Primary Motor Cortex LTP/LTD-like Plasticity in Probable Cortico-basal Syndrome

A. Suppa 1-2* MD, PhD, F. Di Stasio 2* MD, L. Marsili 1 MD, N. Upadhyay 1 PhD, D. Belvisi 1-2 MD, A. Conte 1-2 MD, PhD, N. Modugno 2 MD, C. Colosimo 1 MD, A. Berardelli 1-2 MD

1Department of Neurology and Psychiatry, and 2Neuromed Institute
“Sapienza” University of Rome, Italy

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Running Head: M1 LTP/LTD-like plasticity in patients with probable CBS

*These authors contributed equally to the study

Corresponding author:
Prof. Alfredo Berardelli
Department of Neurology and Psychiatry, and Neuromed Institute, Sapienza University of Rome
Viale dell’Università, 30, 00185 Rome, Italy
Telephone number: 0039-06-49914700
Fax: 0039-06-49914700
E-mail: alfredo.berardelli@uniroma1.it
ABSTRACT

Background: Whether the primary motor cortex (M1) contributes to the pathophysiology of cortico-basal syndrome (CBS) remains unclear.

Objective: In this study in patients with probable CBS, we tested whether M1 plasticity contributes to the pathophysiology of symptoms in the contralateral “less affected” limb manifesting only parkinsonism and in the contralateral “more affected” limb manifesting parkinsonism plus other motor and non motor symptoms.

Methods: In experiment 1, we applied intermittent/continuous theta-burst stimulation (iTBS/cTBS) over the M1 contralateral to the “less affected” limb in 17 patients. In experiment 2, we applied iTBS/cTBS over the M1 contralateral to the “more affected” limb in 14 out of the 17 patients. We measured iTBS/cTBS-induced plasticity as reflected by motor-evoked potential (MEP) changes. Data were compared with those obtained in 17 healthy subjects (HS).

Results: In experiment 1, TBS over the M1 contralateral to the “less affected” limb disclosed reduced plasticity in patients than in HS. In experiment 2, in 5 out of 14 patients we recorded abnormally low-amplitude MEPs preventing the evaluation of plasticity in the M1 contralateral to the “more affected” limb. In the remaining 9 out of 14 patients, TBS disclosed abnormal plasticity characterized by high inter-subject variability. In these 9 patients the response to TBS correlated with specific patients’ clinical features.

Conclusions: in patients with probable CBS, we demonstrate heterogeneous abnormalities of M1 which contribute to the pathophysiology of this condition.
ABBREVIATIONS:

AD: Alzheimer disease; AI: asymmetry index; AMT: active motor threshold; ANOVA: analysis of variance; BFMS: Burke-Fahn-Marsden Movement and Disability Scale; CBS: cortico-basal syndrome; cTBS: continuous theta-burst stimulation; EMG: electromyography; FDI: first dorsal interosseous muscle; FTD: fronto-temporal degeneration; HAMD: Hamilton Depression Scale; HS: healthy subjects; I/O curve: input/output curve; iTBS: intermittent theta-burst stimulation; LTD: long-term depression; LTP: long-term potentiation; M1: primary motor cortex; MDS-UPDRS: Movement Disorder Society-Unified Parkinson’s Disease Rating Scale; MEP: motor evoked potential; MMSE: Mini-Mental State Evaluation; MSA: multiple system atrophy; MRI: magnetic resonance imaging; PD: Parkinson’s disease; PSP: progressive supranuclear palsy; RMT: resting motor threshold; SICI: short-interval intracortical inhibition; TMS: transcranial magnetic stimulation; VBM: voxel-based morphometry.
INTRODUCTION

Cortico-basal syndrome (CBS) is characterized by parkinsonism combined with other asymmetric and heterogeneous motor (dystonia and myoclonus) and non-motor symptoms (apraxia, cortical sensory deficit and alien limb phenomena) that affect one body region alone (predominantly the arm) as well as cognitive impairment and behavioral changes (Alexander et al. 2014; Armstrong et al. 2013, 2014; Boeve et al. 2003; Kompoliti et al. 1998; Kouri et al. 2011; Stamelou et al. 2012). Clinico-pathological studies describe CBS as a complex clinical condition comprising cortico-basal degeneration (CBD) and also other neurodegenerative disorders including progressive supranuclear palsy (PSP), Alzheimer disease (AD) and fronto-temporal degeneration (FTD). Conversely, some patients manifesting neuropathological evidence of CBD may have had a life-time diagnosis of PSP, AD and FTD (Boeve et al. 1999; Chahine et al. 2014; Hu et al. 2009; Ling et al. 2010; Ouchi et al. 2014; Shelley et al. 2009).

The pathophysiology of CBS remains largely unknown. Previous neurophysiological studies in patients with CBS investigating primary motor cortex (M1) excitability with transcranial magnetic stimulation (TMS) have demonstrated a number of abnormalities of cortico-spinal excitability including decreased short-interval intracortical inhibition (SICI) suggesting reduced M1 inhibition in CBS and findings pointing to impaired transcallosal inhibition (Hanajima et al. 1996; Lu et al. 1998; Okuma et al. 2000; Pal et al. 2008; Valls-Solé et al. 2001). More recently, a voxel-based morphometry (VBM) study reported a significant correlation between SICI and the degree of M1 atrophy suggesting a pathophysiological role of M1 in motor and non motor symptoms in patients with CBS (Burrel et al. 2014). More advanced TMS techniques are now available to extend TMS studies on M1 excitability to M1 long-term potentiation (LTP)- or depression (LTD)-like plasticity.
In healthy subjects (HS), a TMS technique for investigating LTP/LTD-like plasticity entails examining long-term changes in MEPs (after-effects) after intermittent and continuous theta-burst stimulation (iTBS/cTBS). The iTBS-induced after-effects are thought to reflect LTP-like, whereas the cTBS-induced after-effects depend on LTD-like plasticity in M1 (Huang et al. 2005; Suppa et al. 2008a, 2012; Ziemann et al. 2008). LTP/LTD-like plasticity in M1 serves as a physiological mechanism for motor execution and learning (Rioult-Pedotti et al. 1998, 2000; Sanes and Donoghue 2000). Hence, in CBS, possible abnormalities in M1 LTP/LTD-like plasticity might impair motor execution and learning thus contributing to the pathophysiology of parkinsonism and other motor and non motor symptoms (Suppa et al. 2011, 2012; Belvisi et al., 2013).

