RESEARCH ARTICLE

The Pathophysiological Correlates of Parkinson's Disease Clinical Subtypes

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ABSTRACT: Background: Possible pathophysiological mechanisms underlying Parkinson's disease (PD) clinical subtypes are unknown. The objective of this study was to identify pathophysiological substrate of PD subtypes using neurophysiological techniques.

Methods: One hundred de novo PD patients participated. We collected patient demographic and clinical data, which were used to perform a hierarchical cluster analysis. The neurophysiological assessment tested primary motor cortex excitability and plasticity using transcranial magnetic stimulation. To evaluate motor performance, we performed a kinematic analysis of fast index finger abduction. To investigate sensory function and sensorimotor mechanisms, we measured the somatosensory temporal discrimination threshold at rest and during movement, respectively.

Results: Hierarchical cluster analysis identified 2 clinical clusters. Cluster I ("mild motor-predominant") included patients who had milder motor and nonmotor symptoms severity than cluster II patients, who had a combination of severe motor and nonmotor manifestations (diffuse malignant). We observed that the diffuse malignant subtype had increased cortical excitability and reduced plasticity compared with the mild motor-predominant subtype. Kinematic analysis of motor performance demonstrated that the diffuse malignant subtype was significantly slower than the mild motor-predominant subtype. Conversely, we did not observe any significant differences in sensory function or sensorimotor integration between the two PD subtypes.

Conclusions: De novo PD subtypes showed different patterns of motor system dysfunction, whereas sensory function and sensorimotor integration mechanisms did not differ between subtypes. Our findings suggest that the subtyping of PD patients is not a mere clinical classification but reflects different pathophysiological mechanisms. Neurophysiological parameters may represent promising biomarkers to evaluate PD subtypes and their progression. © 2020 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease subtypes; motor cortex; sensory system; transcranial magnetic stimulation

Parkinson's disease (PD) is a highly heterogeneous disease characterized by a wide range of motor and nonmotor symptoms.[1,2] The first studies investigating PD heterogeneity identified clinical subtypes on the basis of clinical features. For instance, PD patients with a tremor-dominant subtype were frequently differentiated from patients with a clinical picture characterized by bradykinetic-rigid symptoms.[3] A major limitation of this dichotomic monodimensional approach is that patients are assigned to a specific subtype on the basis of a single clinical symptom, without considering the clinical complexity of PD.[4,5] More recently, several new multidimensional PD subtypes have been identified using data-driven clustering techniques, which allow patients to be assigned to a

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specific subtype on the basis of several motor and nonmotor symptoms, without any a priori hypothesis.[6-9] The most recent studies, based on hierarchical cluster analysis, have described a benign subtype characterized by mild motor symptoms ("mild motor-predominant" subtype) and a "diffuse malignant" subtype characterized by the coexistence of severe motor and nonmotor symptoms at disease onset.[10] Longitudinal assessment has validated the clinical relevance of these subtypes and demonstrated that the diffuse malignant subtype has faster progression than the mild motor-predominant subtype.[10,11]

The pathophysiology of PD is complex, with PD now considered a multisystem neurodegenerative disorder with involvement of dopaminergic and nondopaminergic pathways.[1,12] To date, however, the cortical-basal ganglia-thalamic-cortical sensorimotor loop is still considered the major locus of PD-related circuit dysfunction.[13,14] A number of neurophysiological studies have investigated the sensorimotor loop in PD and have consistently demonstrated that PD patients have several abnormalities in the motor[15-20] and sensory systems,[21] as well as in sensorimotor integration mechanisms.[22] However, one limitation of previous neurophysiological studies is that pathophysiological mechanisms of PD have been investigated without differentiating between clinical subtypes. It is therefore unknown whether the described neurophysiological abnormalities are specific to clinical subtypes or shared by the various subtypes.[15-17,19-24]

In the present article, we aimed to investigate the pathophysiological basis of PD subtypes in early disease stages by using neurophysiological parameters previously used to assess sensorimotor circuit pathophysiology in PD. We first classified enrolled PD patients using hierarchical cluster analysis. To avoid the possibility that disease duration[25] or dopaminergic treatment could influence our findings, we included only de novo PD patients with a clinical history shorter than 2 years. We investigated whether neurophysiological parameters that were consistently found to be abnormal in PD were able to discriminate PD subtypes. To assess primary motor cortex (M1) excitability and plasticity, we used various transcranial magnetic stimulation (TMS) paradigms. To evaluate motor performance in PD subtypes, we performed a kinematic analysis of voluntary movement execution. To investigate sensory function, we measured somatosensory temporal discrimination threshold (STDT). Finally, to assess sensorimotor integration we analyzed STDT movement-induced modulation.

We hypothesized that clinical heterogeneity reflects the activity of different pathophysiological mechanisms in early PD stages. According to this perspective, PD subtyping is not a mere clinical classification but a pivotal step to decode the complexity that characterizes PD pathophysiology.

