10.1016/j.clinph.2019.04.720
1388-2457/

associated motor cortices seems to play a role in the emergence of levodopa induced dyskinesias (LID) (Rascol et al., 1998), LF rTMS has been investigated as a potential noninvasive treatment of LID with conflicting results (Brusa et al., 2006; Filipovic et al., 2009; Flamez et al., 2016; Koch et al., 2005; Wagle Shukla et al., 2007). In a previous trial, we investigated the effect of a single motor cortex LF rTMS session on LID, and to overcome the possible low potential of single session paradigms on LID, we extended our study into an accelerated multiple sequential LF rTMS treatment protocol. We failed to alleviate dyskinesias even in the multiple session treatment paradigm (Flamez et al., 2016). Since the pathophysiological mechanisms of levodopa induced dyskinesias in PD are still not fully unraveled, the ideal cortical rTMS target for the treatment of LID in PD remains to be determined. Although hyperactivity of primary (and associated) motor cortices in LID has been documented (Rascol et al., 1998), there is increasing morphometric and functional Magnetic Resonance Imaging (fMRI) evidence of involvement of the prefrontal cortex in the pathophysiology of LID (Cerasa et al., 2012; Cerasa et al., 2013). Pathological and functional MRI data support a role for the pre-Supplementary Motor Area (pre-SMA) in the pathogenesis in different stages of Parkinson's disease (Eckert et al., 2006; MacDonald and Halliday, 2002). A combined resting state fMRI and rTMS study highlights the key role of the right Inferior Frontal Cortex (IFC) in the cortical-subcortical network in dyskinetic Parkinson patients, suggesting an increased connectivity between the right IFC and the right putamen in those patients (Cerasa et al., 2015). Moreover, an fMRI study demonstrated that exposure to levodopa in late stage dyskinetic patients not only led to a putaminal hyperactivation, but also caused an excessive increase in activity in the pre-SMA, highlighting the importance of pre-SMA involvement in late stage PD brain (Herz et al., 2014). In this study, we chose to investigate whether we could influence this above mentioned right centered network by applying inhibitory low frequency rTMS to the anatomically and functionally connected right pre-SMA.

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive brain imaging technique used to measure in vivo metabolites in the brain. Some of the metabolites can be easily quantified such as N-acetylaspartate/N-acetylaspartylglutamate (tNAA), choline containing compounds (tCho) and creatine/phosphocreatine (tCr), reflecting neuronal density and integrity, turnover of cell membranes and energy metabolism, respectively. Since tCr detection is easy and concentration is fairly stable under physiological conditions, it can be used as an internal concentration reference, expressing neurochemical values as tNAA/tCr and tCho/tCr (Rackayova et al., 2017). ^{1}H-MRS has been used to study age related metabolite concentration changes in the normal aging brain (Ding et al., 2016; Schmitz et al., 2018), and has provided insight in neurochemical mapping in pathological brain, including Parkinson's disease (Levin et al., 2014). Conflicting results in early PD studies (i.e. before 2014), mainly focusing on NAA/Cr and Cho/Cr ratios, were reviewed by Ciurleo and colleagues providing an overview of spectroscopy studies in treated and untreated PD patients. Despite methodological differences, overall, it seems that brain NAA levels in PD are reduced in cortical-basal ganglia networks, reflecting neuronal loss, and it is suggested that this could be influenced by dopaminergic therapy or even Deep Brain Stimulation (DBS) of the Subthalamic Nucleus (STN) (Ciurlio et al., 2014). More recent studies highlight the usefulness of measuring NAA/Cr ratio's in basal ganglia network regions (substantia nigra, globus pallidus, prefrontal lobe) as metabolic markers in early diagnosis of Parkinson's Disease (Guan et al., 2017; Wu et al., 2016). Pre-SMA spectroscopy data in PD brain are less straightforward, even though an fMRI study revealed increased pre-SMA activation in early stage PD patients (Eckert et al., 2006). Both disease duration and dopaminergic treatment seem to play an important role in neurochemical concentrations in PD patients' pre-SMA. Compared to healthy controls, the pre-SMA NAA/Cr and Cho/Cr ratios are normal in early untreated PD patients (Martin et al., 2008). Pre-SMA NAA/Cr ratios however are significantly reduced in a more advanced, albeit treated, PD population (Camicioli et al., 2007). Moreover, it has been documented that dopaminergic treatment is able to restore motor cortex Cho/Cr ratio, which is reduced in de novo untreated PD patients compared to controls (Lucetti et al., 2007).

One recent study in healthy humans demonstrated that one session of sham controlled LF rTMS on the left Dorsolateral Prefrontal Cortex is sufficient to influence the relationship between measures of tNAA and tCho (Bridges et al., 2018). The effect of rTMS on brain metabolites has been studied in treatment resistant depression, including tNAA/tCr ratios, (Baeken et al., 2017) and tinnitus (Cacace et al., 2017), however, little is known about the effect of LF rTMS on brain metabolites in late stage PD patients.