No studies have yet investigated LTP/LTD-like plasticity in M1 by testing iTBS/cTBS-induced after-effects in patients with probable CBS. Nor have they compared TBS-induced after-effects in the M1 contralateral to the “less affected” limb manifesting only parkinsonism (predominantly bradykinesia and rigidity) and the “more affected” limb manifesting parkinsonism plus other motor and non motor symptoms, including dystonia, myoclonus, apraxia, cortical sensory deficit and “alien limb” phenomena, and verified the possible relationship between TBS-induced after-effects and specific patients’ clinical features. Overall this information might help in identifying the possible role of M1 plasticity in the pathophysiology of parkinsonism which typically manifest bilaterally, and additional motor or non-motor symptoms which manifest asymmetrically affecting only one limb, in patients with probable CBS.

Our aim in this study was to explore M1 LTP/LTD-like plasticity as reflected by iTBS/cTBS-induced after-effects in patients with probable CBS. We tested iTBS/cTBS-induced after-effects over the M1 contralateral to the “less affected” limb and the “more affected” limb. Finally, we assessed the possible correlation between neurophysiological
variables and specific CBS clinical features including asymmetric motor and non-motor symptoms.

**MATERIALS AND METHODS**

**Subjects**

We recruited 17 patients with probable CBS (6 men, mean age±SD: 67±6.50, range 59-85 years) and 17 age-matched HS (9 men, mean age±SD: 66±6.4, range 62-83 years). All participants were right-handed. Probable CBS was diagnosed using the Armstrong criteria for the diagnosis of CBS (Armstrong et al. 2013). Patients were recruited from the Movement Disorders Clinic at the Department of Neurology and Psychiatry, Sapienza University of Rome, Italy. In all patients magnetic resonance imaging excluded focal brain lesions and showed mild cortical atrophy prominently in frontal brain regions (Table 1). Motor signs were scored using the Movement Disorder Society-Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) (Antonini et al. 2013). The severity of dystonia was assessed with the Burke-Fahn-Marsden Movement and Disability Scale (BFMS) (Burke et al. 1985). Cognitive function was evaluated using the Mini Mental State Evaluation (MMSE) (Folstein et al. 1975) and Frontal Assessment Battery (FAB) (Dubois et al. 2000). Depression was assessed with the Hamilton Depression Rating Scale (HAM-D) (Hamilton and Guy 1976). When studied, none of the patients were receiving L-DOPA or other drugs acting on the central nervous system. Patients’ clinical features are summarized in Table 1. Subjects gave their informed consent, and the study was approved by the institutional review board and conformed with the Declaration of Helsinki.
**Stimulation techniques and Recording**

Single-pulse TMS was delivered through a Magstim 200 stimulator (Magstim Co, UK) connected to a figure-of-eight coil placed over the left or right M1 for eliciting MEPs in the contralateral first dorsal interosseous (FDI) muscle. Resting (RMT) and active (AMT) motor thresholds were calculated according to standardized techniques (Rossini et al. 1994). Twenty single pulses were delivered at the intensity able to evoke baseline MEPs at about 1-mV. MEPs were tested at the same intensity throughout the experiment.

ITBS and cTBS were delivered through a Magstim SuperRapid stimulator connected to a figure-of-eight coil placed over the left or right M1 according to standardized techniques (Huang et al. 2005). The EMG activity from the FDI muscle was recorded using surface electrodes with comparable impedance of the skin-electrode system in all participants. Signals were then amplified (Digitimer D360, Digitimer Ltd, UK) and digitized (CED 1401, Cambridge Electronic Design, UK). We collected 20 MEPs before (T0) and 5 (T1), 15 (T2), and 30 (T3) minutes after TBS. Trials with involuntary peristimulus EMG activity greater than 50 µV (in a time window of 500 ms preceding MEPs) were rejected to exclude the presence of involuntary muscular contraction. MEPs were measured and averaged.

**Experimental Design**

Patients were pseudorandomly assigned and counterbalanced to participate in two experiments (Experiment 1 and 2), each comprising two separate sessions, one for iTBS and the other for cTBS, held at least 1 week apart. In the Experiment 1, we studied the whole group of 17 patients with probable CBS and 17 healthy subjects, whereas in the Experiment 2, we examined a subgroup of 14 patients with probable CBS (patients 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16 and 17) (5 men, mean age±SD: 67±6.50, range 59-85 years) (Table 1).
**Experiment 1: TMS over the M1 contralateral to the “less affected” limb**

In the whole group of 17 patients with probable CBS, we collected 20 MEPs from the FDI muscle before (T0) and after delivering iTBS and cTBS over the M1 contralateral to the “less affected” limb manifesting only parkinsonism, at T1, T2, and T3. In the whole group of 17 HS, we decided a priori to study the left M1 and record 20 MEPs from the right FDI muscle at T0, T1, T2, and T3.

**Experiment 2: TMS over the M1 contralateral to the “more affected” limb**

In a subgroup of patients with probable CBS (n=14), we delivered iTBS and cTBS in separate sessions over the M1 contralateral to the “more affected” limb manifesting parkinsonism plus other motor and non motor symptoms and collected 20 MEPs at the same time points tested in experiment 1 (T0, T1, T2 and T3).

**Statistical analysis**

All the raw data were tested for normality by using Shapiro-Wills test and for sphericity by using Mauchly’s sphericity.

Unpaired Student’s t test or the Mann-Whitney U test (when normality is violated) was used to compare RMTs and AMTs, MEP latency, and stimulus intensity used for evoking MEPs and for TBS in HS and patients with probable CBS.