Methods

Subjects

We consecutively enrolled 100 patients with de novo PD. Fifty healthy controls were also included in the study. The study was approved by Sapienza University of Rome Research Ethics Committee. All participants gave their written informed consent prior to participating in the study, which was conducted in accordance with the latest revision of the Declaration of Helsinki. Inclusion criteria were diagnosis of PD confirmed by a movement disorder expert based on international clinical criteria[26] and a clinical history less than 2 years (see Supplementary Material for the exclusion criteria). The clinical assessment of PD patients and the neurophysiological assessment of patients and controls were performed in a single session on the same day. Neurophysiological tests were performed in a pseudorandomized order. For each parameter, neurophysiological assessment was performed on the most affected side in PD patients.

Clinical Assessment

Clinical assessment included the administration of a number of clinical scales assessing motor as well as nonmotor symptom severity that were used as variables in the cluster analysis (Supplementary Material).

Neurophysiological Assessment

Transcranial Magnetic Stimulation Techniques: Cortical Excitability and Plasticity

Single- and paired-pulse TMSs were delivered using a monophasic MAGSTIM 200 stimulator connected to a figure-of-eight 70-mm diameter coil. To measure cortical excitability, we tested the input/output (I/O) curve by calculating motor-evoked potential (MEP) amplitude at 100%, 120%, and 140% of the resting motor threshold (RMT) and by measuring the I/O slope (Supplementary Material). The short intracortical inhibition (SICI) and intracortical facilitation (ICF) were also tested (Supplementary Material). Intermittent theta burst stimulation (iTBS) was used to test cortical plasticity (Supplementary Material). To test iTBS effects, we recorded 20 MEPs tested at an intensity equal to 120% active motive threshold before (T0) and 510 (T1), 15-20 (T2), and 25-30 (T3) minutes after iTBS.

Kinematic Recording of Motor Performance

A SMART analyzer motion system was used to record the passive movement of 1 optical marker placed

over the distal phalanx of the index finger during fast finger abduction (Supplementary Material). The range of motion (ROM), that is, the displacement of the index finger around its metacarpophalangeal joint expressed as the degree of the angle and mean velocity (in degrees).

STDT Testing

STDT was measured according to standardized protocols as the interval needed to recognize 2 consecutive electric stimuli as separate in time (Supplementary Material).[21,27-29]

Sensorimotor Integration

Paired stimuli for STDT were then triggered by fast index finger abduction at the onset of electromyography activity and 0, 100, and 200 milliseconds after movement onset. Because movement duration exceeded 200 milliseconds, STDT was tested during movement at all times. The kinematic analysis of movement execution was performed using the SMART analyzer motion system and following the same methodology used to record and analyze motor performance (Supplementary Material).

Statistical Analysis

Cluster Analysis

We used the SPSS 25.0 toolbox (version 25; IBM, New York, USA) for all statistics. Cluster analysis was performed in agglomerative hierarchical clustering, and Euclidean distance calculation was applied (Supplementary Material).

Univariate Analysis

We evaluated differences in demographics, clinical characteristics, and neurophysiological parameters between PD subtypes and controls. Univariate statistical tests were either parametric or nonparametric as appropriate. Spearman's correlation coefficient was used to investigate possible correlations between neurophysiological and clinical variables. Results are reported as significant when $P < 0.05$ after false discovery rate (FDR) correction for multiple comparisons.

Multivariate Analysis

To assess which neurophysiological variable best discriminated between subtypes, we designed a multivariate regression model with subtype as the dependent variable. As independent variables, we included neurophysiological variables that significantly differed between subtypes in univariate analysis.

Results

Demographic and Clinical Data of PD Patients (Whole Group)

A total of 100 patients with de novo PD were included in this study (65% male); mean age was 63.4 ± 9.3 years and mean disease duration from motor symptom onset was 1.3 ± 0.6 years. Fifty healthy controls were also included (64% male; mean age: 63.1 ± 8.8 years). Patients and controls did not differ in age and sex ($P > 0.05$). Fifty-eight percent of patients had a diagnosis of clinically probable PD, whereas 42% had a diagnosis of clinically established PD. The

TABLE 1. Demographic and clinical data of Parkinson's disease subtypes

	Mild-motor predominant subtype	Diffuse malignant subtype	Statistics		
			U	Z	*P*
Demographic and clinical data					
Age	62 ± 9	69 ± 7	520.5	−3.615	0.0003*
Disease duration	1.2 ± 0.5	1.4 ± 0.6	777	−0.727	0.4
Male sex (%)	61%	69%	815	−1.691	0.09
Clinically established PD (%)	42.1%	41.7%	908	−0.38	0.9
Clinically probable PD (%)	57.9%	58.3	911	−0.48	0.8
Motor domain					
MDS-UPDRS parts II + III score	17 ± 8	33 ± 10	218	−5.964	0.000000001*
H&Y score	1.2 ± 0.5	1.8 ± 0.8	568	−3.057	0.002*
Tremor-dominant (%)	54%	26%	714,000	−2614	0,009
Nonmotor domain					
MDS-UPDRS I score	4.1 ± 3.6	7.1 ± 5.2	434.5	−2.365	0.01
NMSS total score	18 ± 15	48 ± 21	239.5	−5.794	0.00000001*
RBDSQ score	4.2 ± 2.1	6.1 ± 2.3	766	−1.987	0.04
Cognitive composite score	185 ± 21	141 ± 22	144	−6.53	0.000000006*

