Primary motor cortex LTP/LTD-like plasticity in probable corticobasal syndrome

Antonio Suppa,1,2* Flavio Di Stasio,2* Luca Marsili,1 Neeraj Upadhyay,1 Daniele Belvisi,1,2 Antonella Conte,1,2 Nicola Modugno,2 Carlo Colosimo,1 and Alfredo Berardelli1,2

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

Submitted 30 July 2015; accepted in final form 10 November 2015

Corticobasal syndrome (CBS) is characterized by parkinsonism combined with other asymmetric and heterogeneous motor (dystonia and myoclonus) and nonmotor 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 2014; Armstrong et al. 2013; Boeve et al. 2003; Kompoliti et al. 1998; Kouri et al. 2011; Stamelou et al. 2012). Clinicopathological studies describe CBS as a complex clinical condition comprising corticobasal degeneration (CBD) and also other neurodegenerative disorders, including progressive supranuclear palsy (PSP), Alzheimer’s disease (AD), and frontotemporal degeneration (FTD). Conversely, some patients manifesting neuropathological evidence of CBD may have had a lifetime 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 corticospinal excitability including decreased short-interval intracortical inhibition (SICI) suggesting reduced M1 inhibition in CBS and findings pointing to impaired transcortical 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 nonmotor symptoms in patients with CBS (Burrell 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 (aftereffects) following intermittent and continuous theta-burst stimulation (iTBS/cTBS). The iTBS-induced aftereffects are thought to reflect LTP-like plasticity, whereas the cTBS-induced aftereffects depend on LTD-like plasticity, in M1 (Huang et al. 2005; Suppa et al. 2008a; Suppa and Berardelli 2012; Ziemann et al. 2008). LTP/LTD-like plasticity in M1 serves as a physiological mechanism for motor execution and learning (Rioul-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 nonmotor symptoms (Belvisi et al. 2013; Suppa et al. 2011; Suppa and Berardelli 2012).

No studies have yet investigated LTP/LTD-like plasticity in M1 by testing iTBS/cTBS-induced aftereffects in patients with probable CBS; nor have they compared TBS-induced aftereffects 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 nonmotor symptoms, including dystonia, myoclonus, apraxia, cortical sensory deficit, and “alien limb” phenomena, and verified the possible relationship between TBS-induced aftereffects and specific patients’ clinical

* A. Suppa and F. Di Stasio contributed equally to this work.
Address for reprint requests and other correspondence: Alfredo Berardelli, Dept. of Neurology and Psychiatry, Neuromed Institute, Sapienza Univ. of Rome, Viale dell’Università, 30, 00185 Rome, Italy (e-mail: alfredo.berardelli@uniroma1.it).
MATERIALS AND METHODS

Subjects

We recruited 17 patients with probable CBS (6 men; age 67 ± 6.50 yr, mean ± SD; range 59–85 yr) and 17 age-matched HS (9 men; age 66 ± 6.4 yr, range 62–83 yr). 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. In all patients, magnetic resonance imaging (MRI) excluded focal brain lesions and showed mild cortical atrophy prominently in frontal brain regions (see 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). While participating in the study, none of the patients were receiving l-dihydroxyphenylalanine (l-DOPA) or other drugs acting on the central nervous system. Patients’ clinical features are summarized in Table 1. Subjects gave 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, Whitland, 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 motor thresholds (AMT) 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.

tBS 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 electromyographic (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, Welwyn Garden City, UK) and digitized (CED 1401; Cambridge Electronic Design, Cambridge, UK). We collected 20 MEPs before (T0) and 5 (T1), 15 (T2), and 30 min (T3) after TBS. Trials with involuntary peristimulus EMG activity >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.

Table 1. Demographic and clinical features in our cohort of 17 patients with probable CBS

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Duration, yr</th>
<th>Side of Disease onset</th>
<th>Disease course</th>
<th>Parkinsonism</th>
<th>Apraxia</th>
<th>Myoclonus</th>
<th>Dystonia</th>
<th>BFMS</th>
<th>MDS-UPDRS</th>
<th>MMSE</th>
<th>FAB</th>
<th>HAM-D</th>
<th>MRI (atrophy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>68</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>73</td>
<td>3</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>62</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>60</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>62</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>65</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>68</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>68</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>65</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>60</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>68</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>65</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>63</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>59</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>68</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>65</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>69</td>
<td>4</td>
<td>R</td>
<td>Part II, limb</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>1</td>
<td>26</td>
<td>10/12</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>Left Frontal</td>
</tr>
</tbody>
</table>

CBS, corticobasal syndrome; 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; P.1., parkinsonism; M, Motor symptoms.