To test MEP changes in HS and patients with probable CBS after iTBS and cTBS over the M1 contralateral to the “less affected” limb and the “more affected” limb, we used a separate between-group analysis of variance (ANOVA) with “Group” (HS/CBS patients) and “Time” (T0/T1, T2 and T3) as main factors of analysis or the Kruskal-Wallis test (when normality is violated).
In the 14 patients with probable CBS participating at both Experiment 1 and 2, we evaluated the individual responses to iTBS and cTBS and calculated a ratio between MEP amplitude at T2/MEP amplitude at T0*100, after TMS over the M1 contralateral to the “less affected” limb (ΔPark) and the “more affected” limb (ΔPark+), in each CBS patient. We included the MEP amplitude at T2 in our ratio because this time-point is considered to be the time window for highest TBS-induced after-effects (Huang et al. 2005; Ziemann et al. 2008).

Next, we calculated a M1 LTP/LTD-like plasticity “asymmetry index” (AI) (Savic and Lindström 2008) for responses to iTBS and cTBS, for each patient with probable CBS, defined by AI = ΔPark− ΔPark+ /ΔPark+ ΔPark−. AI values close to 0 are considered low AI, whereas AI values close to 1 or -1 are considered high AI.

Spearman’s rank-correlation test was used to assess correlation between patients’ clinical features (age, disease duration, BFMS, MDS-UPDRS, MMSE, FAB and HAM-D scores), and neurophysiological variables including RMT, AMT, amount of TBS-induced plasticity (MEP amplitudes at T2) and M1 LTP/LTD-like plasticity AI.

Tukey’s honestly significant difference test was used for post hoc analysis.

P values <0.05 were considered to indicate statistical significance.

RESULTS

None of the participants experienced any adverse effects during or after TBS.

According to the criteria used for rejecting trials with involuntary peristimulus EMG activity greater than 50 µV, only a few trials were rejected (less than 5% on average) in HS and patients. A similar number of trials were rejected in Experiments 1 and 2.
MEP amplitudes recorded at (T0), (T1), (T2), and (T3) in HS and in patients with probable CBS, in all the experiments (Experiment 1 and 2) and sessions (iTBS and cTBS) are shown in Table 3.

*Experiment 1: TMS over the M1 contralateral to the “less affected” limb*

Unpaired t test showed comparable RMTs and AMTs, MEP latency, intensity for eliciting MEPs at T0, and TBS in the whole group of 17 patients with probable CBS and 17 HS (P>0.05 for all comparisons; Table 2) suggesting normal baseline excitability in the M1 contralateral to the “less affected” limb in CBS patients.

TBS-induced changes in MEPs differed significantly in the whole group of 17 patients with probable CBS and 17 HS, as shown by a significant “Group×Time” interaction in the iTBS (F3.96 =26.66; P<0.01) and cTBS (F3.96 = 20.14; P<0.01) sessions and a significant effect of factors “Groups” and “Time” in the iTBS (F1.32 =12.13; P<0.01 and F3.96 =34.78; P<0.01) and cTBS (F1.32 =14.21; P<0.01 and F3.96 =20.14; P<0.01) sessions. Despite similar MEPs at T0 (P>0.05 for all comparisons), after conditioning iTBS and cTBS, MEPs differed in CBS patients and HS at T1, T2, and T3 (P<0.01 for all comparisons). In HS, the effect of factor “Time” was significant after iTBS (F3.48 = 16.66; P<0.01) and cTBS (F3.48 = 27.04; P<0.01); MEPs increased after iTBS and decreased after cTBS at T1, T2, and T3 (P<0.01 for all comparisons). By contrast, in patients with probable CBS, the factor “Time”, had a non significant effect in iTBS (F3.48 = 0.95; P=0.43) and cTBS (F3.48 = 0.64; P=0.6) sessions (Fig. 1A and 1B).

*Experiment 2: TMS over the M1 contralateral to the “more affected” limb*

When considering the 14 patients with probable CBS participating at Experiment 2, RMT values increased significantly in 5 patients (patient 1, 2, 3, 5 and 12; see Table 1) compared
with values obtained in the whole group of 17 HS (P<0.01 for all comparisons) and low-amplitude MEPs (<200 µV) were virtually unrecordable and typically polyphasic in shape even at maximum stimulator output. The Mann-Whitney U test showed that RMTs were higher in these 5 patients than in HS (z=-3.24; P=0.001) and in the remaining 9 CBS patients participating in experiment 2 (z=-3.003; P=0.003). Accordingly, RMTs from these 5 patients were excluded from the subsequent analysis comparing RMT in the remaining 9 CBS patients and HS.

When we compared variables reflecting M1 excitability in the remaining 9 patients with probable CBS (patient 6, 7, 8, 9, 10, 11, 15, 16 and 17; see Table 1) and in the whole group of 17 HS, the Mann-Whitney U test showed similar RMTs and AMTs, MEP latency, intensity for eliciting MEPs at T0, and conditioning TBS (P>0.05 for all comparisons; Table 2).

In the iTBS session, the Kruskal-Wallis test comparing MEPs in the 9 patients with MEPs recorded in the whole group of 17 HS, showed similar MEPs at T0 (H=0.08; P=0.76), T1 (H=0.18; P=0.66), T2 (H=0.79; P=0.37), and T3 (H=0.26; P=0.61). In both study groups, MEPs increased in amplitude at T1, T2, and T3 (Fig. 2A). When we evaluated by visual inspection individual responses to iTBS in the 9 patients participating at Experiment 2, we found that after iTBS, MEPs increased in amplitudes in patients 9, 10, 11 and 16, whereas MEPs remained almost unchanged in patients 6, 7, 8, 15 and 17 (Fig. 2C and 2E). Accordingly, patients 9, 10, 11 and 16 were characterized by high AI, whereas patients 6, 7, 8, 15 and 17 were characterized by low AI (Table 4).

