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

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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 (1H-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.

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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
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 dyskineticains even in the multiple session treatment paradigm (Flamez et al., 2016). Since the pathophysiological mechanisms of levodopa induced dyskinetias 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 cortico-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 increased 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 (1H-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 biochemical values as tNAA/tCr and tCho/tCr (Rackayova et al., 2017). 1H-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 were reviewed by Ciurleo and colleagues providing an overview of the pre-SMA NAA/Cr and Cho/Cr ratios were reduced in cortical-basal ganglia networks, reflecting 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 (Camicioi 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-control 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 dyskinetias, 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 (UPDRS III) 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 (UPDRS IV) (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 dyskinetias were rated prior to the imaging procedure, using the UPDRSIII and UPDRS IV scales, respectively (Fahn and Elton, 1987). Handness 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 1H 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 1H MRS session. Following a cross-over design, patients were randomized to first receive real
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 supplementary motor area. The position of the TMS coil on the participant’s head to target the pre-SMA, was determined using the supplementary motor area. The position of the TMS coil on the par-tial 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 1H MR spectroscopy measurements, only a priori hypotheses were tested. First, we calculated the total tNAA/tCr ratio (t(NAA + NAAG)/(tCr + PCr)) and the total tCho/tCr ratio (t(GPC + PCh)/(tCr + 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. Dyskine-sia (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 (Camiacioli 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, dis-ease duration, UPDRSIII in OFF and equivalent levodopa dosages between patients with or without dyskinesias (p’ > 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 = 0.08), 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.

### Table 1

<table>
<thead>
<tr>
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<th>All</th>
<th>Dyskinesia</th>
<th>No dyskinesia</th>
<th>p-values</th>
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<tbody>
<tr>
<td>Gender (female/male)</td>
<td>6/11</td>
<td>4/5</td>
<td>2/6</td>
<td>0.40**</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.94 (10.17)</td>
<td>72.89 (9.85)</td>
<td>68.75 (10.71)</td>
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<td>Disease duration (years)</td>
<td>10.59 (5.18)</td>
<td>10.11 (4.37)</td>
<td>11.13 (6.24)</td>
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<td>Hoehn and Yahr OFF</td>
<td>2 (2)</td>
<td>3 (2)</td>
<td>2 (1)</td>
<td>0.335</td>
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<td>UPDRSIII OFF</td>
<td>23.00 (6.98)</td>
<td>25.78 (7.79)</td>
<td>19.87 (4.58)</td>
<td>0.081</td>
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<tr>
<td>Daily equivalent levodopa dose (mg)</td>
<td>880.59 (413.77)</td>
<td>876.67 (410.09)</td>
<td>885.00 (446.19)</td>
<td>0.969</td>
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<tr>
<td>RMT (%)</td>
<td>56.76 (4.33)</td>
<td>55.22 (3.77)</td>
<td>58.50 (4.50)</td>
<td>0.12*</td>
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<tr>
<td>Baseline tNAA/tCr</td>
<td>1.15 (0.11)</td>
<td>1.11 (0.10)</td>
<td>1.21 (0.11)</td>
<td>0.06*</td>
</tr>
<tr>
<td>Baseline tCho/tCr</td>
<td>0.22 (0.03)</td>
<td>0.22 (0.03)</td>
<td>0.22 (0.03)</td>
<td>0.57*</td>
</tr>
</tbody>
</table>

*These values are calculated as the mean of the 2 baselines measures.

**Chi-square test.

* Independent t-test.

* Mann-Whitney test.
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/ 
\[ tCr = \text{real post tCho/tCr} - \text{real pre tCho/tCr}; \]
\[ \text{sham delta tCho/tCr} = \text{sham post tCho/tCr} - \text{sham pre tCho/tCr}, \]
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).

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 neuronemetabolites examined. Although we observed a marginally main effect of Stimulation - with increased tNAA/tCr ratio's 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 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 (Camicioni 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 TMS 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 track 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 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.

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