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Stimulation over the cerebellum with a regular figure-of-eight coil induces reduced motor cortex inhibition in patients with progressive supranuclear palsy

Alberto Benussi [a], Valentina Dell'Era [a], Valentina Cantoni [a, b], Rosanna Turrone [c], Andrea Pilotto [a, d], Antonella Alberici [c], Maria Sofia Cotelli [e], Cristina Rizzetti [d], Alessandro Padovani [a], Barbara Borroni [a, *]

[a] Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[b] Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Italy
[c] Neurology Unit, Spedali Civili di Brescia, Brescia, Italy
[d] Parkinson's Disease Rehabilitation Centre FERB ONLUS S. Isidoro Hospital, Trescore Balneario, Italy
[e] Neurology Unit, Valle Camonica Hospital, Brescia, Italy

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ABSTRACT

Objective: To determine whether motor cortex inhibition by stimulation over the cerebellum with a figure-of eight coil (MISC8) may be reduced in patients with Progressive Supranuclear Palsy (PSP).
Methods: Paired pulse TMS was used to evaluate MISC8, in patients with different forms of parkinsonism and dementia. The primary outcome measures were sensitivity and specificity of motor cortex inhibition, derived from receiver operator curve analysis, in discriminating PSP from other neurodegenerative disorders.
Results: A total of 150 participants met inclusion criteria. According to clinical criteria, the study population included 19 PSP, 26 Parkinson's disease, 25 dementia with Lewy bodies, 15 corticobasal syndrome, 25 frontotemporal dementia and 15 Alzheimer's disease patients, and 25 healthy controls. PSP patients were characterized by a specific impairment of MISC8 (0.99 ± 0.08) compared to the healthy control group and to other neurodegenerative disorders (mean range = 0.63–0.80, all p-values<0.001). Using the best cut-off index, MISC8 differentiated PSP from other diagnoses with an overall sensitivity of 100%, a specificity of 94%, and an accuracy of 97%.
Conclusions: TMS is a non-invasive procedure which reliably distinguishes PSP from other neurodegenerative disorders. MISC8 could represent a useful additional diagnostic tool to be used in clinical practice.

Introduction

Progressive supranuclear palsy (PSP) is an atypical parkinsonian disorder associated with ocular motor dysfunction, postural instability, akinesia and cognitive dysfunction [1].

Despite the recent establishment of reliable clinical criteria [2], the diagnosis of PSP still remains challenging, particularly in the early disease stage [3] and, to date, no biomarker or genetic feature has been found to reliably predict a definite PSP diagnosis [4]. With the development of new disease modifying treatments in the near future, it remains essential to accurately identify patients with PSP, particularly in the early disease stages, when the burden of pathological accumulations might still be reversible.

Although PSP rarely presents with cerebellar signs and symptoms, a significant involvement of cerebellar structures, mainly the dentate nucleus, has been reported in pathological studies [5,6]. Indeed, recent imaging studies have shown a persistent impairment in the structural and functional integrity of the dentato-rubro-thalamic tract in patients with PSP [7–9]. Accordingly, transcranial magnetic stimulation (TMS) studies using the cerebellar inhibition (CBI) protocol have identified a significant impairment in the dentato-thalamo-cortical pathway in PSP compared to Parkinson's disease patients [10,11].

* Corresponding author. Clinica Neurologica, Università degli Studi di Brescia, P.le Spedali Civili 1, 25123, Brescia, Italy.
E-mail address: bborroni@inwind.it (B. Borroni).

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However, little is known if CBI may differentiate PSP from other parkinsonisms, including dementia with Lewy bodies (DLB) and corticobasal syndrome (CBS) or other dementing disorders, including frontotemporal dementia (FTD) and Alzheimer's disease (AD).

Considering the difficulty in applying the classical CBI protocol with a double-cone-coil in patients with dementia because of the considerable distress induced by this type of coil (see **Supplementary Materials** for details), we used a different protocol which induces motor cortex inhibition by stimulation over the cerebellum with a figure-of-eight coil (MISC8).

All the above observations defined the objective of this work, aimed at assessing the diagnostic accuracy of MISC8 in the differential diagnosis of PSP and other neurodegenerative disorders.

Methods

Standard protocol approvals, registrations, and patient consents

Full written informed consent was obtained from all participants according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee (Brescia Hospital), #NP1965 approved 25.01.17.

Primary research questions/classification of evidence

Our primary research question was to determine whether TMS can discriminate between PSP and other parkinsonisms, including PD, DLB, CBS, or other dementing disorders, including AD and FTD, or healthy controls (HC). This study provides Class III evidence that TMS measures can distinguish patients with PSP from those with other neurodegenerative disorders with high diagnostic accuracy.