Evaluation of FAB, Frontal Assessment Battery; HAM-D, Hamilton Depression Rating Scale; MMSE, Mini Mental State Evaluation; MRI, magnetic resonance imaging showing prominent atrophy in specific brain regions in each patient; Avg, average.
Experimental Design

Patients were pseudorandomly assigned and counterbalanced to participate in two experiments (Experiments 1 and 2), each comprising two separate sessions, one for iTBS and the other for cTBS, held at least 1 wk apart. In Experiment 1, we studied the whole group of 17 patients with probable CBS and 17 healthy subjects, whereas in 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; age 67 ± 6.50 yr, range 59–85 yr; 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 nonmotor 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 the Shapiro-Wilk 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 in both Experiments 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+). 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 aftereffects (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.

Spearmann’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 >50 μV, only a few trials were rejected (<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 (Experiments 1 and 2) and sessions (iTBS and cTBS), are shown in Table 2.

Experiment 1: TMS over the M1 Contralateral to the Less Affected Limb

Unpaired t-tests 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 3), 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 sessions (F3,96 = 20.14; P < 0.01) and a significant effect of the factors Groups and Time in the iTBS (F1,32 = 12.13; P < 0.01 and F3,96 = 34.78; P < 0.01, respectively) and cTBS sessions (F1,32 = 14.21; P < 0.01 and F3,96 = 20.14; P < 0.01, respectively). 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 the 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 nonsignificant effect in iTBS (F3,48 = 0.95; P = 0.43) and cTBS sessions (F3,48 = 0.64; P = 0.6; Fig. 1, A and B).

Experiment 2: TMS over the M1 Contralateral to the More Affected Limb

When considering the 14 patients with probable CBS participating in Experiment 2, RMT values increased significantly in 5 patients (patients 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 five patients than in HS (z = -3.24; P = 0.001) and in the remaining nine CBS patients participating in Experiment 2 (z = -3.003; P = 0.003). Accordingly, RMTs from these five 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 (patients 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 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 in 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. 2, D and F). 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 patient subgroups (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 Figs. 3, 4, and 5, respectively.

Clinico-neurophysiological Correlations in Patients with Probable CBS

Spearman’s rank correlation test in the 17 patients with probable CBS participating in Experiment 1, in whom we examined TBS-induced plasticity over the less affected limb,
found no clinico-neurophysiological correlations. By contrast, in the nine patients participating in 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. 6A; cTBS: $r = -0.88; P = 0.002$; Fig. 6B), a positive correlation.

An M1 LTP/LTD-like plasticity “asymmetry index” (AI) was determined in 9 patients with probable CBS participating in both Experiments 1 and 2. A ratio was calculated as MEP amplitude at T2/MEP amplitude at T0 × 100, after TMS over the M1 contralateral to the “less affected” limb ($\Delta_{\text{park}}$) and the “more affected” limb ($\Delta_{\text{park}+}$), in each CBS patient. The AI for responses to iTBS and cTBS was calculated, for each CBS patient, as defined by $\Delta_{\text{park}} - \Delta_{\text{park}+} / \Delta_{\text{park}} + \Delta_{\text{park}+}$.
between MMSE and M1 LTP/LTD-like plasticity AI (iTBS: $r = 0.93; P < 0.001$; Fig. 6C; cTBS: $r = 0.92; P < 0.001$; Fig. 6D), 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. 6E; cTBS: $r = -0.98; P < 0.001$; Fig. 6F).

**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 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 of 14 patients in whom we investigated LTP/LTD-like plasticity, we found altered iTBS- and cTBS-induced aftereffects characterized by high intersubject 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 pseudo-randomly 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 HS. 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 wk elapsed between the different experimental sessions, we excluded possible homeostatic or nonhomeostatic 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 aftereffects reflect LTP-like plasticity, whereas the cTBS-induced aftereffects 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 arise 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 nonprimary motor areas including the dorsal-premotor cortex or nonmotor 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 hypotheses 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 in patients with probable CBS in the M1 contralateral to the less affected limb resemble those previously demonstrated in patients with PD and multiple system atrophy (MSA) who manifest prominent parkinsonism (Eggers et al. 2010; Huang et al. 2011; Kishore et al. 2012; Suppa et al. 2011, 2014).