H&Y, Hoehn and Yahr; MDS-UPDRS, International Parkinson and Movement Disorder Society–Unified Parkinson's Disease Rating Scale; NMSS, Nonmotor Symptoms Scale for Parkinson's disease; PD, Parkinson's disease; RBD, REM sleep behavior disorder screening questionnaire.

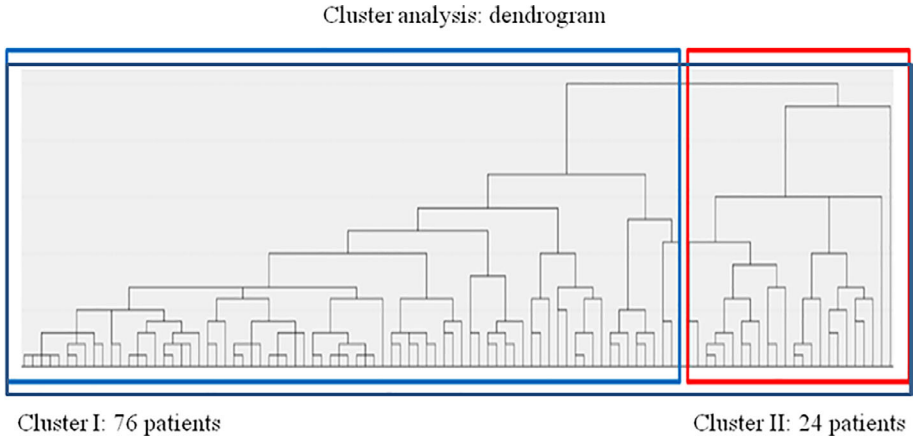

FIG. 1. Dendrogram of the cluster analysis. Cluster I: mild motor-predominant; cluster II: diffuse malignant subtype. [Color figure can be viewed at wileyonlinelibrary.com]

demographic and clinical features of PD patients are reported in Table 1.

Cluster Analysis: Identification of Clinical PD Subtypes

Hierarchical cluster analysis revealed 2 distinct clusters of PD patients with similar disease duration (Table 1). Cluster I included 76 PD patients, whereas cluster II included 24 patients (Fig. 1). Patients assigned to cluster I were significantly younger than those assigned to cluster II. Motor score (expressed by the combination of International Parkinson and Movement Disorder Society-Unified Parkinson's Disease Rating Scale [MDS-UPDRS] parts II and III) was significantly lower in cluster I than in cluster II. Nonmotor severity (as tested by the Nonmotor Symptoms Scale score) was significantly lower in cluster I than in cluster II. In addition, cognitive performance (expressed by a composite score; see Supplementary Material) was worse in cluster II than in cluster I. All statistics and *P* values related to comparisons between patients and controls are reported in Table 1.

In summary, cluster I included patients who were younger and had milder motor and nonmotor symptom severity than cluster II patients, who were characterized by a combination of more severe motor and nonmotor manifestations. According to previous studies,[10,11] we termed cluster I "mild motor predominant" and cluster II "diffuse malignant."

Neurophysiological Findings: Mild Motor-Predominant Subtype Versus Diffuse Malignant Subtype Versus Healthy Controls

Transcranial Magnetic Stimulation Techniques: Cortical Excitability and Plasticity

The RMT was similar in the 3 groups (cluster I, 49% ± 12%; cluster II, 51% ± 13%; controls, 47% ± 13%; Kruskal-Wallis H = 0.21; $P = 0.39$). The I/O curve, determined by measuring single-pulse MEPs tested at an intensity equal to 100%, 120%, and 140% RMT, showed that MEP amplitude differed significantly in the 3 groups at 100% RMT (H = 12.7; $P = 0.001$) and 140% RMT (H = 17.05; $P = 0.0001$), but not at 120% RMT (H = 11.6; $P = 0.003$, not significant after FDR correction). Post hoc analysis showed that MEP amplitude was higher in cluster I at 100% RMT and in cluster II at 140% RMT compared with controls (for all post hoc analysis statistics, see Supplementary Material, Table S1). After correction for multiple comparisons, post hoc analysis also revealed that cluster I and cluster II patients had similar MEP amplitudes (Fig. 2). The input/output (I/O) curve slope was steeper in cluster II than in cluster I and controls, but similar between cluster I and controls.