Participants

One hundred and fifty-eight participants were recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, Neurology Unit, University of Brescia, Italy and entered the study. Three patients were excluded because carrying electronic implants (n = 2) or suffered from epilepsy (n = 1). Of the remaining one hundred and fifty participants, nineteen were diagnosed as PSP Richardson's syndrome (PSP-RS) [2], twenty-six as PD [12], twenty-five as DLB [13], fifteen as CBS [14], twenty-five as FTD [15–17], fifteen as AD [18], and twenty-five healthy controls (HC); of these, five patients with CBS were excluded because of motor cortex unexcitability (see Fig. 1). Each patient fulfilled current clinical criteria for the specific diagnosis.

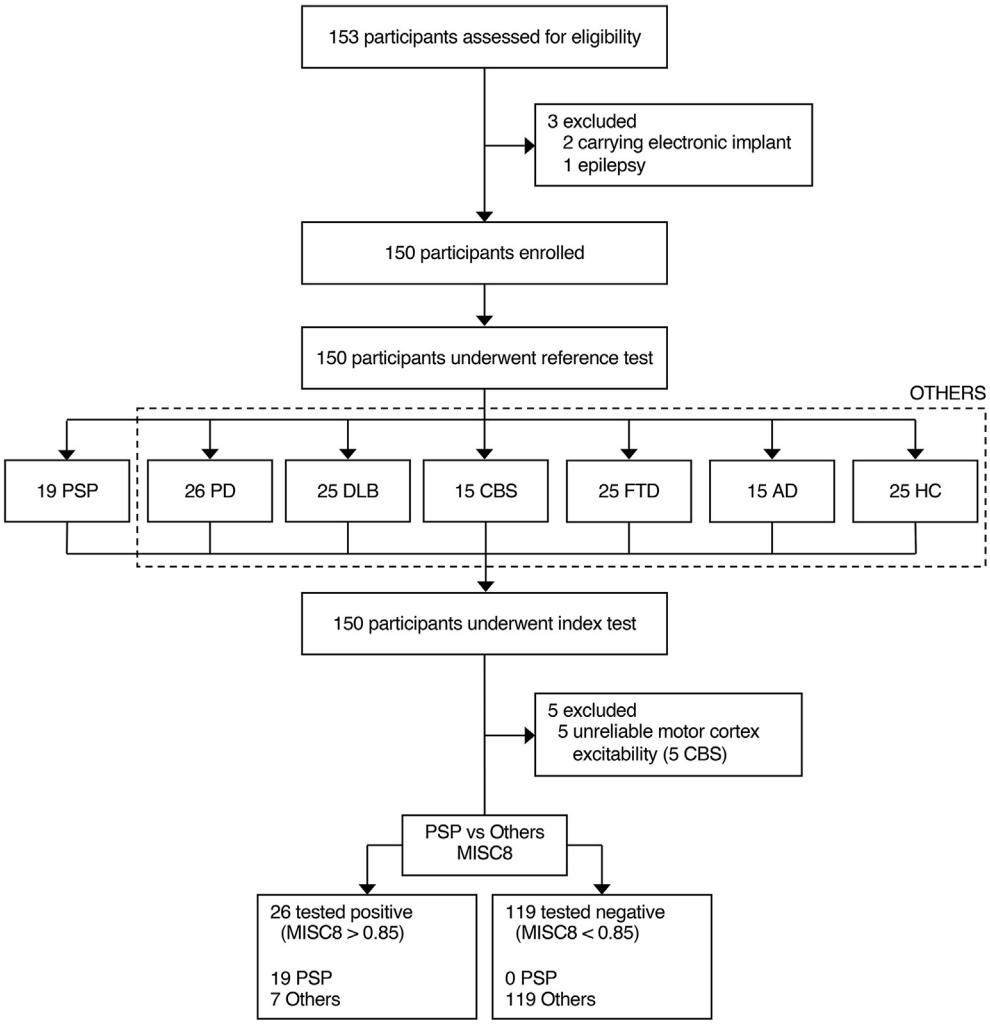

Fig. 1. Flow diagram of the study.
HC = healthy controls; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; PSP = Progressive Supranuclear Palsy; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease; MISC8 = motor cortex inhibition by stimulation over the cerebellum with a figure-of eight coil.