In the cTBS session, the Kruskal-Wallis test comparing MEPs in the 9 patients with MEPs recorded in the whole group of 17 HS, showed that although MEPs were similar at T0 (H=0.123; P=0.70), after cTBS, MEPs differed in patients and HS at T1 (H=14.68; P=0.001), T2 (H=17.03; P=0.0001), and T3 (H=8.18; P=0.004). After cTBS, in HS MEPs decreased at
T1, T2, and T3, whereas in CBS, MEPs increased at T1, T2, and T3 (Fig. 2B). When we evaluated by visual inspection individual responses to cTBS in the 9 patients participating at Experiment 2, we found that after cTBS, MEPs increased in amplitudes in patients 9, 10, 11 and 16, whereas MEPs remained almost unchanged in patients 6, 7, 8, 15 and 17 (Fig. 2D and 2F). As for iTBS, also in the cTBS session, patients 9, 10, 11 and 16 were characterized by high AI, whereas patients 6, 7, 8, 15 and 17 were characterized by low AI (Table 4).

A graphical representation of MEPs recorded in Experiment 2 in the three patients’ subgroup (patients with low-amplitude MEPs at baseline, patients showing increased responses after iTBS and cTBS and finally patients with no MEP changes after iTBS and cTBS) is shown in Figures 4, 5 and 6.

Clinico-neurophysiological correlations in patients with probable CBS

Spearman’s rank correlation test in the 17 patients with probable CBS participating at Experiment 1 in whom we examined TBS-induced plasticity over the “less affected” limb found no clinico-neurophysiological correlations. By contrast, in the 9 patients participating at Experiment 2, in whom we examined TBS-induced plasticity over the “more affected” limb, Spearman’s rank correlation test found a negative correlation between MMSE and amount of TBS-induced plasticity (MEP amplitudes at T2) following TBS over the M1 contralateral to the “more affected” limb (iTBS: r= -0.93; p<0.001; Fig. 3A; cTBS: r= -0.88; p=0.002; Fig. 3B), a positive correlation between MMSE and M1LTP/LTD-like plasticity AI (iTBS: r= 0.93; p<0.001; Fig. 3C; cTBS: r= 0.92; p<0.001; Fig. 3D) and a negative correlation between amount of TBS-induced plasticity (MEP amplitudes at T2) and M1 LTP/LTD-like plasticity AI (iTBS: r= -0.98; p<0.001; Fig. 3E; cTBS: r= -0.98; p<0.001; Fig. 3F).
DISCUSSION

The first new finding in this study investigating M1 LTP/LTD-like plasticity in patients with probable CBS is that, when we tested the M1 contralateral to the “less affected” limb in the whole group of 17 patients, iTBS and cTBS elicited reduced responses. When we tested the M1 contralateral to the “more affected” limb in 14 out of 17 patients with probable CBS, an unexpected finding was that in 5 patients we were unable to evaluate LTP/LTD-like plasticity because TMS elicited abnormally low-amplitude MEP. In the remaining 9 out of 14 patients in whom we investigated LTP/LTD-like plasticity, we found altered iTBS and cTBS-induced after-effects characterized by high inter-subject variability. Overall our findings provide new helpful information on the role of abnormal M1 LTP/LTD-like plasticity in the pathophysiology of CBS.

As measures to guarantee reliable findings, when applying TBS we found that motor thresholds and TMS intensities used for eliciting MEPs were similar in HS and patients thus excluding confounding factors and ensuring that our TBS findings reliably reflected changes in M1 LTP/LTD-like plasticity. Although the present study did not include a sham stimulation, our experimental design implied patients pseudorandomly assigned and counterbalanced to participate in the two experiments thus excluding confounding due to “placebo effects”. In addition results were compared with those obtained in healthy subjects.

By checking that none of the EMG recordings showed muscle activity immediately before, during, or after TBS, we also excluded the possibility that in patients with probable CBS the altered response to TBS reflected interference between TBS and muscle activity (Gentner et al. 2008; Huang et al. 2008; Iezzi et al. 2008). Given that 1 week elapsed between the different experimental sessions, we excluded possible homeostatic or non-homeostatic
interference between sessions (Ziemann et al. 2008). Finally, all participants were right-handed excluding confounding due to hand-dominance.

Neurophysiological abnormalities of the M1 contralateral to the “less affected” limb

Several mechanisms might explain the lack of significant MEP changes after TBS over the M1 contralateral to the “less affected” limb. One factor that might contribute in determining response to TBS is the input/output (I/O) curve (Chen et al. 2008). In CBS, the reduced LTP-like plasticity in the M1 contralateral to the “less affected” limb might be secondary to a reduced I/O curve slope or on a maximal response amplitude already achieved at baseline due to a “ceiling effect”. We consider this explanation unlikely for several reasons. First, patients with probable CBS and HS had similar thresholds and intensities for evoking 1 mV MEPs at baseline. Second, although in our study we did not investigate the I/O curve, a previous observation of normal I/O curve in CBS patients (Pal et al. 2008) makes this hypothesis unlikely. Because the iTBS-induced after-effects reflect LTP-like, whereas the cTBS-induced after-effects depend on LTD-like plasticity in M1 interneurons (Huang et al. 2005; Suppa et al. 2008a, 2012; Ziemann et al. 2008), we think that the altered responses to iTBS and cTBS arises from intrinsic M1 abnormalities in LTP/LTD-like plasticity mechanisms. It is likely that in patients with probable CBS, the abnormal M1 LTP/LTD-like plasticity reflects abnormal motor inputs to M1 from non-primary motor areas including the dorsal-premotor cortex, or non-motor areas including sensory areas (Huang et al. 2008; Iezzi et al. 2011; Ishikawa et al. 2007; Katayama et al. 2010; Suppa et al. 2008b, 2010) in agreement with hypothesis from structural neuroimaging studies (Boxer et al. 2006; Whitwell et al. 2010). Finally, the reduced M1 LTP/LTD-like plasticity we now describe in patients with probable CBS might reflect abnormal motor inputs from basal ganglia to cortical motor areas. This hypothesis is in line with the observation that the reduced responses to iTBS and cTBS we
describe here in patients with probable CBS in the M1 contralateral to the “less affected” limb
resemble those previously demonstrated in patients with Parkinson’s disease (PD) and
multiple system atrophy (MSA) who manifest prominent parkinsonism (Eggers et al. 2010;

When we assessed the possible correlation between patients’ clinical features and the
amount of responses to TBS delivered over the M1 contralateral to the “less affected” limb, in
the whole group of 17 patients with probable CBS, we found no significant clinico-
neurophysiological correlations.

