Metabolic changes induced by theta burst stimulation of the cerebellum in dyskinetic Parkinson's disease patients

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Abstract
Background: Cerebellar repetitive transcranial magnetic stimulation may be effective in reducing peak-dose levodopa induced dyskinesia in Parkinson's disease patients. It was proposed that the antidyskinetic effect could be due to modulation of cerebello-thalamo-cortical pathways. However the neural basis for these clinical effects has not yet been demonstrated.

Methods: We investigated the effects of repeated sessions of cerebellar continuous theta burst stimulation (cTBS) in Parkinson's disease patients with levodopa induced dyskinesia on brain metabolism by means of positron emission tomography scan with fluorodeoxyglucose (18F-FDG) to characterize the specific cerebral network activated by cerebellar stimulation in these patients.

Results: We found that five days of bilateral cerebellar continuous theta burst stimulation (cTBS) were effective in reducing levodopa induced dyskinesia. Clinical changes were paralleled by a reduction of 18F-FDG metabolism in the cerebellum as revealed by positron emission tomography imaging. We found a global decrease in the metabolism of the bilateral cerebellar hemispheres, and a significant decrease in 18F-FDG uptake in correspondence of bilateral dentate nucleus.

Conclusions: Our study demonstrates the antidyskinetic effect of cerebellar cTBS in Parkinson's disease patients with levodopa induced dyskinesia, is paralleled by modulation of the activity of the pathways connecting the cerebellar cortex with the deep cerebellar nuclei, confirming the hypothesis that the motor cerebellar circuit is involved in the generations of levodopa induced dyskinesia.

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1. Introduction

Long term therapy with levodopa and dopamine agonists in Parkinson’s disease (PD) patients is complicated by the development of fluctuations in motor response, such as levodopa induced dyskinesia (LID) [1,2]. Repetitive Transcranial Magnetic Stimulation (rTMS) has been recently put forward as a possible therapeutic tool able to reduce LID in PD. Trains of 1 Hz rTMS applied either over the supplementary motor area (SMA) or the primary motor cortex (M1) were able to induce a transient reduction in the severity of LID, suggesting that an over-activity of these areas plays a crucial role in the pathophysiology of LID [3–7]. However, repeated sessions of rTMS were not effective in inducing persistent beneficial clinical effects [4]. Functional or metabolic changes have been reported in the cerebellum in studies in PD patients treated with procedures known to alleviate LID, such as deep brain stimulation (DBS) or pallidotomy of the Globus Pallidus (Gpi) [8–10]. Furthermore a recent PET study showed in a sample of PD patients that underwent stereotactic pallidotomy, that the level of binding potential of cerebellar sigma-receptors did not correlate with Hoehn and Yahr (H & Y) stages and the Unified Parkinson’s Disease Rating Scale (UPDRS), but a strong positive correlation was seen between the binding potential and the preoperative LID severity score, suggesting that cerebellar sigma-receptors may potentially involve the...
were calculated every 15 min for 1 h (t0, t15, t30, t45, t60). Two blinded raters
top and lower limbs, and was scored as follows: 0, none; 1, mild; 2, moderate; 3,
scale[16]. LID was assessed individually in the face, neck, trunk, and right and left

equivalent dose as immediate release levodopa/carbidopa. The assessment in each

Therapies [CAPSIT]) and had been fasting since the night before. After overnight

rTMS. Patients took regular medications during the study except on days of levodopa

fixation for at least one

month prior and during the study. Inclusion criteria were: stable medication dose

for 4 weeks, and LID for up to four weeks after the end of the daily stimulation

were submitted to a one week course of bilateral cerebellar cTBS. There were

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cTBS induced persistent clinical beneﬁts, reducing peak-dose

magnetic stimulus had a biphasic waveform with a pulse width of about 300 μs.

During the first phase of the stimulus, the current in the center of the coil flowed

toward the handle. Three-pulse burst at 50 Hz repeated every 200 ms for 40 s

(equivalent to continuous theta burst stimulation-cTBS[17] were delivered at 80% of

the active motor threshold (AMT) over the lateral cerebellum (600 pulses)). AMT

for the first dorsal interosseous (FDI) was tested over the motor cortex of the hemi-

sphere contralateral to each individual patient’s side in which dyskininesias were

predominant.

cTBS was applied over the lateral cerebellum using the same scalp co-ordinates.

(1 cm inferior and 3 cm left/right to the inion) adopted in previous studies, in which

MRI reconstruction and neuronavigation systems showed that cerebellar cTBS in

this site predominantly target the posterior and superior lobules of the lateral

cerebellum [18,19]. Although cerebellar stimulation has been originally performed with a
double cone coil [20] we used the ﬁgure-of-eight coil, since this approach has

been adopted in previous investigations in which cerebellar rTMS was shown to be

effective in modulating the excitability of the contralateral motor cortex [13]. The

coil was positioned tangentially to the scalp, with the handle pointing superiorly.