The diagnostic evaluation included a review of the full medical history, a neurological examination, and a brain Magnetic Resonance Imaging scan in all patients. For each patient, a standardized assessment, including the Unified Parkinson's Disease Rating Scale (UPDRS-III) [19], the Mini-Mental State Examination (MMSE) [20], the Instrumental and Basic Activities of Daily Living (IADL and BADL) [21,22], was carried out. Disease stage of PSP patients was evaluated by PSP Rating Scale (PSP-RS) [23].

TMS protocol

Historically, the cerebellar inhibition protocol has been first studied by Ugawa and colleagues by applying an electrical conditioning stimulus over the cerebellum; this led to an inhibition of EMG responses in the first dorsal interosseous muscle (FDI) evoked by a magnetic stimulus to the contralateral motor cortex [24]. Considering that electrical stimulation may be uncomfortable for patients, further studies identified the possibility of delivering the conditioning cerebellar stimulus by transcranial magnetic stimulation, obtaining somewhat contrasting results. In particular, the use of the double-cone coil, which can reach deeper structures but with reduced focality, has shown a consistent inhibition of MEPs [25–27]; on the other hand, the application of a figure-of-eight coil, with a reduced electric field depth, has shown that stimulation may be corrupted by some components, prevalently from sensitive nerve afferents, other than true cerebellar inhibition [28,29]. While the electric field depth-focality tradeoff between these two coils has still to be elucidated [30], it has to be considered that the anatomy of the cerebellum is different from that of the primary motor cortex and the functional topography of the cerebellum and how it is related to the connectivity between cerebellum and primary motor cortex are still not clear. Thus, the focality of the figure-of-eight coil might be considered as a limitation in this context, activating less Purkinje cells that the double-cone coil. In the **Supplementary Materials** we included several premises and preliminary experiments which led us to the application of this particular setup.

Thus, in the present study, TMS was performed with two figure-of-eight coils (Magstim D70^2, each loop diameter of 70 mm) connected to two Magstim stimulators (Magstim Company, Oxford, UK). The magnetic stimuli had a monophasic current waveform (rise time of 100 μs, decaying back to zero over 800 μs). Motor evoked potentials (MEPs) were recorded from the right FDI through surface Ag/AgCl electrodes placed in a belly-tendon montage and acquired using a Biopac MP-150 electromyograph (BIOPAC Systems Inc., Santa Barbara, CA, USA), as previously reported [31].

The TMS coil was held tangentially over the scalp region corresponding to the primary hand motor area contralateral to the target muscle, with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The motor hot spot was defined as the location where TMS consistently produced the largest MEP size at 120% of the resting motor threshold (RMT) in the target muscle and was marked with a felt tip pen on the scalp to ensure constant placement of the coil throughout the experiment.

RMT was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μV in 5 out of 10 consecutive trails during complete muscle relaxation, which was controlled by visually checking the absence of EMG activity at high-gain amplification [32].

Motor cortex inhibition by stimulation over the cerebellum was assessed using previously described techniques [11,33–37].

Briefly, the second coil was used to deliver the conditioning stimuli (CS) which was placed over the contralateral cerebellar hemisphere [25] (1 cm inferior and 3 cm right to the inion), a site corresponding to the posterior and superior lobules of the lateral cerebellum [38,39]. For cerebellar stimulation, the handle was positioned upward with the coil placed tangentially to the skull (see Fig. 2).

The cerebellar CS intensities were set at 110% RMT obtained in the contralateral motor cortex [34] while the test stimulus (TS) was adjusted to evoke a MEP approximately 0.5–1.0 mV peak-to-peak in the relaxed FDI [40,41].

CS preceded the TS by different interstimulus intervals (ISIs) ranging from 3 to 10 ms (3, 5, 10 ms). There were four conditions, corresponding to the three different ISI and the TS alone. Ten responses were collected for each different ISI and fifteen for the TS alone in a pseudorandomized sequence. The amplitude of the conditioning MEPs was expressed as a ratio of the mean unconditioned response. The inter trial interval was set at 5 s (±10%).

Statistical analyses

Clinical characteristics were compared using one-way ANCOVA or Fisher's exact test. TMS measures were compared using a two-way mixed ANCOVA with ISI as within-subjects factor and GROUP as between-subjects factor, including age at evaluation and disease duration as covariates. If a significant main effect was obtained, group differences were examined with *post hoc* tests (Bonferroni correction for multiple comparisons). To check and correct for sphericity violation, Mauchly's test and Greenhouse-Geisser epsilon determination were used.

Correlations between MISC8 and demographic or clinical variables were assessed by Spearman's rank test.

To determine the area under the curve (AUC), receiver operating characteristics (ROC) curves were used, including 95% confidence interval (CI) values. Cut-off points were set to minimize the difference between sensitivity and specificity (Youden's index).