SICI (cluster I, 82% ± 88%; cluster II, 77% ± 84%; controls, 36% ± 24%; H = 11.96; $P = 0.002$, not significant after FDR correction) and ICF (H = 5.48; $P = 0.06$) were similar in the 3 groups. MEP amplitude changes induced by iTBS significantly differed in the 3 groups at T1 (H = 17.8; $P = 0.0001$), T2 (H = 29.5; $P = 0.0000004$), and T3 (H = 14.9; $P = 0.0005$). Post hoc analysis showed that cluster I and controls had similar iTBS-induced MEP amplitude facilitation, whereas cluster II showed a lower extent of MEP facilitation than controls at all timets after iTBS (Supplementary Material, Table S1). We also observed a greater extent of MEP facilitation in cluster I compared with cluster II at T1 and T2 but not at T3 (Supplementary Material, Table S1, and Fig. 3). In controls, iTBS induced significant MEP amplitude facilitation at all times (T1: $Z = -5.54$, $P = 0.000003$; T2: $Z = -5.17$, $P = 0.000002$; T3: $Z = -3.99$, $P = 0.00006$). In cluster I, we observed that iTBS induced significant MEP amplitude facilitation at T1 ($Z = -3.12$, $P = 0.002$) and T2 ($Z = -3.03$, $P = 0.002$), but not at T3 ($Z = -1.24$,

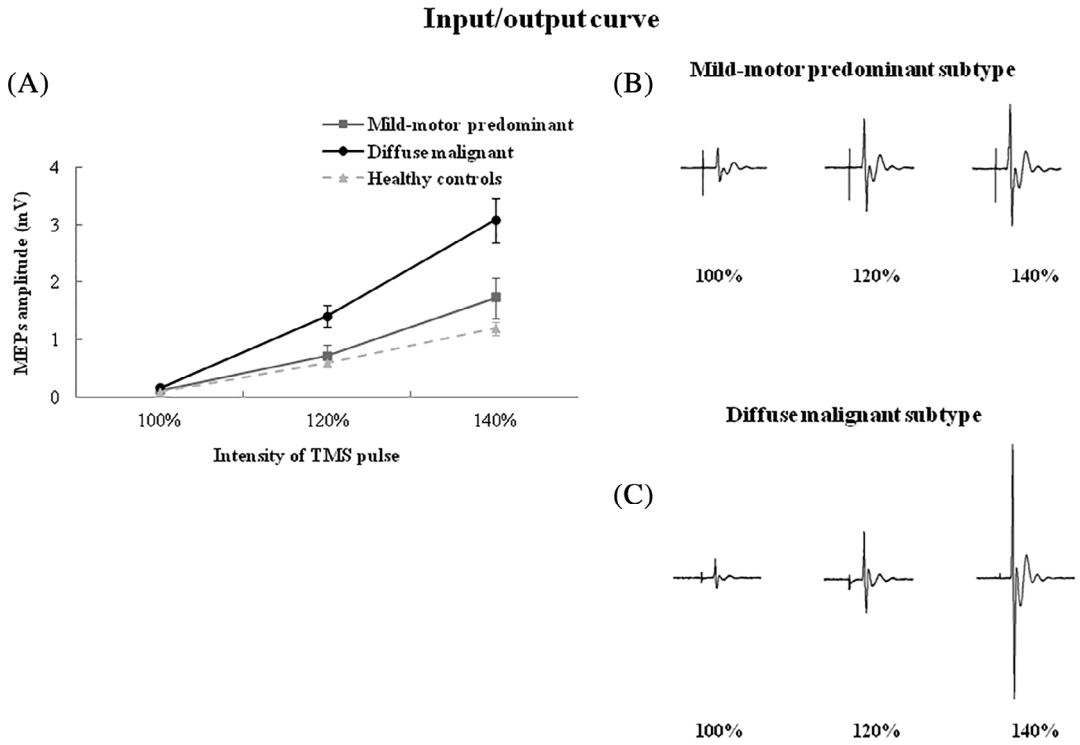

FIG. 2. Primary motor cortex excitability:input/output curve. (**A**) Input/output curve in PD patients with mild motor-predominant subtype (gray line), diffuse malignant subtype (black line), and healthy controls (dotted line). *X* axis, intensity of TMS pulse expressed as percentage of the intensity of resting motor threshold; *Y* axis, MEP amplitude expressed in millivolts. Each point represents mean; bars represent standard error. Note that input/output curve was significantly steeper in malignant diffuse subtype than in mild motor-predominant subtype. (**B**, **C**) Representative trace from mild motor-predominant (**B**) and diffuse malignant (**C**) patients. MEPs, motor-evoked potentials; PD, Parkinson's disease; TMS, transcranial magnetic stimulation.