This orientation is able to modulate contralateral M1 excitability [11]. PD patients

were submitted to a one week course of bilateral cerebellar cTBS. There were

ﬁve days of stimulation (ﬁve days per week, Monday to Friday). Cerebellar cTBS was

applied daily at the same hour in the morning (9 a.m.) for each patient. Two trains of

cTBS (80%AMT, 600 pulses, duration 40 s) were applied over the left and right lateral

cerebellum with a pause of 2 min between the two trains. The order of stimulation

was pseudo-randomized in each subject in every session. The total duration of every
daily session was of approximately 4 min. Sham stimulation was delivered through a

70 mm ﬁgure-of-eight focal coil angled at 90° with only the edge of the coil resting on

the scalp. Stimulus intensity, expressed as a percentage of the maximum stimu-

lator output (MSO), was set only at 40% AMT for the FDI. This stimulation intensity

along with the tilted arrangement of the ﬁgure-of-eight focal coil, while ineffective in

inducing any cortical activation or unpleasant sensations [21–25], ensures an ade-

quate noise and scalp sensation. Evaluation of dyskinesias and UPDRS was per-

formed on day 1, before starting the ﬁrst session of stimulation (pre-cTBS) and the

Monday after the week of stimulation (post-cTBS), in the same days in which PET

scanning was performed. cTBS and sham sessions were performed at least three

months apart in order to allow for repeated PET scanning. The order of presentation

(cTBS or Sham) was counterbalanced across subjects. Therapy was maintained stable
during the entire period of the study.

2.2. PET

Each patient was placed supine on a bed and an intravenous line was established. Patients were scanned in off dopaminergic medications. Blood glucose levels were monitored in all patients before the tracer injection. 18F-ﬂuorodeoxyglucose (200 MBq) was i.v. administered in a dimly lit room with minimal background noise. Scanning began 40 min after injection. Scans were acquired using a PET/CT scanner Discovery ST (GE Medical Systems, Milwaukee, WI), following a standardized procedure [26] A scout scan projection was used to center brain acquisition. Then a low-dose CT scan (60 mA, 120 KV) CT scan was performed for attenuation correction of PET data. A 3D FDG PET scan was acquired for 10 min. PET data were reconstructed using FBP, a 128 × 128 matrix size and a 25 cm FOV (pixel size 1.95 × 1.95 mm and slice thickness 3.27 mm). After reconstruction spatial resolution was approximately 6 mm full-width at half maximum (FWHM) over all planes. Data were then reor-

iented along the anterior commissure–posterior commissure line, as best inferred by visual inspection approximating the orientation of the SPM PET template, and thus improving the registration accuracy. Reoriented transverse slices were ﬁnally exported to a Windows-based personal computer (Microsoft, Redmond, WA, USA) and converted to the Analyze format using the software package MBirco (http://

www.uab.es/comp/medimirco.html). PET scans were performed for each subject in the same experimental paradigm under the following conditions: at baseline (pre-cTBS), and after cerebellar cTBS (post-cTBS); for the same subjects such experimental paradigm was then repeated (at most three months apart) in the daily protocol that included a basal scan cTBS (pre-Sham) and another scan performed after sham cerebellar cTBS (post-Sham).

2.3. Data analysis

Non-parametric Wilcoxon test were applied on mean CAPSIT dyskinesia scale and UPDRS scores for each session. For statistical analyses, a p value of 0.05 was considered to be signiﬁcant. Mauchley’s test examined for sphericity. The Greenhouse-Geisser correction was used for non-spherical data. PET data analysis. Spatial pre-processing and statistical analysis were performed using the SPM2 software (Institute of Neurology, University College of London, London, U.K.) implemented in Matlab 7.3 (The MathWorks, Inc., Natick, MA). For each patient TMS-

### Table 1

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Medication</th>
<th>Duration of PD (years)</th>
<th>Duration of dyskinesia (years)</th>
<th>UPDRS score item 32</th>
<th>UPDRS score item 33</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70/F</td>
<td></td>
<td>Levodopa (600)</td>
<td>14</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>62/M</td>
<td></td>
<td>Levodopa (850)</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>65/M</td>
<td></td>
<td>Levodopa (300)</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>61/F</td>
<td></td>
<td>Levodopa (1000)</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>67/M</td>
<td></td>
<td>Levodopa (800)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>75/M</td>
<td></td>
<td>Levodopa (800)</td>
<td>13</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>63/F</td>
<td></td>
<td>Levodopa (900)</td>
<td>11</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
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<td>59/F</td>
<td></td>
<td>Levodopa (800)</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