Statistical significance was assumed at $p < 0.05$. Data analyses were carried out using SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA).

Data availability

All data, including study protocol, statistical analysis plan and results are available from the corresponding author, BB, upon reasonable request.

Results

Participants and neurophysiological parameters

Nineteen PSP, twenty-six PD, twenty-five DLB, ten CBS, twenty-five FTD and fifteen AD patients, and twenty-five age-matched HC underwent MISC8 assessment with a TMS paired-pulse protocol. Demographic and clinical characteristics of included subjects are reported in Table 1. No adverse events were reported in all participants.

Repeated measures ANCOVA performed on MISC8 measures revealed a statistically significant two-way interaction ($F(11.16,253.01) = 10.56$, $p < 0.001$, partial $\eta^2 = 0.32$, $\varepsilon = 0.93$). *Post hoc* comparisons revealed a significant difference between PSP and DLB, CBS, AD, PD and HC at ISI 3 ms (all $p < 0.001$) but not for FTD ($p = 0.346$), while at ISI 5 ms there was a significant difference between PSP and all other neurodegenerative disorders and HC (all $p < 0.001$). At ISI 10 ms, there was a significant difference between PSP and PD ($p = 0.031$) and HC ($p = 0.007$) but not for other neurodegenerative disorders (all $p > 0.05$) (see Fig. 3**, panel A**).

Regarding FTD patients, we also observed a significant difference at *post hoc* tests at ISI 5 ms compared to PSP, DLB, AD, PD and HC (all $p < 0.05$), but not for CBS patients ($p > 0.05$).

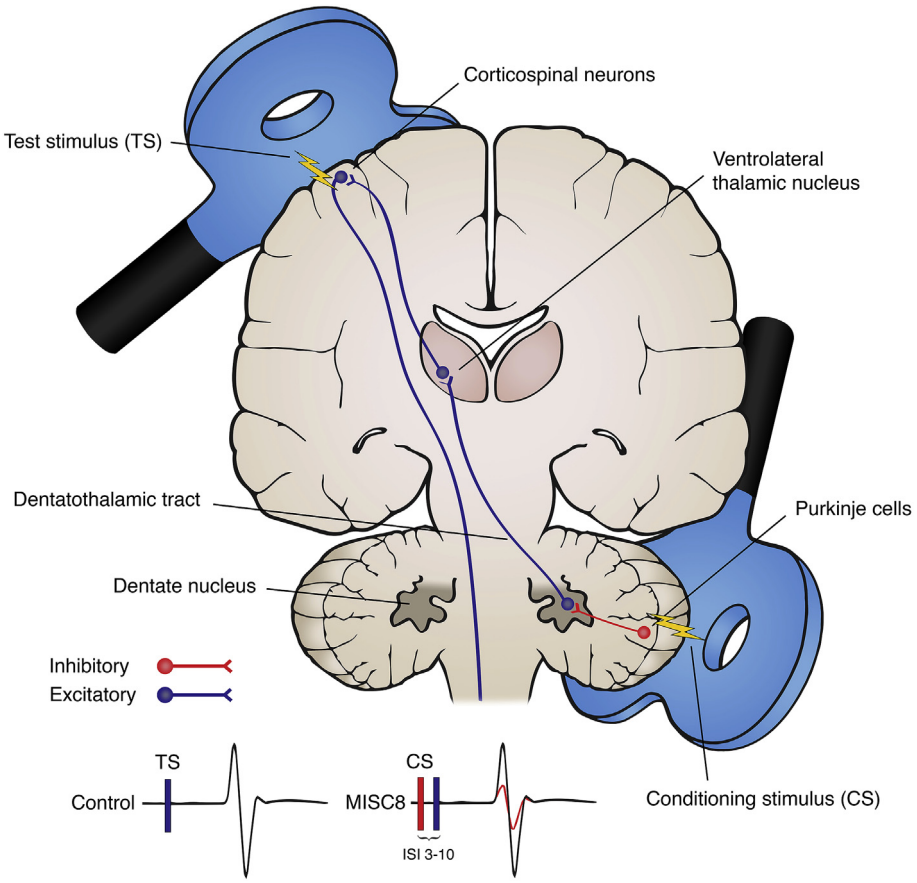

Fig. 2. Stimulation protocol for evoking motor cortex inhibition.
TS = Test stimulus; CS = Conditioning stimulus; MISC8 = motor cortex inhibition by stimulation over the cerebellum with a figure-of eight coil.

Table 1
Demographic and clinical characteristics of included subjects.