Therefore, in our study we chose to evaluate the effect of a single sham-controlled LF rTMS session over the right pre-SMA on tNAA/tCr and tCho/tCr pre-SMA ratios in late stage PD patients, taking into consideration disease duration and the presence of dyskinesias, we expected that one real LF rTMS session and not sham would affect tNAA/tCr and tCho/tCr ratios.

2. Methods

2.1. Study population

Seventeen right handed patients with diagnosis of idiopathic Parkinson's disease, according to the United Kingdom Parkinson's Disease Society Brain Bank Criteria (Hughes et al., 1992), were enrolled. Inclusion criteria were: (a) Hoehn and Yahr scale score of at least 2 in OFF (Hoehn and Yahr, 1967); (b) disease duration of at least 4 years; (c) obligatory treatment with levodopa for at least 1 year, add-on treatment with a dopamine agonist and/or entacapone and/or a MAO-B inhibitor is permitted; (d) no or only slight or intermittent tremor present in OFF according to the Unified Parkinson's Disease Rating Scale III (UPDRSIII) tremor scores (item 20 <=5 and item 21 <=2) (Fahn and Elton, 1987) (e) the presence of LID, according to the Unified Parkinson's Disease Rating Scale IV (UPDRSIV) (Fahn and Elton, 1987) criteria, is permitted but not required; (f) absence of a serious neuropsychiatric disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5: American Psychiatric Association); (g) an optimal and stable medication dose for at least 4 weeks preceding inclusion; (h) no contra-indications for rTMS (Rossi et al., 2009). Patient demographics and characteristics are listed in Table 1.

The Ethics Committee of UZ Brussel approved the study, and written informed consent was obtained from all patients. Treatment remained unchanged throughout the study period.

2.2. Experimental procedures

Each experimental condition (real and sham) was performed after a 12 hours overnight dopaminergic treatment withdrawal (practically defined OFF). Patients' motor disability and dyskinesias were rated prior to the imaging procedure, using the UPDRSIII and UPDRSIV scales, respectively (Fahn and Elton, 1987). Handedness was determined according to the Edinburgh Handedness Inventory (Oldfield, 1971). Subsequently, patients were accompanied to the radiology department and prepared for their structural Magnetic Resonance Imaging (MRI) and baseline ^{1}H Magnetic Resonance Spectroscopy (MRS) session (see below). Upon completion, LF rTMS was delivered to the right pre-SMA according to our rTMS protocol (see below), followed by a second ^{1}H MRS session. Following a cross-over design, patients were randomized to first receive real

Table 1
Patients' baseline characteristics.

	All	Dyskinesia	No dyskinesia	*p*-values
Gender (female/male)	6/11	4/5	2/6	0.40[a]
Age (years)	70.94 (10.17)	72.89 (9.85)	68.75 (10.73)	0.42[b]
Disease duration (years)	10.59 (5.18)	10.11 (4.37)	11.13 (6.24)	0.70[b]
Hoehn and Yahr OFF[†]	2 (2)	3 (2)	2 (1)	0.335[c]
UPDRSIII OFF	23.00 (6.98)	25.78 (7.79)	19.87 (4.58)	0.081[b]
LID Yes/No	9/8	9	8	
Daily equivalent levodopa dose (mg)	880.59 (413.77)	876.67 (410.09)	885.00 (446.19)	0.969[b]
RMT (%)	56.76 (4.33)	55.22 (3.77)	58.50 (4.50)	0.12[b]
Baseline tNAA/tCr[††]	1.15 (0.11)	1.11 (0.10)	1.21 (0.11)	0.06[b]
Baseline tCho/tCr[††]	0.22 (0.03)	0.22 (0.03)	0.22 (0.03)	0.57[b]

[†] Data are presented as mean (SD) or median values (range).
[††] These values are calculated as the mean of the 2 baselines measures.
[a] Chi-square test.
[b] Independent *t*-test.
[c] Mann-Whitney test.

or sham rTMS by flipping a coin. To avoid carry-over effects of rTMS a time window of at least 1 week was introduced between sessions (Rossi et al., 2009). Medical treatment was kept stable throughout the duration of the trial.

2.2.1. rTMS stimulation protocol

1 Hz rTMS was delivered with a Magstim high-speed magnetic stimulator (Magstim Company Limited, Wales, UK) through a 70 mm figure-of eight-shaped coil to the right pre-supplementary motor area. The position of the TMS coil on the participant's head to target the pre-SMA, was determined using the 3D-MRI localization method (Peleman et al., 2010) at [5, −2, 62] (MNI coordinates). Before applying rTMS and acquiring MRS imaging, the patient's Resting Motor Threshold (RMT) was determined in the Abductor Pollicis Brevis (APB) muscle, contralateral to the magnetic stimulation of the motor cortex. The RMT for eliciting responses in the relaxed muscle was defined as the minimal intensity of stimulation capable of inducing motor evoked potentials greater than 50 μ V peak-to-peak in at least 5 of 10 consecutive trials. Upon completion of the baseline MRS session (T0) a series of 1000 pulses at a rate of 1 Hz (duration of 16 minutes) with an intensity of 90% of the RMT were delivered to the right pre-SMA, followed by the second MRS session (T1). During sham stimulation, a sham coil identical to the real coil was used providing the same auditory stimuli, but no cortical stimulation.