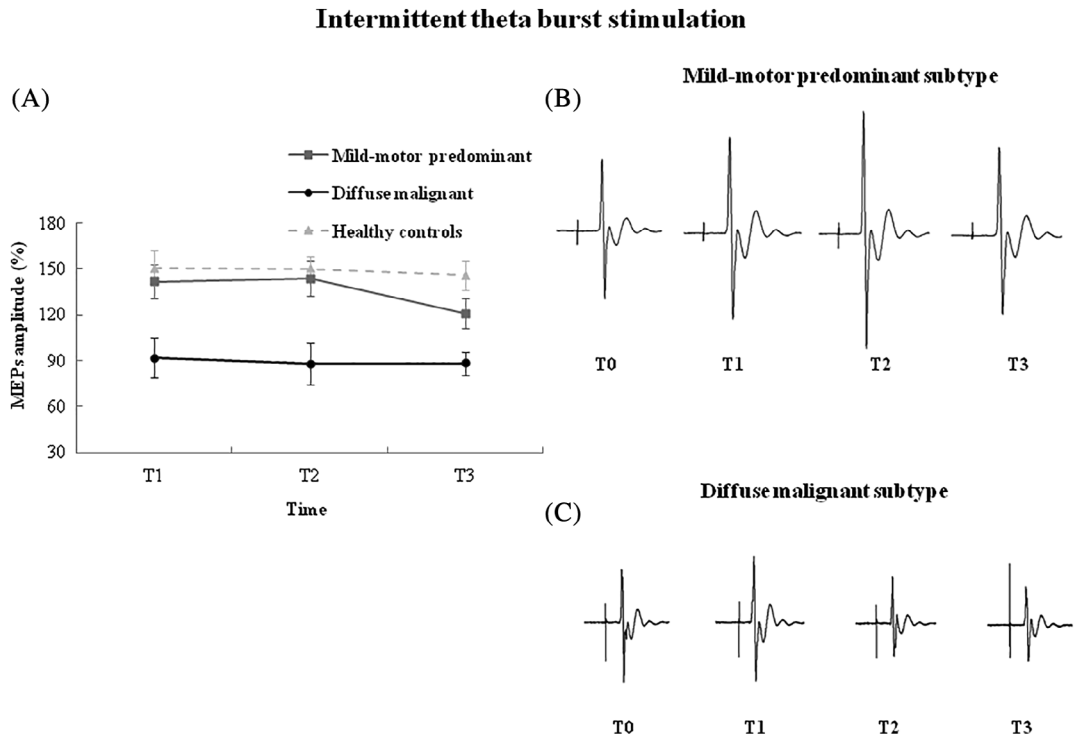

FIG. 3. Primary motor cortex plasticity: intermittent theta burst stimulation. (**A**) Effects of intermittent theta burst stimulation on MEP amplitude in PD patients with mild motor-predominant (gray line) and diffuse malignant (black line) subtypes and healthy controls (dotted line). *X* axis, time; *Y* axis, MEP size expressed as percentage of MEP size at T0. Note that the increase in MEP size is significantly higher in mild motor-predominant than in diffuse malignant subtype at T1 and T2. Each point represents mean; bars represent standard error. (**B**, **C**) Representative trace from mild motor-predominant (**B**) and diffuse malignant (**C**) patients. MEPs, motor-evoked potentials; PD, Parkinson's disease.