	HC	PSP	AD	FTD	CBS	DLB	PD
Patients (n)	25	19	15	25	10	25	26
Age (yrs)	68.8 ± 8.4	73.8 ± 7.2	73.5 ± 6.6	66.4 ± 8.6	69.5 ± 12.0	72.6 ± 6.5	71.4 ± 7.7
Age at onset (yrs)	–	70.0 ± 7.5	70.8 ± 6.7	63.3 ± 8.5	66.7 ± 11.6	70.4 ± 6.4	65.6 ± 9.1
Gender (% female)	64.0	36.8	60.0	44.0	30.0	28.0	30.8
Education	9.7 ± 3.5	8.5 ± 4.5	9.1 ± 2.8	10.3 ± 4.5	7.5 ± 3.2	8.5 ± 3.5	8.2 ± 4.2
MMSE	29.9 ± 0.2	26.7 ± 3.0	24.5 ± 2.5	24.3 ± 5.9	26.6 ± 2.7	24.0 ± 3.1	27.5 ± 2.8
BADL	0.0 ± 0.0	1.6 ± 1.7	0.2 ± 0.6	0.6 ± 1.3	1.1 ± 1.9	1.3 ± 1.9	0.1 ± 0.3
IADL	0.0 ± 0.0	2.5 ± 2.8	1.2 ± 1.1	1.5 ± 2.2	1.4 ± 1.6	2.8 ± 2.5	0.4 ± 0.8
UPDRS-III	–	24.2 ± 16.7	–	–	21.2 ± 16.0	15.0 ± 11.8	17.6 ± 8.1
PSP-RS	–	28.8 ± 11.6	–	–	–	–	–
RMT (% MSO)	41.1 ± 10.5	38.8 ± 9.1	37.8 ± 9.0	38.2 ± 7.2	43.1 ± 12.9	39.3 ± 11.0	41.3 ± 8.9
TS amplitude (mV)	0.75 ± 0.13	0.85 ± 0.14	0.82 ± 0.10	0.75 ± 0.20	0.87 ± 0.34	0.87 ± 0.16	0.82 ± 0.22

Demographic and clinical characteristics, and neurophysiological parameters are expressed as mean ± SD.
HC = healthy controls; PSP = Progressive Supranuclear Palsy; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease; MMSE = Mini-Mental State Examination; BADL = Basic activities of daily living; IADL = Instrumental activities of daily living; UPDRS-III = Unified Parkinson's disease rating scale part III; PSP-RS = PSP-Rating scale; yrs = years; RMT = resting motor threshold; % MSO = % maximum stimulator output.; TS = test stimulus; mV = millivolt.

For the purpose of the present study, peak motor cortex inhibition (ISI 5 ms) was considered. As shown in Table 2 and Fig. 3 **(panel B)**, peak motor cortex inhibition was significantly reduced in patients with PSP (0.99 ± 0.08) when compared to the other diagnostic groups (mean range = 0.63–0.80, all $p < 0.001$).

Peak motor cortex inhibition did not correlate with age, gender, age at onset or disease duration.

In patients with PSP, peak motor cortex inhibition did not correlate with both PSP-RS or UPDRS-III scores ($p > 0.05$).

Diagnostic accuracy of MISC8

A ROC curve was plotted in order to evaluate how effectively neurophysiological measures differentiated patients with PSP from