2.2.2. ^{1}H MR spectroscopy

Imaging data were obtained using a Philips 3 T MRI scanner (Ingenia, Best, The Netherlands). First an anatomical scan was taken (3D T1 TFE, FOV = 240 × 240 × 200 mm^3, resolution = 1 × 1 × 2 mm^3, TR = 12 ms, TE = 3.85 ms, flip angle = 10°, turbo factor = 240). Second, a ^{1}H MRS scan was taken of the right pre-SMA region (SV PRESS, VOI = 20 × 20 × 20 mm^3, TR = 2000 ms, TE = 38 ms, NSA = 128, sample frequency = 2000, samples = 1024) (Fig. 1A and B). The pre-SMA was visually determined and we used a voxel size of 20 × 20 × 20 mm^3. MRS data were analyzed using TARQUIN following the default processing pipeline (Wilson et al., 2011). The basic brain metabolite set of TARQUIN consisted of -CrCH2, alanine, aspartate, creatine (Cr), GABA, Glycerophosphocholine (GPC), glucose, glutamine, glutamate, gua , inositol, lactate, 4 lipids, 5 macromolecules, N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), Phosphocholine (PCh), Phosphocreatine (PCr), scyllo-inositol and taurine. The results for the metabolites coming out of TARQUIN were referenced to an unsuppressed water spectrum. Since it was not possible to trace back the spectro volume on the anatomical scan, we did not correct for partial volume effects. Besides the individual water-referenced metabolite estimates, TARQUIN calculated total NAA (tNAA) as NAA + NAAG, total Cho (tCho) as GPC + PCh and total Cr (tCr) as Cr + PCr.

2.3. Statistical analysis

All analyses were carried out in SPSS 24 (Statistical Package for the Social Sciences; IBM SPSS Statistics for Windows, Version 24.0, IBM Corp., Armonk, NY). The significance level was set at $p < 0.05$, two-tailed, for all analyses. Where necessary, we applied the Greenhouse–Geisser correction to ensure the assumption of sphericity. Baseline characteristics were evaluated using parametric and non-parametric statistics where appropriate.

For the effects of one LF-rTMS session on ^{1}H MR spectroscopy measurements, only a priori hypotheses were tested. First, we calculated the total tNAA/tCr ratio (t(NAA + NAAG)/t(Cr + PCr)) and the total tCho/tCr ratio (t(GPC + PCh) /t(Cr + PCr)) for the right pre-SMA ROI. Second, to evaluate baseline differences between PD patients with and without dyskinesia we calculated the mean of the two baseline measurements for a given patient (before the sham and real LF-rTMS session) for tNAA/tCr and tCho/tCr levels. Third, to examine the effect of one session of LF-rTMS on right pre-SMA metabolism, we performed two separate mixed ANCOVA's including the individual neurometabolite ratios as the dependent variables and with Time (before vs. after LF-rTMS) and Stimulation (real vs. sham) as the within subjects factors. Dyskinesia (yes vs. no) was the between-subjects factor and given that Parkinson's disease duration seems to play an important role in pre-SMA's neurochemistry, the latter was introduced as covariate (Camicioli et al., 2007; Martin et al., 2008). Significant interaction effects were followed-up with separate univariate analyses of variance, correlation analyses, and T-tests where appropriate.

3. Results

We found no baseline statistical differences concerning age, disease duration, UPDRSIII in OFF and equivalent levodopa dosages between patients with or without dyskinesias (p's > 0.05). Also the time interval (in days) between the number of PD patients first receiving real (n = 8, 13.13 days (SD = 6.94)) and those patients first receiving sham (n = 9, 13.22 days (SD = 6.50)) was not significant t (15) = 0.03, p = 0.98). We did however observe a trend that PD patients with dyskinesia had lower mean baseline tNAA/tCr levels compared to PD patients without dyskinesia (p = 0.06), but not for tCho/tCr (p = 0.57). Patients' characteristics are listed in Table 1. tNAA/tCr and tCho/tCr ratios for the right pre-SMA are provided in Table 2.