Neurophysiological abnormalities of the M1 contralateral to the “more affected” limb
The experiment testing iTBS and cTBS-induced after-effects over the M1 contralateral the
“more affected” limb in 14 patients with probable CBS, unexpectedly disclosed in 5 patients,
abnormally low-amplitude MEPs reflecting exceptionally decreased M1 excitability, a finding
that prevented us from examining M1 LTP/LTD-like plasticity over the M1 contralateral to
the “more affected” limb. Precisely why it did so remains open to discussion. The small
amplitude MEPs recorded in 5 patients might merely reflect a technical error due to high
impedance secondary to widespread cortical atrophy rather than disclosing a specific
pathophysiological mechanism in M1. This hypothesis is unlikely as a recent magnetic
resonance imaging (MRI) study (Burrel et al. 2014) found no differences in the amount of
cortical and subcortical atrophy when comparing CBS patients with and without recordable
MEPs. A possible hypothesis for explaining the abnormally low-amplitude MEPs in these 5
patients with probable CBS is M1 neuronal loss including Betz neuron (Boelmans et al. 2009;
Burrell et al. 2014; Gibb et al. 1989; Tsuchiya et al. 2005). Given that no studies have
reported abnormally low-amplitude MEPs in other neurodegenerative movement disorders
including PD (Eggers et al. 2010; Huang et al. 2011; Kishore et al. 2012; Suppa et al. 2011),
PSP (Conte et al. 2012) and MSA (Suppa et al. 2014), we suggest that this M1 excitability abnormality might be a specific pathophysiological mechanism in patients with probable CBS.

When we examined the remaining 9 out of 14 patients in which we investigated M1 LTP/LTD-like plasticity, iTBS- and cTBS-induced abnormal after-effects characterized by high inter-subject variability suggesting heterogeneous TBS features in CBS. When we looked at the individual responses to TBS in these 9 patients, we found that in 5 patients (patient 6, 7, 8, 15 and 17, Table 1; Figure 2) iTBS and cTBS elicited homogeneous reduced responses leading a low M1 LTP/LTD-like plasticity “asymmetry index ” (Table 4). Accordingly, these 5 patients had reduced responses to TBS regardless of whether TBS was delivered over the M1 contralateral to the “less affected” or “more affected” limb. These 5 patients in addition to parkinsonism, manifested dystonia and myoclonus. Again, the reduced iTBS and cTBS-induced after-effects we report in these 5 patients resemble those previously collected in patients with PD and MSA (Eggers et al. 2010; Huang et al. 2011; Kishore et al. 2012; Suppa et al. 2011, 2014). Hence, our findings might support the hypothesis that in patients with probable CBS, reduced TBS-induced responses arises from impaired LTP/LTD-like plasticity in M1 possibly secondary to abnormal motor inputs from non primary motor and non motor areas or from the basal ganglia. In the remaining 4 out of 9 patients (patients 9, 10, 11 and 16, Table 1; Figure 2), iTBS and cTBS induced increased rather than decreased responses regardless of whether patients received iTBS or cTBS. In these 4 patients TBS we found high M1 LTP/LTD-like plasticity “asymmetry index ” (Table 4) because these 4 patients manifested reduced responses to TBS when given over the M1 contralateral to the “less affected” limb, and increased responses to TBS when delivered over the M1 contralateral to the “more affected” limb. These 4 patients in addition to parkinsonisms...
manifested non-motor symptoms including apraxia, cortical sensory deficit, “alien limb” phenomena and moderate executive dysfunction (Table 1).

The heterogeneous TBS features observed in our cohort of patients with probable CBS undergoing Experiment 2 might reflect the lateralization (right or left) of upper limb motor symptom. In CBS, a recent MRI study of Jütten et al. (2014) demonstrated that compared with patients with right side disease onset, those with left side disease onset manifested earlier and had a more prominent cortical grey matter loss leading to more severe motor and non motor symptoms. The observation of Jütten et al. (2014) is in line with a previous report in a cohort of right-handed patients with PD demonstrating that patients who manifested left side motor symptom onset developed earlier motor disability (Marras et al., 2011). In our cohort of right-handed patients with probable CBS, we found that the lateralization (right or left) of upper limb motor symptom onset is homogeneously present in the three subgroups of CBS patients who manifested different neurophysiological patterns of response to TBS in the Experiment 2 (see Table 1). We suggest therefore that the lateralization (right or left) of upper limb motor symptom onset unlikely explain the specific pattern of response to TBS observed in our patients participating at Experiment 2.