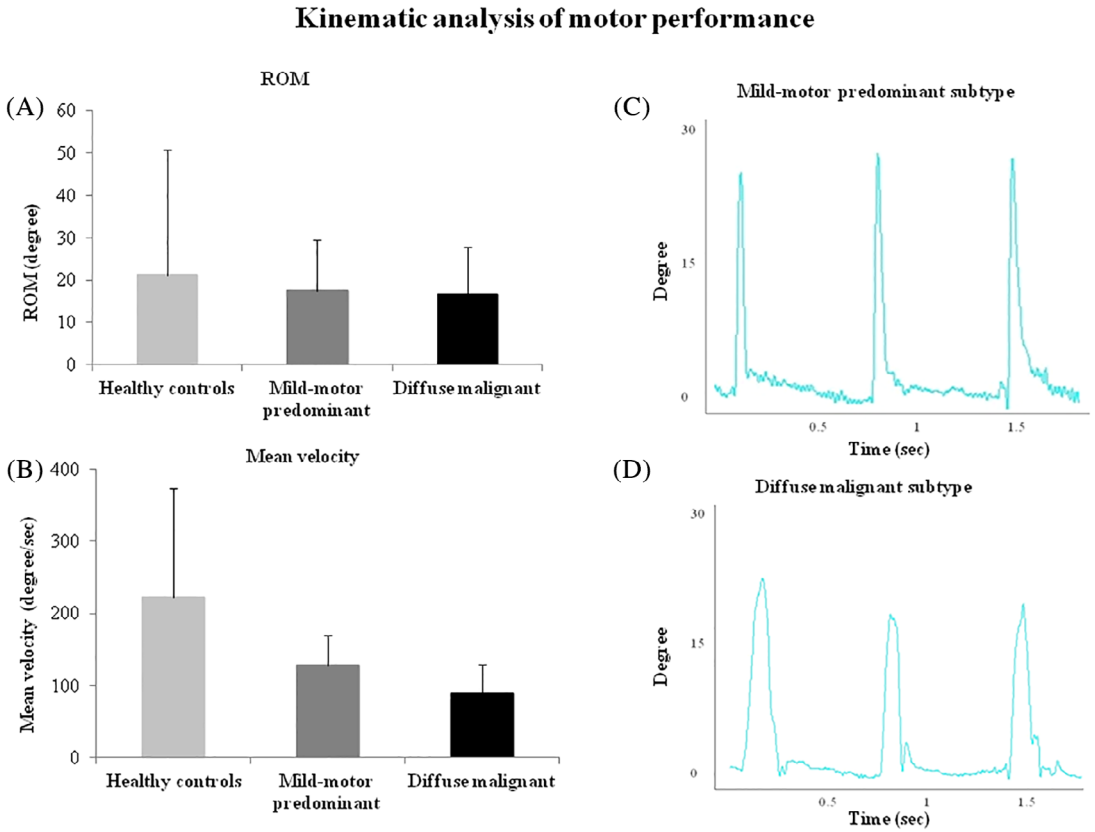

FIG. 4. Kinematic analysis of motor performance. (**A**, **B**) ROM (A) and mean velocity (**B**) of index finger abduction in PD patients with mild motor-predominant subtype (dark gray rectangle), diffuse malignant subtype (black rectangle), and healthy controls (light gray rectangle) subtype. *X* axis, subtype; *Y* axis, ROM expressed in degrees (**A**) and mean velocity expressed in degree/s (**B**). Note that mean velocity was significantly higher in mild motor-predominant than in diffuse malignant subtype. Bars represent standard error. (**C**, **D**) Representative trace from mild motor-predominant (**C**) and diffuse malignant (**D**) patients. ROM, range of motion; PD, Parkinson's disease. [Color figure can be viewed at wileyonlinelibrary.com]

P = 0.2). Conversely, MEP amplitude was unchanged after iTBS at T1 (Z = −1.55, P = 0.1), T2 (Z = −1.9, P = 0.06), and T3 (Z = −1.7, P = 0.09) in cluster II.

Kinematic Recording of Motor Performance

The 3 groups showed a similar ROM (H = 1.93, P = 0.38), whereas mean velocity differed significantly (H = 42.9, P = 0.0000000004). Post hoc analysis showed that mean velocity was significantly higher in controls than in either patient cluster. However, cluster I showed significantly higher mean velocity (U = 196, Z = −4.18, P = 0.00002) than cluster II (Supplementary Material, Table S1, and Fig. 4).

STDT

Neither the electrical stimulation intensity used to measure STDT (H = 4.1, P = 0.06) nor STDT (H = 3.16, P = 0.2) differed in the 3 groups.

Sensorimotor Integration

The extent of STDT changes during movement was similar in the 3 groups at 0 milliseconds (H = 0.47, P = 0.78), 100 milliseconds (H = 0.22, P = 0.89), and 200 milliseconds (H = 0.17, P = 0.41). In controls, movement induced significant STDT facilitation at 0 milliseconds (Z = −6.12, P = 0.000000001), 100 milliseconds (Z = −4.9, P = 0.0000004), and 200 milliseconds (Z = −4.5, P = 0.000005), similar to cluster I at 0 milliseconds (Z = −6.55, P = 0.000001), 100 milliseconds (Z = −5.76, P = 0.00001), and 200 milliseconds (Z = −4.76, P = 0.0001). In cluster II, movement induced significant STDT facilitation at 0 milliseconds (Z = −3.82, P = 0.0001), 100 milliseconds (Z = −3.38, P = 0.001), and 200 milliseconds (Z = −3.29, P = 0.001).

During the sensorimotor integration task, we also measured kinematic movement parameters using the SMART analyzer motion system. We observed that the modulation of ROM and mean velocity was similar in the 3 groups at 0 milliseconds (H = 3.15, P = 0.89), 100 milliseconds (H = 5.06, P = 0.7), and 200 milliseconds (H = 3.71, P = 0.0.1).

Multivariate Logistic Regression Analysis

The multivariate logistic regression analysis showed that MEP amplitude changes after iTBS at T2 were

the best discriminant between mild motor-predominant and diffuse malignant subtypes (Exp [B] = 1.032, $P = 0.01$).

Relationship Between Neurophysiological and Clinical Variables

Because the neurophysiological variables that significantly differed between the 2 subtypes were all related to the motor system, we investigated possible correlations between these neurophysiological variables and clinical scales assessing motor symptoms, that is, MDS-UPDRS parts II and III. In the PD patient group as a whole, we observed significant correlation between MDS-UPDRS part III and index finger abduction mean velocity ($r = 0.0.37$, $P = 0.004$), I/O slope ($r = 0.31$, $P = 0.003$), and MEP amplitude change at T2 after iTBS ($r = -0.21$, $P = 0.04$).