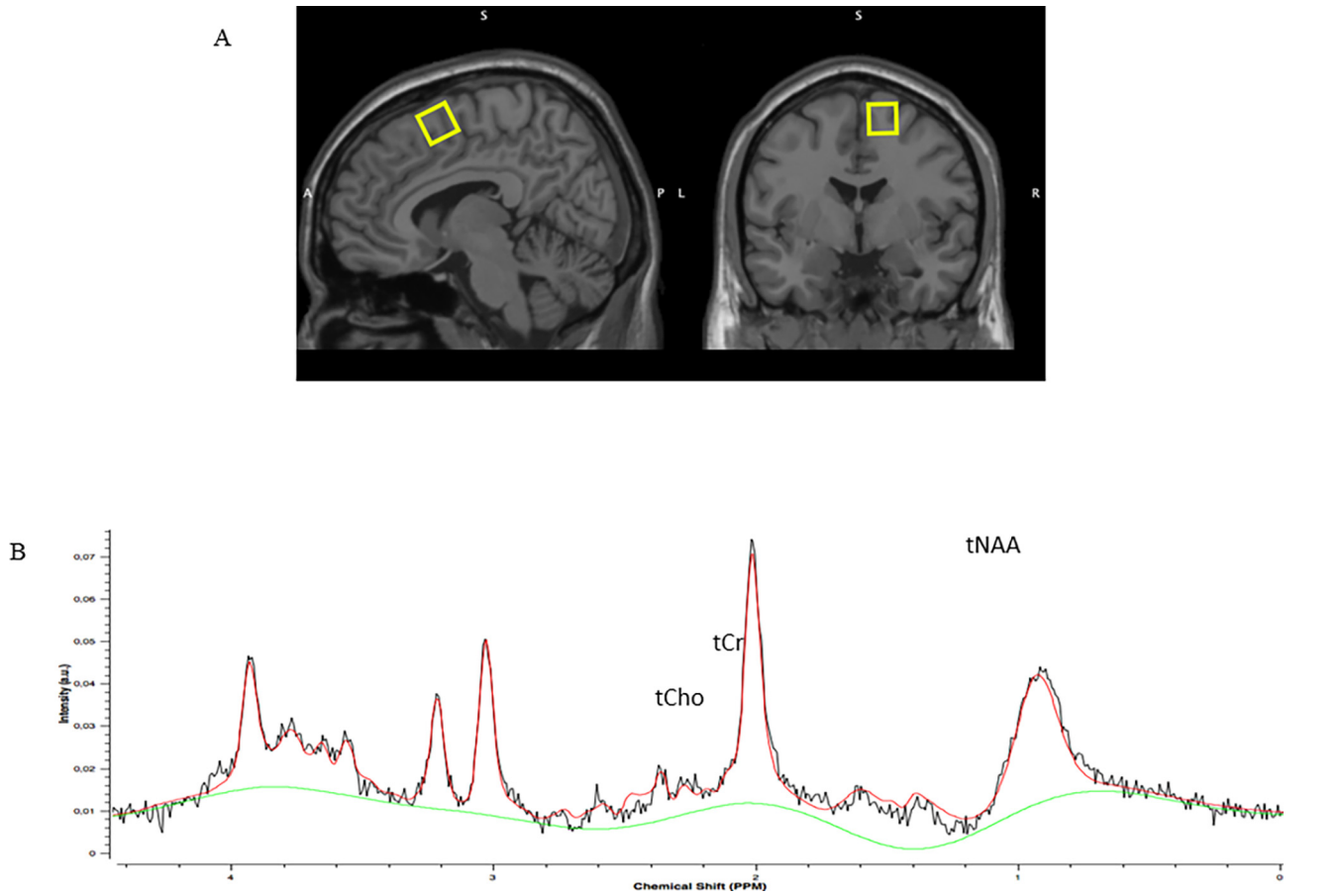

Fig. 1. (A) Example of pre-SMA voxel placement, and (B) of a typical spectrum obtained in one of our patients. Depiction of plots showing the original measured spectrum (black lines), the result of the model fitting (green lines) and the fitting results for some individual metabolites (red lines). TARQUIN calculates total NAA (tNAA) as NAA + NAAG, total Cho (tCho) as GPC + PCh and total Cr (tCr) as Cr + PCr. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Right pre-SMA tNAA/tCr and tCho/tCr ratio's.

	Sham baseline	Sham post LF rTMS	Real baseline	Real post LFrTMS
All				
tNAA/tCr	1.12 (0.18)	1.13 (0.21)	1.19 (0.15)	1.24 (0.13)
tCho/tCr	0.22 (0.04)	0.21 (0.04)	0.22 (0.02)	0.23 (0.04)
Dyskinesia				
tNAA/tCr	1.07 (0.15)	1.04 (0.17)	1.14 (0.15)	1.21 (0.14)
tCho/tCr	0.21 (0.04)	0.21 (0.05)	0.22 (0.03)	0.22 (0.04)
No Dyskenisia				
tNAA/tCr	1.17 (0.21)	1.23 (0.22)	1.24 (0.14)	1.27 (0.12)
tCho/tCr	0.23 (0.04)	0.21 (0.03)	0.22 (0.03)	0.23 (0.04)

Presented values are means and standard deviations of the ratio's of following metabolites: tNAA (sum of the absolute concentrations N-acetylaspartate + N-acetylaspartylglutamate); tCho (sum of absolute concentrations phosphocholine and glycerophosphocholine); tCr (sum of the absolute concentrations creatine + phosphocreatine).

The mixed ANCOVA for the tNAA/tCr ratio in the right pre-SMA did not show a main effect of Time and Disease duration (p's > 0.05). However, we did observe a significant main effect of Dyskinesia ($F(1,14) = 6.58$, $p = 0.02$) and Stimulation ($F(1,14) = 8.49$, $p = 0.01$). Pairwise comparisons showed a marginal significant difference ($p = 0.06$) between sham (1.13, SE = 0.03) and real stimulation (1.22, SE = 0.03) and a significant difference ($p = 0.02$) between PD patients with Dyskinesia (1.12, SE = 0.03) and those without Dyskinesia (1.23, SE = 0.03). Besides a significant 2-way interaction effect between Stimulation and Disease duration ($F(1,14) = 5.14$, $p = 0.04$), the other 2-way interactions between Time and Disease duration, between Time and Dyskinesia, between Stimulation and Dyskinesia, between Time and Stimulation, and between Dyskinesia and Disease duration were not significant (p's > 0.05). Also, the 3-way interaction effects between Time, Stimulation, and Disease duration, and between Time, Stimulation, and Dyskinesia were not significant (p's > 0.05).