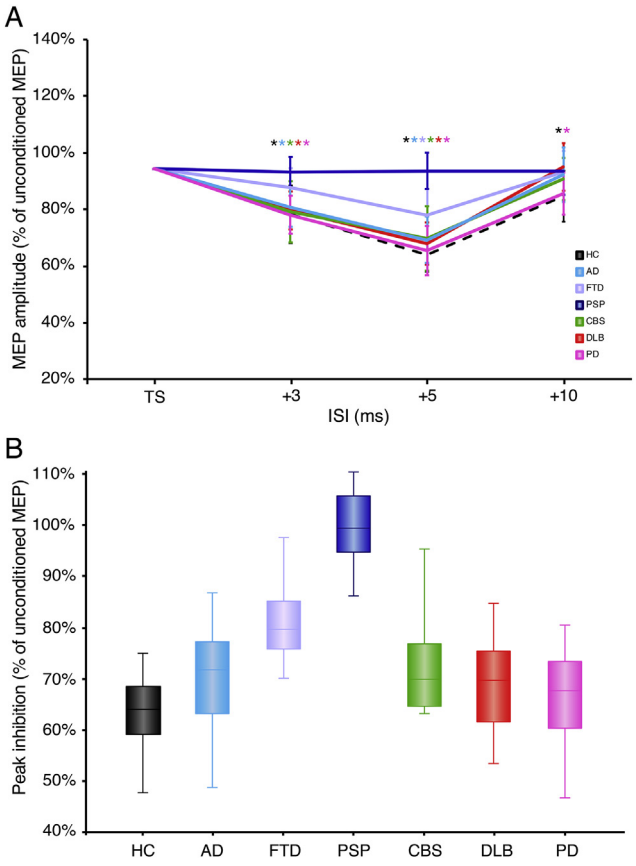

Fig. 3. Neurophysiological parameters in PSP and other groups.
Panel A. Motor cortex inhibition at ISI 3, 5 and 10 ms in HC, AD, FTD, PSP, CBS, DLB and PD patients. Data are represented as a ratio to the unconditioned motor evoked potential amplitude; error bars represent standard deviations. $^{*}p < 0.05$ vs PSP using one-way ANCOVA (*post hoc* tests with Bonferroni correction for multiple comparisons).
Panel B. Peak motor cortex inhibition (ISI 5 ms) in HC, AD, FTD, PSP, CBS, DLB and PD. Results are expressed as first and third quartiles (boxes) and minimum and maximum values (whiskers). HC = healthy controls; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; PSP = Progressive Supranuclear Palsy; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease.

Table 2
Peak motor cortex inhibition by stimulation over the cerebellum scores in patients with PSP and in other groups.

Diagnosis	Mean ± SD	95% Confidence Intervals		p^{*} vs PSP
		Lower	Upper	
HC	0.63 ± 0.07	0.60	0.66	<0.001
PSP	0.99 ± 0.08	0.95	1.03	–
AD	0.70 ± 0.10	0.64	0.75	<0.001
FTD	0.80 ± 0.11	0.75	0.85	<0.001
CBS	0.70 ± 0.14	0.60	0.80	<0.001
DLB	0.68 ± 0.09	0.64	0.72	<0.001
PD	0.65 ± 0.10	0.61	0.69	<0.001

$^{*}p$-value of ANCOVA and Bonferroni *post-hoc* analysis of each group vs progressive supranuclear palsy (PSP); no other significant differences among groups were found. HC = healthy controls; PSP = Progressive Supranuclear Palsy; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease.

those with other diagnoses (see Fig. 4). Peak motor cortex inhibition showed an AUC of 0.984 (p < 0.001, 95% CI 0.97–1.00). At the best cut-off score of 0.86 for motor cortex inhibition, sensitivity was 100%, specificity 94.4%, positive predictive value 73.1%, negative predictive value 100%, and accuracy 95.2%. Furthermore, as shown

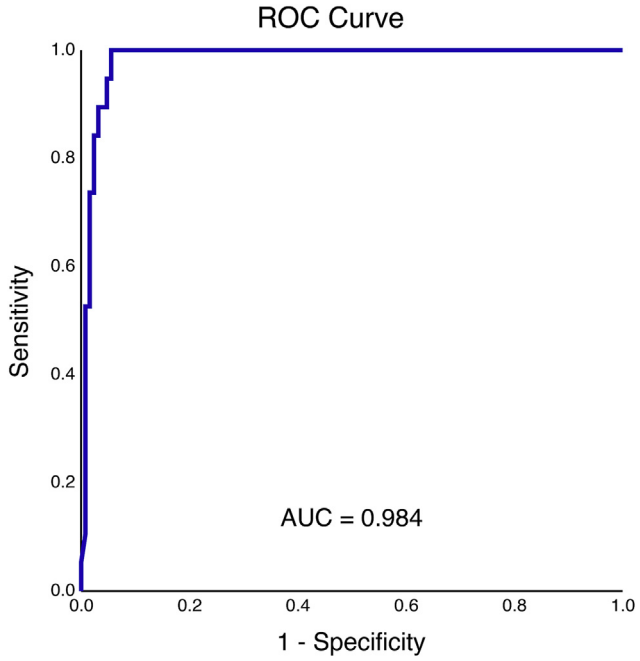

Fig. 4. Receiver Operating Characteristic curve for neurophysiological parameters in differentiating PSP from other groups. HC = healthy controls; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; PSP = Progressive Supranuclear Palsy; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease.

in Table 3, sensitivity, specificity, and accuracy values were still high when the PSP group was compared to each group of patients and to controls.

MISC8 in PSP patients with milder disease

We considered PSP patients with milder disease (median value PSP-RS≤28, n = 12). In these patients peak motor cortex inhibition was comparable to that found in the entire PSP sample (0.99 ± 0.08), and was significantly increased when compared to the other diagnostic groups (all $p < 0.001$).