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 aftereffects over the M1 contralateral to the more affected limb in 14 patients with probable CBS unexpectedly disclosed in five 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 five 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 because a recent MRI study (Burrell 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 five patients with probable CBS is M1 neuronal loss, including Betz neurons (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 of 14 patients in which we investigated M1 LTP/LTD-like plasticity, iTBS and cTBS induced abnormal aftereffects characterized by high intersubject variability, suggesting heterogeneous TBS features in CBS. When we looked at the individual responses to TBS in these nine patients, we found that in five patients (patients 6, 7, 8, 15, and 17; Table 1 and Fig. 2), iTBS and cTBS elicited homogeneous reduced responses leading to a low M1 LTP/LTD-like plasticity AI (Table 4). Accordingly, these five 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 five patients, in addition to parkinsonism, manifested dystonia and myoclonus. Again, the reduced iTBS- and cTBS-induced aftereffects we report in these five patients resemble those previously collected in patients with probable CBS. 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 nonprimary motor and nonmotor areas or from the basal ganglia. In the remaining four of nine
patients (patients 9, 10, 11, and 16; Table 1 and Fig. 2), iTBS and cTBS induced increased rather than decreased responses regardless of whether patients received iTBS or cTBS. In these four patients’ responses to TBS, we found high M1 LTP/LTD-like plasticity AI (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 four patients, in addition to parkinsonisms, also manifested nonmotor 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 gray matter loss, leading to more severe motor and nonmotor 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 Experiment 2 (see Table 1). We suggest therefore that the lateralization (right or left) of upper limb motor symptom onset is unlikely to explain the specific pattern of response to TBS observed in our patients participating in 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 we found abnormally low amplitude in 5 of the 14 patients participating in the experiment testing iTBS- and cTBS-induced aftereffects over the M1 contralateral to the more affected limb, preventing us from evaluating M1 LTP/LTD-like plasticity, we assessed the clinico-neurophysiological correlations only in the remaining 9 patients. In these nine 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 AI values. Overall, these findings suggest a close relationship between the amount of responses to TBS, the M1 LTP/LTD-like plasticity AI, 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 four 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 (Boeke et al. 1999; Chahine et al. 2014; Hu et al. 2009; Ling et al. 2010; Ouchi et al. 2014; Shelley et al. 2009; Respondek et al. 2014; Whitwell et al. 2010). The possibility that some of the patients with probable CBS in this study who manifested 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 2014; Armstrong et al. 2013). Second, conventional MRI showed asymmetric frontoparietal cortical atrophy in all the patients we studied (see Table 1). In CBD/PSP, MRI commonly discloses asymmetric frontal cortical atrophy predominantly involving the prefrontal cortex, supplementary motor area, and posterior cingulate cortex (Aminoff and Chang 1982; Boeke et al. 1999; Chahine et al. 2014; Hu et al. 2009; Ling et al. 2010; Ouchi et al. 2014; Shelley et al. 2009; Whitwell et al. 2010). Third, all the patients with probable CBS that we studied have been clinically reevaluated periodically, and

**Fig. 6.** Clinico-neurophysiologic correlations in the subgroup of 9 of 14 patients with probable CBS participating in both Experiments 1 and 2 in whom we examined TBS-induced plasticity over the less affected limb and the more affected limb. Note the negative correlation between Mini Mental State Evaluation (MMSE) and MEP amplitudes (in mV) recorded at T2 following iTBS (A) and cTBS (B) over the M1 contralateral to the more affected limb, the positive correlation between MMSE and the M1 LTP/LTD-like plasticity asymmetry index (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).
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 the possibility, 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 noninvasive brain-stimulating protocols for improving motor and nonmotor symptoms in patients with probable CBS (Civardi et al. 2015; Shehata et al. 2015).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


J Neurophysiol • doi:10.1152/jn.00755.2015 • www.jn.org