The mixed ANCOVA for the tCho/tCr ratio in the right pre-SMA showed a significant main effect of Time ($F(1,14) = 4.70$, $p = 0.048$), but not for Stimulation, Dyskinesia, and Disease duration (p's > 0.05). We observed a significant 2-way interaction effect between Time and Disease duration ($F(1,14) = 6.95$, $p = 0.02$) and between Time and Stimulation ($F(1,14) = 19.30$, $p < 0.01$), but not for the remaining 2-way interaction effects (p's > 0.05). However,

although the 3-way interaction effect between Time, Stimulation, and Dyskinesia was not significant ($F(1,14) = 1.95$, $p = 0.18$), we found a significant 3-way interaction effect between Time, Stimulation, and Disease duration ($F(1,14) = 16.46$, $p < 0.01$).

To further examine this significant three-way interaction effect, we calculated the differences in tCho/tCr changes before and after stimulation for real and sham LF-rTMS separately (real delta tCho/tCr = real post tCho/tCr – real pre tCho/tCr; sham delta tCho/tCr = sham post tCho/tCr – sham pre tCho/tCr), and the neurometabolic differences between real and sham stimulation separately (delta pre tCho/tCr = real pre tCho/tCr – sham pre tCho/tCr; delta post tCho/tCr = real post tCho/tCr – sham post tCho/tCr). Correlation analyses showed a significant negative association between Disease duration and the change after one real LF-rTMS session (real delta tCho/tCr) ($r = -0.73$, $p < 0.01$) (see Fig. 2A), indicating that the longer duration of PD, the less this affects tCho/tCr in the right pre-SMA. In line with this assumption, the correlation analysis between Disease duration and the difference between having had one real or one sham LF-rTMS session (delta post tCho/tCr) showed that the longer the presence of PD the less the effect of one real LF-rTMS session on the right pre-SMA tCho/tCr ratio ($r = -0.59$, $p = 0.01$) (see Fig. 2B). No significant correlations were observed between Disease duration and the change after one sham LF-rTMS session (sham delta tCho/tCr) ($r = -0.26$, $p = 0.31$).

4. Discussion

Although the effect of LF rTMS on brain metabolites has been studied in healthy volunteers and tinnitus (Cacace et al., 2017; Bridges et al., 2018), to our knowledge, this is the first study investigating the effects of LF rTMS on brain metabolism in PD patients. We evaluated the effect of a single session of right pre-SMA LF rTMS on tNAA/tCr and tCho/tCr pre-SMA ratios in late stage Parkinson's disease patients in their practically defined OFF state.

Although we found a main effect of Stimulation and Dyskinesia, there was no significant interaction with Time, suggesting that one session of LF rTMS does not affect tNAA/tCr ratios. These observations imply that, at least in this population, one LF rTMS session appears to be a safe intervention showing no negative effects on neuronal integrity. The significant 2-way interaction effect between Stimulation and Disease duration insinuates that unrelated to Time, the duration of PD illness is related to a difference between sham and real stimulation. However, because we always measured before and after LF-rTMS stimulation (real and sham), and we used a within subject's design (so no difference in groups), this can only be coincidence (probably due to a random difference between sham and real tNAA/tCr ratios). Since we did not include a matched healthy control population in our study, we cannot comment on whether the tNAA/tCr ratio in our patients is different from controls. We did in our population, however, find a trend towards lower baseline pre-SMA tNAA/tCr ratios in PD patients with dyskinesias. Since it has been shown that the tNAA/tCr ratio in the pre-SMA in early untreated PD patients is not different from healthy controls (Martin et al., 2008), but that it is significantly reduced in a more advanced treated PD population (Camicioli et al., 2007), our findings indicate that our patients suffering from dyskinesias are in a slightly more advanced PD stage, which is not reflected in their other baseline characteristics (see Table 1).

The effect of one session of 1 Hz rTMS at the right pre-SMA on pre-SMA tCho/tCr ratio is more complex. Unrelated to the presence of dyskinesias, real LF rTMS (and not sham) significantly affected the tCho/tCr ratio, but only when taking disease duration into account. The shorter the PD duration, the stronger the observed effects. This indicates that in the early stages of the advanced stages of PD, membrane turnover (breakdown and/or synthesis) at the pre-SMA could still be influenced by one session of real LF rTMS, suggesting that at least some brain plasticity is still preserved. Most of the brain's choline is bound to membrane phospholipids, such as phosphatidylcholine, and not moving freely in solution, therefor largely invisible on ^{1}H MRS (Klein, 2000). Increased phosphocholine formation from free choline may indicate de novo membrane biosynthesis, but also an increased membrane breakdown, releasing membrane bound choline into the cytosol. This assumption is in line with the observation that a dopaminergic treatment is able to restore the Cho/Cr ratio in the motor cortical regions (Lucetti et al., 2007). Our LF rTMS findings also indicate that not the presence of levodopa induced dyskinesias is of importance but the disease duration. This is aligned with findings in other neuropsychiatric illnesses that disease duration is a negative influential factor for beneficial clinical outcome after rTMS (Wu and Baeken, 2017).