Discussion

In the present study we performed a controlled neurophysiological assessment of PD clinical subtypes, as identified by cluster analysis. Hierarchical cluster analysis identified a mild motor-predominant subtype and a diffuse malignant subtype. Using TMS techniques, we tested M1 excitability and observed that patients with the diffuse malignant subtype had a steeper I/O curve than patients with the mild motor-predominant subtype. The assessment of M1 plasticity, as tested by iTBS, also showed that patients with the diffuse malignant subtype had a significantly reduced response to iTBS compared with patients with the mild motor-predominant subtype. Similarly, kinematic analysis of motor performance demonstrated that patients with the diffuse malignant subtype were significantly slower than patients with the mild motor-predominant subtype. Conversely, we did not observe any significant differences in sensory function (as tested by STDT) or sensorimotor integration (as tested by STDT movement-induced modulation) between subtypes.

In our study, we used hierarchical cluster analysis to identify PD subtypes. To perform hierarchical cluster analysis, we chose the motor and nonmotor clinical variables that best discriminated between subtypes according to the results of previous studies.[10,11] In these studies, the authors used 2-,[7,30,31] 3-,[10,11] and 4-[9,32,33]cluster solutions. In the present study, we chose a 2-cluster solution to maximize the comparison between subtypes. By using this approach, we confirmed the presence of mild motor-predominant and diffuse malignant subtypes, the latter of which is characterized by the coexistence of severe motor and nonmotor symptoms. Most PD patients who participated in the study had the mild motor-predominant subtype.[10,11] Although the 2 subtypes identified differed in several clinical features (Table 1), disease duration was similar in the 2 groups of de novo patients. This excluded the possibility that differences we found were because of differences in disease duration.

To the best of our knowledge, this is the first investigation of neurophysiological correlates of PD clinical subtypes. We found that the I/O curve slope was significantly steeper for the diffuse malignant than for the mild motor-predominant subtype. The I/O curve, that is, the increase in MEP amplitude with increasing TMS intensity, is a measure of M1 excitability and assesses the recruitment of neurons that are intrinsically less excitable or spatially farther from the center activated by TMS.[34,35] Increased excitability of the motor cortex, as tested by the I/O curve, has previously been reported in PD patients,[20,36,37] although other authors have observed a normal I/O curve in PD patients.[38-41] Our findings also showed that iTBS response was significantly reduced in the diffuse malignant compared with the mild motor-predominant subtype. This was also confirmed by logistic regression analysis, which showed that MEP amplitude changes after iTBS significantly discriminated between the 2 subtypes. iTBS is a repetitive TMS technique that is able to induce long-lasting enhancement of motor cortex excitability, as tested by the facilitation of MEP amplitude.[20,42] The aftereffects of iTBS are thought to resemble long-term potentiation (LTP) mechanisms observed in animal models and are likely mediated by the activity of N-methyl-D-aspartate (NMDA) glutamatergic receptors.[43,44] Most previous studies have reported that PD patients have a reduced response to iTBS[15-17,19,20] and that the extent of iTBS response in PD may depend on the disease stage.[18] However, the correlation between iTBS aftereffects and PD clinical features is unclear,[16,19] and studies have reported inconsistent findings regarding the possible effects of L-dopa therapy on iTBS response in PD.[15-17,19,20] A further main finding of our study was that the execution of voluntary movement, as tested by repetitive fast index finger abduction recorded by a kinematic analysis system, was significantly slower in the diffuse malignant subtype than in the mild motor-predominant subtype. Bradykinesia is considered a clinical hallmark of PD and is the only sign that must be present for PD diagnosis.[26,45] Several previous neurophysiological studies investigating motor execution of the upper limb in PD have consistently demonstrated the presence of bradykinesia,[46] even in early disease stages.[23,47-49]

Overall, we observed that patients with the diffuse malignant subtype had increased M1 excitability, reduced M1 plasticity, and worse motor performance compared with mild motor-predominant patients. Because abnormal M1 excitability and plasticity in PD are thought to reflect the effects of dopaminergic loss on the cortical-basal ganglia-thalamic-cortical motor

loop,[15,17,19,20] it is plausible that the extent of involvement of this loop is greater in the diffuse malignant subtype than in the mild motor-predominant subtype. This hypothesis is in line with the neuroimaging observation that in early stages more atrophy occurs in structures functionally connected to the substantia nigra in the diffuse malignant subtype than in the mild motor-predominant subtype.[10] The increase in M1 excitability in the diffuse malignant subtype may therefore be a possible compensatory mechanism for defective basal ganglia function.[14,50-54] At the same time, given that dopamine promotes NMDA-dependent LTP plasticity mechanisms,[55-58] the reduced M1 plasticity that characterizes the diffuse malignant subtype may directly reflect the effect of dopaminergic denervation on M1. Similarly, the observation that bradykinesia is more evident in the diffuse malignant subtype than in the mild motor-predominant subtype seems to confirm that dopaminergic loss differs between the 2 subtypes. Indeed, bradykinesia in PD has traditionally been associated with dopamine striatal depletion.[1,59-61]