When we assessed the possible correlation between patients’ clinical features and the amount of responses to TBS delivered over the M1 contralateral to the “more affected” limb, we found several new important findings. Given that in 5 out of the 14 patients participating at the experiment testing iTBS and cTBS-induced after-effects over the M1 contralateral to the “more affected” limb we found abnormally low- amplitude preventing us from evaluating M1 LTP/LTD-like plasticity, we assessed the clinico-neurophysiological correlations only in the remaining 9 patients. In these 9 patients, we found a significant correlation between MMSE scores, the amount of TBS- induced plasticity following both iTBS and cTBS delivered over the M1 contralateral to the “more affected” limb and M1 LTP/LTD-like
plasticity “asymmetry index” values. Overall these findings suggest a close relationship between the amount of responses to TBS, the M1 LTP/LTD-like plasticity “asymmetry index” and the degree of cognitive impairment as tested by the MMSE. Given that CBS implies widespread, asymmetric and prominent neuronal loss in a number of cortical structures including the premotor cortex, supplementary motor area, superior parietal cortex and posterior cingulate cortex (Borroni et al. 2008; Boxer et al. 2006; Huey et al. 2009; Jütten et al. 2014; Lee et al. 2011; Whitwell et al. 2010) we suggest that the abnormally increased responses to TBS delivered over the M1 contralateral to the “more affected” limb might reflect a cortico-cortical disconnection syndrome due to a broader and asymmetric cortical degeneration process occurring in a specific subgroup of patients with probable CBS. The unexpected pattern of abnormally increased responses to TBS observed in the 4 patients (Table 1) resembles that previously reported in PSP (Conte et al. 2012). Because neuropathological studies show that CBS may arise from clinical entities other than CBD, including PSP (Boeve et al. 1999; Chahine et al. 2014; Hu et al. 2009; Ling et al. 2010; Ouchi et al. 2014; Shelley et al. 2009) some of the patients with probable CBS here studied manifesting abnormally increased responses to TBS might have had PSP with a life-time diagnosis of CBS or differently, might have CBD with neurodegenerative processes similar to those underlying PSP including tau-pathology-related neuronal loss of M1 interneurons (Boelmans et al. 2009; Boeve et al. 1999; Boxer et al. 2006; Chahine et al. 2014; Halliday et al. 2005; Hu et al. 2009; Ling et al. 2010; Ouchi et al. 2014; Respondek et al. 2014; Shelley et al. 2009; Whitwell et al. 2010). The possibility that some of the patients with probable CBS here studied manifesting abnormally increased responses to TBS are affected by AD or FTD is unlikely for several reasons. First, we enrolled patients according to the current consensus for a diagnosis of probable CBS (Alexander et al. 2014; Armstrong et al. 2013, 2014). Second, our conventional MRI imaging showed asymmetric fronto-parietal cortical atrophy in
all the patients we studied (see Table 1). In CBD/PSP, MRI commonly discloses asymmetric frontal cortical atrophy predominantly involving the premotor and supplemental motor area, whereas in FTD/AD, frontal cortical atrophy is more frequently symmetric and widespread (Josephs et al., 2008; Whitwell et al., 2010). Third, all the patients with probable CBS we studied have been clinically re-evaluated periodically and the diagnosis of probable CBS was confirmed in all patients. Finally, our cohort did not include patients in whom TBS induced responses similar to those previously observed in AD, namely reduced responses to iTBS and normal responses to cTBS (Koch et al. 2012). We cannot fully exclude however, that at least some part of the neurophysiological heterogeneity observed in our study might also reflect neuropathological conditions other than CBD. A final comment is that the high M1 LTP/LTD-like plasticity asymmetry index in some patients with probable CBS manifesting abnormally increased responses to TBS might reflect specific asymmetric pathophysiological mechanisms in the two hemispheres in line with neuropathological and neuroimaging evidence of asymmetric neurodegenerative processes in widespread cortical regions in addition to M1 and basal ganglia pathology (Borroni et al. 2008; Boxer et al. 2006; Huey et al. 2009; Jütten et al. 2014; Katayama et al. 2010; Lee et al. 2011).

CONCLUSIONS

In patients with probable CBS, we found specific LTP/LTD-like plasticity abnormalities in the M1 contralateral to the “less affected” limb and specific excitability and LTP/LTD-like plasticity changes in the M1 contralateral to the “more affected” limb. Our findings help to identify different neurophysiological subgroups of patients with probable CBS possibly explaining the clinical heterogeneity of this condition. Finally, we suggest that an abnormal
M1 plasticity plays a role in the pathophysiology of CBS. Our observation will help in designing new non-invasive brain stimulating protocols for improving motor and non-motor symptoms in patients with probable CBS (Civardi et al, 2015; Shehata et al, 2015).

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**FIGURE LEGENDS**

**Fig. 1** Effect of iTBS and cTBS delivered over M1 in the whole group of 17 patients with probable CBS and 17 HS (Experiment 1). In patients, iTBS/cTBS were delivered over the M1 contralateral to the “less affected” limb. In HS, iTBS/cTBS were delivered over the left M1. MEPs elicited at T0 and T1-T3 after iTBS (A) and cTBS (B). Each point corresponds to the mean MEP amplitude expressed as absolute values; vertical bars denote SD. MEP amplitudes after iTBS/cTBS differed significantly in patients with probable CBS and in HS.

**Fig. 2** Effect of iTBS and cTBS delivered over M1 in 9 out of 14 patients with probable CBS who participated at Experiment 2 and allowed the investigation of M1 plasticity. In patients, iTBS and cTBS were delivered over the M1 contralateral to the “more affected” limb. The figure also includes data recorded in the whole group of 17 HS in Experiment 1. MEPs elicited at T0 and T1-T3 in the subgroup of 9 patients after iTBS (A) and cTBS (B). MEPs elicited at T0 and T1-T3 in individual patients after iTBS (C) and cTBS (D) are also shown. Each point corresponds to the mean MEP amplitude expressed as absolute values; vertical bars denote SD. MEP amplitudes after iTBS/cTBS differed significantly in HS and patients with probable CBS despite high inter-subject variability. Lower panels also show the mean MEP amplitude expressed as a percentage of the responses obtained at T0 in individual patients after iTBS (E) and cTBS (F).

**Fig. 3** Clinico-neurophysiologic correlations in the subgroup of 9 out of 14 patients with probable CBS participating at both Experiment 1 and 2 in whom we examined TBS-induced plasticity over the “less affected” limb and the “more affected” limb. MMSE: Mini Mental
State Evaluation; MEP amplitude at T2: amplitude of MEPs (mV) recorded at T2 after iTBS or cTBS; AI: M1 LTP/LTD-like plasticity asymmetry index. Note the negative correlation between MMSE and MEP amplitudes at T2 following iTBS (A) and cTBS (B) over the M1 contralateral to the “more affected” limb, the positive correlation between MMSE and AI after iTBS (C) and cTBS (D) and finally the negative correlation between MEP amplitudes at T2 and AI after iTBS (E) and cTBS (F).