There are several limitations to our study. First, we chose to evaluate the effect of an intervention (before and after LF rTMS)

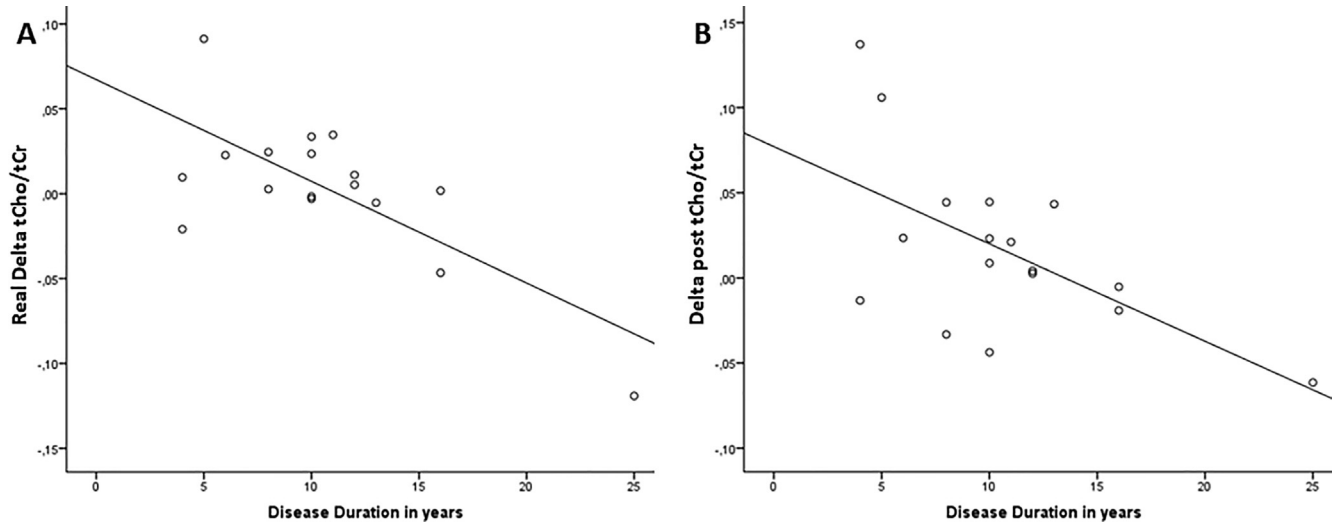

Fig. 2. Influence of disease duration on the effect of one session of real LF rTMS on pre-SMA tCho/tCr ratios. (A) Significant negative association between Disease duration and the change after one real LF-rTMS session (real delta tCho/tCr) (r = −0.73, p < 0.01). (B) Significant negative correlation between Disease duration and the difference between having had one real or one sham LF-rTMS session (delta post tCho/tCr) (r = −0.59, p = 0.01).

on pre-SMA tNAA/tCr and tCho/tCr ratio's in late stage Parkinson's disease patients, in their practically defined OFF state. Because we did not include a group of PD patients on levodopa during the experiment, we cannot comment on its influence on the neurometabolites examined. Although we observed a marginally main effect of Stimulation - with increased tNAA/tCr ratios after real LF rTMS - there was no significant interaction with Dyskinesia. This indicates that one session of LF rTMS does not affect patients with or without levodopa induced dyskinesias differently. Notwithstanding that this observation may suggest that real LF-rTMS on the right preSMA could locally enhance metabolic processes, whether this results in clinical improvements remains to be determined in larger patient samples treated with multiple LF rTMS sessions. It has been demonstrated that in early untreated and more advanced, but treated, PD patients the pre-SMA Cho/Cr ratio is not different from healthy controls (Camicioli et al., 2007; Martin et al., 2008). Since we did not include a healthy control population, comparison with previous studies is difficult. Second, although the selection of one a priori chosen brain area implicated in the pathophysiology of PD and the well-defined selection of our PD sample must be considered as study strengths, the relatively small sample limits general conclusions. Third, a multiple session rTMS approach could have yielded larger effects on metabolites. Fourth, although we deduce that tCho/tCr ratio increases are related to potential neuroplastic processes, this increased ratio could also point to increased membrane breakdown, releasing membrane bound choline into the cytosol. And last, since it was not possible to trace back the spectro volume on the anatomical scan, we did not correct for partial volume effects.