The increase in M1 excitability, reduction in M1 plasticity, and bradykinesia that characterize the diffuse malignant subtype therefore seem to be related to the same pathological mechanism, that is, the effect of dopaminergic loss on the cortical-basal ganglia-thalamic-cortical motor loop. This unifying pathophysiological hypothesis is strongly supported by a recent article that reported on an investigation of the neurophysiological correlates of bradykinesia in PD.[36] The authors found that in moderate/advanced PD, increased M1 excitability and reduced M1 plasticity (ie, the same neurophysiological abnormalities that we found in the diffuse malignant subtype) significantly correlated with bradykinesia features. Intriguingly, the authors observed that dopaminergic treatment simultaneously improved movement velocity and normalized M1 excitability and plasticity.[36] This observation corroborates the hypothesis that dopaminergic loss is at a crossroads between M1 abnormalities and bradykinesia in PD, and we now suggest that this pathophysiological pattern is already present in early disease stages in the diffuse malignant subtype.

In the present study we observed that SICI did not differ between PD subtypes A number of previous studies investigating SICI in PD have reported contrasting results, although these discrepancies are likely because of methodological differences.[24] We included a large number of de novo PD patients and observed normal SICI. This suggests that SICI does not play a major role in PD pathophysiology in the early stages, but it does not exclude the possibility that SICI abnormalities may differentiate subtypes in more advanced disease stages.

Our findings also showed that sensory function and sensorimotor integration mechanisms were normal in de novo PD and that they did not differ between subtypes in early disease stages. It is possible that sensory function abnormalities predominantly intervene in moderate/advanced stages of PD and that sensory system involvement is transversal in PD and thus independent of the various subtypes. In line with these hypotheses, it has been demonstrated that STDT parallels PD severity and duration[62,63] and that STDT does not differ between tremor-dominant and bradykinetic-rigid subtypes.[29] Therefore, the presence of sensory abnormalities in PD should be considered a marker of longitudinal intraindividual heterogeneity represented by clinical and pathophysiological evolution during disease progression, rather than of interindividual heterogeneity, as suggested by the presence of PD subtypes.

One strength of our study was that we identified clusters using a multidimensional approach that included motor and nonmotor clinical variables. The observation that neurophysiological parameters assessing motor systems significantly differed in subtypes that were also identified using nonmotor variables suggests that different degrees of cortical-basal ganglia-thalamic-cortical motor loop dysfunction play a pivotal role in PD heterogeneity. A further strength of our study was that we used validated and reliable neurophysiological techniques that have been repeatedly used in PD, and we performed our assessment in a large and controlled population of de novo PD patients. However, our study has some limitations. We included clinically diagnosed de novo PD patients with a short disease duration and without DATSCAN or FDG PET (DAT, dopamine transporter imaging; FDG-PET, fluorine-18 deoxyglucose positron emission tomography). By including only early-stage de novo patients, we avoided the possibility that disease duration and dopaminergic treatment could have influenced our findings. However, it was not possible to fully exclude misdiagnosis bias (for instance, atypical parkinsonisms) because diagnostic accuracy is lowest in the first years, particularly when treatment response is unknown. Nevertheless, it is important to highlight that PD diagnosis is currently based on clinical criteria, which we strictly followed.[26,45] Future longitudinal clinical and neurophysiological assessments of our findings are needed to test the reliability of our results over time. Another possible limitation of the present study was that our assessment investigated only motor and sensory functions. However, it is known that PD pathophysiology affects several systems, including the autonomic nervous system, with consequent cardiovascular, gastrointestinal, and genitourinary dysfunction.[64] Because several nonmotor symptoms, including orthostatic hypotension[10] and urinary dysfunction,[65] differ in clinical subtypes, future studies providing a quantitative assessment of these functions in PD subtypes are needed. A further limitation of our study was that we did not test some TMS cortical parameters that are known to be altered

in PD, such as short-latency afferent inhibition and short-interval intracortical facilitation.

In conclusion, we provided the first experimental evidence that PD data-driven subtyping is not a mere clinical classification, but that the different clinical subtypes have specific pathophysiological mechanisms. This information bridges the gap between the clinical heterogeneity and pathophysiological complexity that characterize PD and represents the first step toward new individualized therapeutic strategies in PD. Second, we demonstrated that in PD, the neurophysiological abnormalities are associated with specific subtypes. Third, this is the first neurophysiological controlled study that in a large number of de novo PD patients demonstrated that motor and sensory systems play different roles in the pathophysiology of PD in the early stages.

One remaining question is how long pathophysiological mechanisms and pathological involvement differ between subtypes. If we consider our present findings together with those provided by Fereshtehnejad et al in 2017,[10] we may speculate that neurophysiological, neuroimaging, and cerebrospinal fluid markers clearly differentiate PD subtypes in early disease stages. Indeed, this multidisciplinary data set strongly supports the nonclinical substrate of PD subtypes and the existence of useful quantitative and reliable tools to distinguish between subtypes. Longitudinal studies will clarify whether these differences are still present in moderate and advanced stages of PD. ●

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Supporting Data

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