Peak motor cortex inhibition in the milder PSP disease stage showed an AUC of 0.982 ($p < 0.001$, 95% CI 0.96–1.00). At the best cut-off score of 0.86 for MISC8, sensitivity was 100%, specificity was 94.4%, and accuracy was 94.9%.

Discussion

There is urgent need for diagnostic markers to identify PSP even in the initial phases of disease, allowing an earlier diagnosis and interventions to optimally benefit from disease-modifying therapies. Over the past decade, several potential neuroimaging, biological and neurophysiological markers have been reported as potentially helpful in differentiating PSP-RS from other neurodegenerative parkinsonisms and dementing disorders [42,43].

In the present study, we evaluated the inhibition of the motor cortex induced by stimulation of the cerebellum with a figure-of-eight coil (MISC8), observing a significant impairment of motor cortex inhibition in PSP compared to other neurodegenerative disorders. These findings have been observed for the first time in a rather large group of patients, which had a predictable defined neuropathological process. Indeed, the involvement of the dentate nucleus, and thus of the dentato-thalamo-cortical pathway, has been systematically observed in patients with PSP, both pathologically [5–9] and, just recently, with Tau PET tracers [44–47]. On the

Table 3
Comparison between patients with PSP and other groups: receiver operating characteristic (ROC) curve analysis.

ROC curve	PSP vs CON	PSP vs AD	PSP vs FTD	PSP vs CBS	PSP vs DLB	PSP vs PD	PSP vs all
AUC	1.000	0.996	0.929	0.974	1.000	1.000	0.984
95% CI	1.00–1.00	0.99–1.00	0.85–1.00	0.92–1.00	1.00–1.00	1.00–1.00	0.96–1.00
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cut-off	0.82	0.87	0.86	0.83	0.85	0.83	0.86
Sensitivity, %	100	94.7	100	100	100	100	100
Specificity, %	100	100	80	90.0	100	100	94.4
Accuracy, %	100	97.1	88.6	96.6	100	100	95.2

HC = healthy controls; AD = Alzheimer's Disease; FTD = Frontotemporal Dementia; PSP = Progressive Supranuclear Palsy; CBS = Corticobasal syndrome; DLB = Dementia with Lewy bodies; PD = Parkinson's disease; AUC = Area under the curve; ROC = Receiver operating characteristic; CI = Confidence Interval.

other hand, the involvement of the dentate nucleus is not a prominent feature of dementing disorders, such as AD [48], nor of parkinsonisms, such as PD [46] or CBD [49]. Nevertheless, we also observed a significant decrease in motor cortex inhibition at ISI 5 ms in FTD patients, which however differed significantly from PSP patients. Several reports have now shown that that cerebellar structures may be involved to a certain degree in both sporadic and genetic forms of FTD [50,51].

Indeed, our data confirm previous investigations performed in relatively smaller samples, showing that PSP patients are characterized by a specific impairment of CBI [10,11]. From a neurophysiological perspective, CBI, which results in the inhibition of motor evoked potentials induced in the primary motor cortex, is believed to be a result of activation of Purkinje cells in the cerebellar cortex [52]. Indeed, Purkinje cells exert a suppression of excitatory outputs from cerebellar nuclei, including the dentate nucleus, resulting in inhibition of excitatory signals passing from the ventrolateral thalamic nucleus to neurons in the contralateral motor cortex [41,53].

Compared to the study by Shirota and colleagues, where a significant deficit in cerebellar inhibition was observed at ISI 5–7 ms in PSP patients with a double-cone coil, in this study we observed a significant difference in motor cortex inhibition also at ISI 3 ms and, only partially, at ISI 10 ms, with the use of a figure-of-eight coil. As outlined in the **Supplementary Materials**, it seems that there are different components that can be evoked by magnetic stimulation over the cerebellum with the particular setup used in this study. The first, with an onset at 3 ms, may represent a direct suppression of spinal cord circuits [25,54], a second at 5–7 ms representing a true cerebellar inhibition of motor cortical activity [25,27] and a third, beginning at 7 or 8 ms, possibly due to stimulation of afferent fibers in the peripheral nerves [27,29]. The decreased inhibition here observed in PSP patients might be due to the impairment not only of the cerebellum and of the cerebello-thalamo-cortical tract, but also of other brainstem structures, which have been showed to be impaired in PSP patients [2,42]. However, a direct evidence of a cerebellar stimulation with this type of setup is currently lacking, also considering that conditioning stimulus with three different intensities (90%, 110%, 130% RMT, showed in the **Supplementary Materials**) produced similar MEP inhibition, with only a significant difference between 90% RMT and the double-cone coil at ISI 5 and 6 ms; this could be simply explained by a maximum inhibition with peripheral inputs reached at low stimulus intensities.