To summarize, one LF rTMS session at the right pre-SMA in late stage Parkinson patients does not alter neuronal integrity, but influences membrane turnover, inversely correlated with disease duration. Importantly, this neurophysiological effect was not dependent on the presence of dyskinesias. Given the relatively small sample size, even if we did not observe serious adverse advance clinically and on the brain level, we cannot firmly conclude that our LF-rTMS is a safe procedure. Although our sample was very similar to other studies applying single rTMS sessions in PD patients, clearly larger clinical studies with different neurobiological measurements are needed. Because the effect of LF rTMS on tCho/tCr ratio seems more important in patients with shorter disease duration, PD patients may benefit more from this non-invasive neurostimulation in the earlier stages of the disease. Of course, larger LF rTMS treatment studies applying multiple sessions, targeting the pre-SMA are needed to substantiate these assumptions.

Declaration of Competing Interest

None.

Acknowledgements and study funding

This research was supported by a grant from the Scientific Fund Willy Gepts UZBrussel.

Ethical standards

This study has been reviewed by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients gave their informed consent prior to their inclusion in the study.

References

Baeken C, Lefaucheur JP, Van Schuerbeek P. The impact of accelerated high frequency rTMS on brain neurochemicals in treatment-resistant depression: insights from 1H MR spectroscopy. Clin. Neurophysiol. 2017;128:1664–72.

Bridges NR, McKinley RA, Boeke D, Sherwood MS, Parker JG, McIntire LK, Nelson JM, Fletchall C, Alexander N, Mc Connell A, Goodyear C, Nelson JT. Single session low frequency left Dorsolateral Prefrontal Transcranial Magnetic Stimulation changes in neurometabolite relationships in healthy humans. Front. Hum. Neurosci. 2018;26(12):1–13. https://doi.org/10.3389/fnhum.2018.00077.

Brusa L, Versace V, Koch G, Iani C, Stanzione P, Bernardi G, Centonze D. Low frequency rTMS of the SMA transiently ameliorates peak-dose LID in Parkinson's disease. Clin. Neurophysiol. 2006;117:1917–21.

Brys M, Fox MD, Agarwal S, Biagoni M, Dacpano G, Kumar P, Pirraglia E, Chen R, Chin MBB, Wu A, Fernandez H, Wagle Shukla A, Lou JS, Gray Z, Simon DK, Di Rocco A, Pascual-Leone A. Multifocal repetitive TMS for motor and mood symptoms of Parkinson disease. Neurology 2016;87:1907–15.

Cacace AT, Hu J, Romero S, Xuan Y, Burkard RF, Tyler RS. Glutamate is down-regulated and tinnitus loudness-levels decreased following rTMS over auditory cortex of the left hemisphere: a prospective randomized single-blinded sham-controlled cross-over study. Hear. Res. 2017;S0378–5955(17):30487–92. https://doi.org/10.1016/j.heares.2017.

Camicioli RM, Hanstock CC, Bouchard TP, Gee M, Fisher NJ, Martin WR. Magnetic resonance spectroscopic evidence for presupplementary motor area neuronal dysfunction in Parkinson's disease. Mov. Disord. 2007;22:382–6.

Cerasa A, Koch G, Donzuso G, Mangone G, Morelli M, Brusa L, et al. A network centred on the inferior frontal cortex is critically involved in levodopa-induced dyskinesias. Brain 2015;138:414–27.

Cerasa A, Morelli M, Augimeri A, Salsone M, Novellino F, Gioia MC, Arabia G, Quattrone A. Prefrontal thickening in PD with levodopa-induced dyskinesias: New evidence from cortical thickness measurement. Park. Relat. Disord. 2013;19:123–5.

Cerasa A, Pugliese P, Messina D, Morelli M, Gioia MC, Salsone M, Novellino F, Nicoletti G, Arabia G, Quattrone A. Prefrontal alterations in Parkinson's disease with levodopa-induced dyskinesia during fMRI motor task. Mov. Disord. 2012;27:364–71.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997;48:1398–403.

Ciurleo R, Di Lorenzo G, Bramanti P, Marino S. Magnetic Resonance Spectroscopy: an in vivo molecular imaging biomarker for Parkinson's Disease? Biomed Res. Int. 2014:519816. https://doi.org/10.1155/2014/519816.

Cohen OS, Rigbi A, Yahalom G, Warman-Alaluf N, Nitsan Z, Zangen A, Hassin-Baer S. Repetitive deep TMS for Parkinson Disease: a 3-month double-blind, randomized sham-controlled study. J. Clin. Neurophysiol. 2018;35:159–65.

Ding XQ, Maudsley AA, Sabati M, Sheriff S, Schmitz B, Schütze M, Bronzlik P, Kahl KG, Lanfermann H. Physiological neuronal decline in healthy aging human brain-an in vivo study with MRI and short echo time whole brain 1HMR spectroscopic imaging. Neuroimage 2016;137:45–51.

Eckert T, Peschel T, Heinze HJ, Rotte M. Increased pre-SMA activation in early PD patients during simple self-initiated hand movements. J. Neurol. 2006;253:199–207.

Fahn S, Elton RL. UPDRS Development Committee. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, editors. Recent Developments in Parkinson's Disease. Florham Park, NJ: Macmillan; 1987. p. 153–63.

Filipovic SR, Rothwell JC, van de Warrenburg BP, Bhatia K. Repetitive transcranial magnetic stimulation for levodopa-induced dyskinesias in Parkinson's disease. Mov. Disord. 2009;24:246–53.