**Fig. 4** MEPs in the subgroup of 5 patients with CBS showing abnormally low-amplitude MEPs at baseline (patients number 1, 2, 3, 5 and 12; see Table 1). MEPs were evoked by transcranial magnetic stimulation (TMS) delivered at the maximum stimulator output over the M1 contralateral to the “more affected limb”. Note abnormally low-amplitude (<200 µV) and polyphasic MEPs in all patients.

**Fig. 5** MEPs in the subgroup of 4 patients with CBS showing abnormally increased responses after iTBS and cTBS (patients number 9, 10, 11 and 16; see Table 1). MEPs were recorded for each patient before (T0) and at 15 minutes (T2) after iTBS and cTBS delivered over the M1 contralateral to the “more affected” limb. Note that MEPs abnormally increased in amplitude at T2 compared to T0 after iTBS and cTBS in all patients. HS: representative MEPs in healthy subjects.

**Fig. 6** Motor evoked potentials (MEPs) in the subgroup of 5 patients with Cortico-basal syndrome (CBS) showing no changes in MEP amplitudes after intermittent or continuous theta burst stimulation (iTBS and cTBS, respectively) (patients number 6, 7, 8, 15 and 17; see
Table 1). MEPs were recorded for each patient before (T0) and at 15 minutes (T2) after iTBS and cTBS both delivered over the primary motor cortex (M1) contralateral to the “more affected” limb. Note comparable amplitude MEPs at T0 and T2 after iTBS and cTBS in all patients. HS: representative MEPs in healthy subjects.
Table 1. Demographic and clinical features in our cohort of 17 patients with probable CBS.
BFMS: Burke-Fahn-Marsden Movement and Disability Scale; MDS-UPDRS: Movement Disorder Society-Unified Parkinson’s Disease Rating Scale; MMSE: Mini Mental State Evaluation; FAB: Frontal Assessment Battery; HAM-D: Hamilton Depression Rating Scale; Park + limb: “more affected” limb. MRI: magnetic resonance imaging showing prominent atrophy in specific brain regions in each patient. Av: Average; SD: standard deviation.

Table 2. TMS data for HS and patients with probable CBS in the experiment testing LTP/LTD-like plasticity over the M1 contralateral to the “less affected” limb (Park Limb) and the “more affected” limb (Park + Limb). Table includes variables measured in patients in whom we evaluated M1 LTP/LTD-like plasticity (n = 17 in Experiment 1 and n = 9 in Experiment 2). iTBS: intermittent theta-burst stimulation; cTBS: continuous theta-burst stimulation; RMT: resting motor threshold; AMT: active motor threshold; 1 mV: TMS intensity used for evoking MEPs of about 1 mV at baseline; TBS%: intensity used for delivering iTBS and cTBS. RMT, AMT, 1 mV, and TBS are expressed as percentages of the maximum stimulator output (average ± SD). Note that RMT values are lower than AMT values in HS and patients with probable CBS because these thresholds are calculated with different type of magnetic stimulators (monophasic and biphasic, respectively).

Table 3. MEP amplitudes recorded before (T0) and 5 (T1), 15 (T2), and 30 (T3) minutes after intermittent and continuous theta-burst stimulation (iTBS and cTBS), in HS and in patients with probable CBS, in the different experiments (Experiment 1 and 2). Each value shows the averaged amplitude of 20 MEPs recorded at all time points (T0, T1, T2 and T3).
Table 4. M1 LTP/LTD-like plasticity “asymmetry index” (AI) in 9 patients with probable CBS participating at both Experiment 1 and 2. We calculated a ratio between MEP amplitude at T2/MEP amplitude at T0*100, after TMS over the M1 contralateral to the “less affected” limb (ΔPark) and the “more affected” limb (ΔPark +), in each CBS patient. We then calculated the AI for responses to iTBS and cTBS, for each CBS patient, defined by AI = ΔPark - ΔPark+/ΔPark + ΔPark +. iTBS: intermittent theta-burst stimulation; cTBS: continuous theta-burst stimulation.
A. iTBS
\[ r = -0.93; \quad p < 0.001 \]