Indeed, it may be disputable that what we observed here might not be all “true CBI” but may be “contaminated” by other components; nevertheless, these results highlight how PSP is possibly characterized by a pronounced degeneration of structures of the posterior fossa including the brainstem and how this particular protocol makes them emerge.

The dentato-thalamo-cortical alterations observed in PSP patients might explain early postural instability features compared to other neurodegenerative diseases and thus open windows of intervention by using stimulation techniques over cerebellum, as recently demonstrated for ataxia patients [37,55]. On the other hand, our findings do not show an impairment of motor cortex inhibition in a wide series of different neurodegenerative disorders, which represent the most common differential diagnoses in clinical practice.

Taking into consideration these findings, we observed a specific impairment in MISC8, obtaining high levels of diagnostic accuracy, with overall sensitivity of 100% and specificity ranging between 80% and 100% in differentiating PSP from each of the other diagnostic groups. Moreover, we obtained a negative predictive value of 100%, thus making PSP diagnosis unlikely in those patients who tested negative at MISC8. Furthermore, MISC8 was already impaired in mild PSP patients, thus arguing for a potential diagnostic usefulness even in the early phases of disease.

The differential diagnosis between PSP and either parkinsonisms or dementing disorders is still challenging. In the last years, several neuroimaging markers have been proposed, with the most robust evidence being represented by the assessment of midbrain atrophy measurements [56]. Cerebrospinal fluid (CSF) markers, such as CSF Tau fragments [57,58], have also been described as potentially helpful in identifying PSP but with a drawback due to the invasiveness of the procedure. Quantification of blood neurofilament light chain concentration can be used to distinguish PSP from PD but not from other parkinsonisms [59]. Furthermore, tau-specific PET imaging ligands [46] and tau RT-QuIC [60] hold the potential to be used in the clinical setting in the near future, but insufficient clinical data exist at this time to gauge their potential utility. Each of the proposed markers actually has advantages and disadvantages, which are not based solely on diagnostic accuracy values, but also on invasiveness, availability not limited to tertiary referral centers, cost, time and ability to differentiate PSP from all possible differential diagnoses. Herein, we propose MISC8 as a useful diagnostic marker with several advantages, as this is: *a)* non-invasive, reliable, easy to apply and with a relatively low cost; *b)* not time-consuming (the procedure lasts up to 15 min); *c)* free from strict exclusion criteria (the exclusion rate in our sample was 2.03%); and *d)* able to differentiate PSP from other parkinsonisms and other dementing disorders with high accuracy. For a disease still orphan of any evidence based-treatment, the selective involvement of dentato-thalamo-cortical pathway could be considered as a specific target for future pharmacological and non-pharmacological interventions. Further studies should determine whether the impairment of CBI could also provide relevant prognostic information, as potential surrogate marker to predict beneficial effects in clinical trials [37].

We acknowledge that this work entails some limitations. First, PSP is a rare disorder, and larger, multicenter studies are needed to confirm the present findings. Second, other possible differential diagnoses, such as multisystem atrophy type P and C [61], should be taken into account. Moreover, in the present study we included only patients with PSP-RS, without neuropathological confirmation. Finally, we used a protocol to induce motor cortex

inhibition which is different from that originally described by Shirota et al. [10], which involves the use of a double-cone coil and not a figure-of-eight coil, which is however difficult to apply in patients with dementia because it is uncomfortable for the patients [41,62]. A direct evidence that the stimulation of the cerebellum with this particular setup is conceivable is currently lacking, and further studies should be performed in patients with pure cerebellar deficits (as in cerebellar infarctions or cerebellar agenesis) or in patients with a selective involvement of the motor thalamic tracts. The systematical assessment of the different effects of coil orientation with this setup, which was oriented to produce a downward current in the brain, somewhat in contrast to several previous studies [41], should be carried out in future studies.

Nevertheless, motor cortex inhibition induced with the figure-of-eight coil as the conditioning coil over the cerebellum has been successfully applied in several other published studies [11,34,37,63,64].

Despite these limitations, the MISC8 has been shown to reliably distinguish PSP from other neurodegenerative disorders and, if these findings are replicated in larger samples, could represent a useful additional diagnostic tool to be used in clinical practice and, eventually, as outcome measures in clinical trials.

Author contributions

Conception and design of the study: AB and BB. Acquisition and analysis of data: AB, VD, VC, RT, AP, AA, MSC, CR, AP and BB. Drafting the manuscript and figures: AB and BB.

Relevant conflicts of interest/financial disclosures

Nothing to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.05.017.

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