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 genesis of LID in advanced PD [11]. Therefore, the effects of rTMS applied over the lateral cerebellum have been recently tested in patients with LID. A two-week course of bilateral cerebellar continuous theta burst stimulation (cTBS), a novel form of rTMS, induced persistent clinical beneficial effects, reducing peak-dose LID for up to four weeks after the end of the daily stimulation period [12]. Moreover cerebellar cTBS changed the profile of activation of intracortical circuits in the contralateral primary motor cortex (M1), reﬂecting the long lasting modulation of motor cortical excitability driven by activation of cerebello-thalamo-cortical pathways [13,14]. Thus the effects of cerebellar cTBS were hypothesized to depend on the above described proﬁle of motor cortical excitability, that could represent the cortical reorganization that is associated with a reduction of LID. However, cerebellar cTBS may have expressed its antidyskinetic effect through a direct modulation of the excitability of the cerebellar cortex or through remote changes in other interconnected brain areas. Therefore, in the present study, we aimed to investigate the effects of repeated sessions of cerebellar cTBS in dyskinetic PD on brain metabolism by means of PET scan with FDG at the aim to characterize the speciﬁc cerebral network activated by cerebellar stimulation in these patients.

2. Materials and methods

2.1. Cerebellar cTBS

Eight advanced PD patients suffering from disabling peak-dose dyskinesias following levodopa ingestion were enrolled (see Table 1). Diagnosis of idiopathic PD was made according with Brain Bank Criteria [15]. Anti-parkinsonian medications producing the best control of PD and LID symptoms were ﬁxed for at least one month prior and during the study. Inclusion criteria were: stable medication dose for 4 weeks, and LID >25% of waking hours (item 32 of UPDRS ≥ 2) and bothothersme (item 33 ≥ 2). Exclusion criteria were previous PD surgery and contraindications to rTMS. Patients took regular medications during the study except on days of levodopa challenge test. Informed consent and Ethics Board approval were obtained. Patients were in withdrawal of therapy (Core Assessment Program for Surgical Interventional Therapies [CAPSIT]) and had been fasting since the night before. After overnight medication withdrawal, patients received 125% of their usual morning levodopa equivalent dose as immediate release levodopa/carbidopa. The assessment in each video-recorded session consisted of a complete UPDRS III and CAPSIT dyskinesia scale [16]. LID was assessed individually in the face, neck, trunk, and right and left upper and lower limbs, and was scored as follows: 0, none; 1, mild; 2, moderate; 3, severe; and 4, extreme (0–28). After levodopa administration, UPDRS III and CAPSIT were calculated every 15 min for 1 h (t0, t15, t30, t45, t60). Two blinded raters
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of FDG PET data were spatially realigned to TMS-on data by using a rigid body transformation as well as sham-TMS-off realigned to sham-TMS-On FDG PET data. Each realigned PET volumes were then spatially normalized into the Montreal Neurological Institute (McGill University, Montreal, Quebec, Canada) standard templates by affine transformation (12 parameters for rigid transformations, zooms, and shears) and nonlinear transformations using the relative mean volume as source. Normalized data were smoothed by convolution with an isotropic Gaussian kernel with a 8-mm full-width at half maximum to increase the signal-to-noise ratio. The effects of global metabolism were removed by normalizing the count of each voxel to the total count of the brain using proportional scaling. The following comparisons (post-cTBS versus pre-cTBS; pre-Sham versus post-Sham images) were performed by using a paired t test based on 2 contrasts (1–1) to detect any regional increase or decrease in metabolism in relation to the two explored different conditions. At a voxel height threshold that had a probability value \( p < 0.005 \) without a correction for multiple comparisons, clusters consisting of a minimum of 100 contiguous voxels were considered significantly different. Results were displayed on the three orthogonal planes of a MRI template.