B. cTBS
\[ r = -0.88; \quad p = 0.002 \]

C. iTBS
\[ r = 0.93; \quad p < 0.001 \]

D. cTBS
\[ r = 0.92; \quad p < 0.001 \]

E. iTBS
\[ r = -0.98; \quad p < 0.001 \]

F. cTBS
\[ r = -0.98; \quad p < 0.001 \]
| Case | Gender | Age (years) | Disease Duration (years) | Side of Disease onset | Park + Limb | Dystonia (BFMS) | Myoclonus | Apraxia | Cortical Sensory Deficit | Alien Limb Phenomena | MDS UPDRS | MMSE | FAB | HAM-D | MRI (atrophy) |
|------|--------|-------------|-------------------------|----------------------|------------|----------------|-----------|---------|------------------------|---------------------|-----------|------|-----|------|-------------|-------------|
| 1    | F      | 68          | 2                       | R                    | R          | 10/12          | -         | +/-     | +                      | -                   | 46        | 26   | 16  | 17   | Left Frontal |
| 2    | M      | 74          | 2                       | L                    | L          | -              | +         | +       | +                      | +                   | 26        | 27   | 15  | 13   | Right Fronto-parietal |
| 3    | F      | 85          | 3                       | R                    | R          | -              | +         | +       | +                      | +                   | 30        | 25   | 14  | 12   | Left Fronto-parietal |
| 4    | M      | 60          | 3                       | L                    | L          | -              | +         | +/-    | +/-                   | -                   | 26        | 27   | 10  | 16   | Right Fronto-temporo-parietal |
| 5    | M      | 62          | 4                       | L                    | L          | -              | +         | +       | +                      | +                   | 36        | 26   | 14  | 14   | Right Frontal |
| 6    | M      | 63          | 5                       | R                    | R          | 7/8            | +         | +/-    | +/-                   | -                   | 8         | 27   | 15  | 7    | Left Fronto-temporo-parietal |
| 7    | F      | 61          | 1                       | L                    | L          | 11/17          | +         | +/-    | +/-                   | -                   | 37        | 27   | 16  | 13   | Right Fronto-parietal |
| 8    | F      | 68          | 2                       | L                    | L          | 4/4            | +         | +/-    | +/-                   | -                   | 16        | 28   | 16  | 10   | Right Fronto-parietal |
| 9    | F      | 65          | 2                       | L                    | L          | -              | +         | +       | +                      | +                   | 14        | 23   | 14  | 6    | Right Frontal |
| 10   | F      | 63          | 2                       | R                    | R          | -              | +         | +       | +                      | +                   | 15        | 24   | 8   | 13   | Left Fronto-temporo-parietal |
| 11   | F      | 69          | 3                       | L                    | L          | 5/4            | -         | +       | +                      | +                   | 22        | 25   | 16  | 14   | Right Fronto-parietal |
| 12   | M      | 68          | 2                       | R                    | R          | -              | +         | +/-    | +/-                   | +                   | 20        | 26   | 15  | 8    | Left Fronto-parietal |
| 13   | F      | 69          | 4                       | L                    | L          | 15/24          | -         | +       | +                      | -                   | 63        | 25   | 15  | 16   | Right Fronto-temporal |
| 14   | F      | 78          | 4                       | L                    | L          | -              | +         | +       | +                      | -                   | 30        | 27   | 16  | 17   | Right Fronto-parietal |
| 15   | F      | 65          | 3                       | R                    | R          | 6/8            | +         | +/-    | +/-                   | -                   | 30        | 27   | 16  | 8    | Left Frontal |
| 16   | M      | 59          | 3                       | R                    | R          | -              | +         | +       | +                      | +                   | 13        | 23   | 14  | 14   | Left Frontal |
| 17   | F      | 70          | 1                       | L                    | L          | 13/22          | +         | +/-    | +/-                   | -                   | 77        | 28   | 17  | 16   | Right frontal |
| AV   |        |              |                          |                      |            |                |           |         |                       |                     | 29.9      | 25.9 | 14.5| 12.6 |              |
| SD   |        |              |                          |                      |            |                |           |         |                       |                     | 18.2      | 16.5 | 2.3 | 3.6  |              |

Table 1

<p>| Case | Gender | Age (years) | Disease Duration (years) | Side of Disease onset | Park + Limb | Dystonia (BFMS) | Myoclonus | Apraxia | Cortical Sensory Deficit | Alien Limb Phenomena | MDS UPDRS | MMSE | FAB | HAM-D | MRI (atrophy) |
|------|--------|-------------|-------------------------|----------------------|------------|----------------|-----------|---------|------------------------|---------------------|-----------|------|-----|------|-------------|-------------|
| 1    | F      | 68          | 2                       | R                    | R          | 10/12          | -         | +/-     | +                      | -                   | 46        | 26   | 16  | 17   | Left Frontal |
| 2    | M      | 74          | 2                       | L                    | L          | -              | +         | +       | +                      | +                   | 26        | 27   | 15  | 13   | Right Fronto-parietal |
| 3    | F      | 85          | 3                       | R                    | R          | -              | +         | +       | +                      | +                   | 30        | 25   | 14  | 12   | Left Fronto-parietal |
| 4    | M      | 60          | 3                       | L                    | L          | -              | +         | +/-    | +/-                   | -                   | 26        | 27   | 10  | 16   | Right Fronto-temporo-parietal |
| 5    | M      | 62          | 4                       | L                    | L          | -              | +         | +       | +                      | +                   | 36        | 26   | 14  | 14   | Right Frontal |
| 6    | M      | 63          | 5                       | R                    | R          | 7/8            | +         | +/-    | +/-                   | -                   | 8         | 27   | 15  | 7    | Left Fronto-temporo-parietal |
| 7    | F      | 61          | 1                       | L                    | L          | 11/17          | +         | +/-    | +/-                   | -                   | 37        | 27   | 16  | 13   | Right Fronto-parietal |
| 8    | F      | 68          | 2                       | L                    | L          | 4/4            | +         | +/-    | +/-                   | -                   | 16        | 28   | 16  | 10   | Right Fronto-parietal |
| 9    | F      | 65          | 2                       | L                    | L          | -              | +         | +       | +                      | +                   | 14        | 23   | 14  | 6    | Right Frontal |
| 10   | F      | 63          | 2                       | R                    | R          | -              | +         | +       | +                      | +                   | 15        | 24   | 8   | 13   | Left Fronto-temporo-parietal |
| 11   | F      | 69          | 3                       | L                    | L          | 5/4            | -         | +       | +                      | +                   | 22        | 25   | 16  | 14   | Right Fronto-parietal |
| 12   | M      | 68          | 2                       | R                    | R          | -              | +         | +/-    | +/-                   | +                   | 20        | 26   | 15  | 8    | Left Fronto-parietal |
| 13   | F      | 69          | 4                       | L                    | L          | 15/24          | -         | +       | +                      | -                   | 63        | 25   | 15  | 16   | Right Fronto-temporal |
| 14   | F      | 78          | 4                       | L                    | L          | -              | +         | +       | +                      | -                   | 30        | 27   | 16  | 17   | Right Fronto-parietal |
| 15   | F      | 65          | 3                       | R                    | R          | 6/8            | +         | +/-    | +/-                   | -                   | 30        | 27   | 16  | 8    | Left Frontal |
| 16   | M      | 59          | 3                       | R                    | R          | -              | +         | +       | +                      | +                   | 13        | 23   | 14  | 14   | Left Frontal |
| 17   | F      | 70          | 1                       | L                    | L          | 13/22          | +         | +/-    | +/-                   | -                   | 77        | 28   | 17  | 16   | Right Frontal |
| AV   |        |              |                          |                      |            |                |           |         |                       |                     | 29.9      | 25.9 | 14.5| 12.6 |              |
| SD   |        |              |                          |                      |            |                |           |         |                       |                     | 18.2      | 16.5 | 2.3 | 3.6  |              |</p>
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