Flamez A, Cordenier A, De Raedt S, Michiels V, Smetcoren S, Van Merhaegen-Wieleman A, Parys E, De Keyser J, Baeken C. Bilateral low frequency rTMS of the primary motor cortex may not a be suitable treatment for levodopa-induced dyskinesias in late stage Parkinson's disease. Park. Relat. Disord. 2016;22:54–61.

Guan J, Rong Y, Wen Y, Wu H, Qin H, Zhang Q, Chen W. Detection and application of neurochemical profile by multiple regional 1H-MRS in Parkinson's disease. Brain Behav. 2017;7:e00792. https://doi.org/10.1002/brb3.792.

Herz DM, Haagensen BN, Christensen MS, Madsen KH, Rowe JB, Lokkegaard A, Siebner HR. The acute brain response to levodopa heralds dyskinesias in Parkinson's disease. Ann. Neurol. 2014;75:829–36.

Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. Neurology 1967;17:427–42.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinical pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 1992;55:181–4.

Klein J. Membrane breakdown in acute and chronic neurodegeneration: focus on choline-containing phospholipids. J. Neural Transm. 2000;107:1027–63.

Koch G, Brusa L, Caltagirone C, Peppe A, Oliveri M, Stanzione P, Centonze D. rTMS of supplementary motor area modulates therapy-induced dyskinesias in Parkinson disease. Neurology 2005;65:623–5.

Lefaucheur JP, Drouot X, Von Raison F, Ménard-Lefaucheur I, Cesaro P, Nguyen JP. Clin. Neurophysiol. 2004;115:2530–41.

Levin BE, Katzen HL, Post J, Myerson C, Govindaraju V, Maudsley A, Nahab F, Scanlon B, Mittel A. Whole-brain MR spectroscopic imaging in Parkinson's Disease. J. Neuroimaging 2014;24:39–44.

Lucetti C, Del Dotto P, Gambaccini G, Ceravolo R, Logi C, Berti C, Rossi G, Bianchi MC, Tosetti M, Murri L, Bonuccelli U. Influences of dopaminergic treatment on motor cortex in Parkinson disease: a MRI/MRS study. Mov. Disord. 2007;22:2170–5.

MacDonald V, Halliday GM. Selective loss of pyramidal neurons in the Pre-supplementary motor cortex in Parkinson's disease. Mov. Disord. 2002;17:1166–73.

Martin WR, Wieler M, Gee M, Hanstock CC, Camicioli RM. Intact Presupplementary motor area function in early untreated Parkinson's disease. Mov. Disord. 2008;23:1756–9.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9(1):97–113.

Peleman K, Van Schuerbeek P, Luypaert R, Stadnik T, De Raedt R, De Mey J, Bossuyt A, Baeken C. Using 3D-MRI to localize the dorsolateral prefrontal cortex in TMS research. World J. Biol. Psychiatry 2010;11:425–30.

Rascol O, Sabatini U, Brefel C, Fabre N, Rai S, Senard JM, Celsis P, Viallard G, Montastruc JL, Chollet F. Cortical motor overactivation in parkinsonian patients with L-dopa-induced peak-dose dyskinesia. Brain 1998;121:527–33.

Rackayova V, Cudalbu C, Pouwels PJW, Braissant O. Creatine in the central nervous system: from magnetic resonance spectroscopy to creatine deficiencies. Anal. Biochem. 2017;529:144–57.

Rossi S, Hallett M, Rossini PM. Pascual-Leone and the Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 2009;120:2008–39.

Schmitz B, Wan X, Barker PB, Pilatus U, Bronzlik P, Dadak M, Kahl KG, Lanfermann H, Ding XO. Effects of aging on the human brain: a proton and phosphorus MR spectroscopy study at 3T. J. Neuroimaging 2018;28:416–21.

Wagle-Shukla A, Angel MJ, Zadikoff C, Enjati M, Gunraj C, Lang AE, Chen R. Low-frequency repetitive transcranial magnetic stimulation for treatment of levodopa-induced dyskinesias. Neurology 2007;68:704–5.

Wilson M, Reynolds G, Kauppinen RA, Arvanitis TN, Peet AC. A constrained least-squares approach to the automated quantitation of in vivo ^{1}H magnetic resonance spectroscopy data. Magn. Reson. Med. 2011;65:1–12. https://doi.org/10.1002/mrm.22579.

Wu GR, Baeken C. Longer depressive episode duration negatively influences HF-rTMS treatment response: a cerebellar metabolic d eficiency? Brain Imaging Behav. 2017;11:8–16.

Wu G, Shen YJ, Huang MH, Xing Z, Liu Y, Chen J. Proton MR Spectroscopy for monitoring pathologic changes in the substantia nigra and globus pallidus in Parkinson disease. AJR Am. J. Roentgenol. 2016;206:385–9.

Zamir O, Gunraj C, Ni Z, Mazzella F, Chen R. Effects of theta burst stimulation on motor cortex excitability in Parkinson's disease. Clin. Neurophysiol. 2012;123:815–21.