3. Results

The procedure was well tolerated by the PD patients. No adverse effect was reported. The mean patients’ AMT taken with the Mag-Stim Super Rapid magnetic stimulator was 43 ± 2.4% MSO. We found in this experiment that bilateral cerebellar cTBS was effective in reducing LIDs when applied with repeated sessions during one week. Wilcoxon test revealed that after one week of bilateral cerebellar cTBS global CAPSIT dyskinesia scale scores were decreased in comparison with baseline pre-cTBS evaluation at t15 (\( Z = -2.53; p = 0.011 \)), at t30 (\( Z = -2.72; p = 0.006 \)), t45 (\( Z = -2.69; p = 0.007 \)) and at t60 (\( Z = -2.53; p = 0.011 \)) (Fig. 1a). No changes were observed in the patients group submitted to sham TBS (Fig. 1b). Motor abilities scored by the mean UPDRS section III were not modified by any cTBS condition as revealed by Wilcoxon test. SPM analysis of post-cTBS versus pre-cTBS scans disclosed a change in a cluster of cerebellar domains including bilateral cerebellar hemispheres and the deep cerebellar nuclei. A significant decreased glucose utilization (post-cTBS versus pre-cTBS) was found in a cluster of voxels within inferior vermis (46, –68, –34) and bilaterally the inferior semilunar lobule (46, –64, –38 right; –51, –65, –38 left), and dentate nucleus (10, –54, –30 right; –16, –54, –21 left) (Fig. 2). No changes were detected in other cerebral areas interconnected with the cerebellum. We did not find any significant correlation between the individual changes in glucose utilization in the cerebellar cluster of voxels within the inferior vermis, the inferior semilunar lobules and the dentate nucleus and the decrease of the global CAPSIT dyskinesia scale scores. No significant change (neither increase or decrease) of FDG uptake was observed when post-Sham condition was compared with pre-Sham condition.

4. Discussion

The present study confirms and extends our previous results demonstrating the antidysskinetic effect of cerebellar cTBS in PD patients with LID [12]. We found that a week of bilateral cerebellar cTBS was able to induce a reduction of LID; clinical changes were paralleled by a reduction of FDG metabolism in the cerebellum as revealed by PET imaging. Here, we addressed the question of whether cerebellar cTBS may have expressed its antidysskinetic effect through a direct modulation of the cerebellum or through remote changes in other interconnected brain areas. The current findings seem to indicate that cerebellar cTBS exerts its clinical effect through substantial changes in the metabolism of cerebellar areas. In particular we found a global decrease in the metabolism of the bilateral cerebellar hemispheres, with a strong significant decrease in FDG uptake in correspondence of the dentate nuclei. Therefore, it seems likely that cTBS may have modulated the activity of the pathways connecting the cerebellar cortex with the deep cerebellar nuclei. This is consistent with the physiology of the cerebellar-thalamo-cortical pathway activated by magnetic stimulation, that has been recently clarified [20,27]. In fact, it has been proposed that cerebellar TMS activates the Purkinje cells of the superior cerebellum; such activation results in an inhibition of the
dentate nucleus, which is known to exert a background tonic facilitatory drive onto the contralateral cerebral cortex (M1) through synaptic relay in the ventral lateral thalamus [4]. This in turn leads to an inhibition of the contralateral cerebral cortex, due to synaptic relay in the ventral lateral thalamus [4]. Therefore, our findings seem to confirm the hypothesis that the motor cerebellar circuit is involved in the generations of LIDs [12]. One intriguing possibility is that cerebellar cTBS may have expressed its antidyskinetic effect trough a direct modulation of the excitability of the cerebellar cortex. In analogy with the long term effects of cTBS when applied over the primary motor cortex [17], it is possible that cerebellar cTBS may have induced Long Term Depression (LTD)-like [29] effects in the cerebellar cortex, that could have counteracted an abnormal state of excitability. However, it has to be considered that we were not able to detect changes in other interconnected cerebral areas that were expected to be involved in the LID mechanisms, on the basis of previous imaging and neurophysiological investigations. In fact, LID can be considered the consequence of an abnormal pattern or code of activity that originates and is conveyed from the basal ganglia to the thalamus and the cortical motor areas, leading to overactivation of cortical motor and premotor areas such as the supplementary motor areas, the M1, and the premotor cortex [30–32]. It may well be that these areas indeed might contribute to the onset of LID, but could not be affected by cerebellar stimulation. In alternative, there could be subtle changes in their function that cannot be detected by FDG PET scanning following cerebellar cTBS. For instance there may very well be changes in the motor cortex (or basal ganglia) but changes in cortical inhibition and facilitation may not be be detected with FDG PET scan. In addition, it should be noted that there was a slight asymmetry of the metabolic changes induced by cTBS between the cerebellar hemispheres. There were also changes in the vermis, an area of the cerebellum might be more expected to be involved in oculomotor changes rather than those measured in a forelimb muscle. These modifications are not likely to be ascribed to the observed clinical changes. Moreover, it has to be considered that we were not able to demonstrate a direct correlation between the clinical reduction of dyskinesia and the PET metabolic changes that occurred in the cerebellum, given the relatively low number of subjects that took part in the study. Further studies using more specific PET ligands for dopaminergic receptors such as raclopride would allow to deeply investigate remote effects of cerebellar cTBS on the basal ganglia activity [33].

**Documentation of author roles**

Research project: LB, GK, PS Conception, OS, PS Organization, FM, OS Execution.


Manuscript Preparation: LB, GK Writing of the first draft, PS, CI, RC, LC Review and Critique.

**Disclosure**

The authors have no financial disclosure.

**References**


