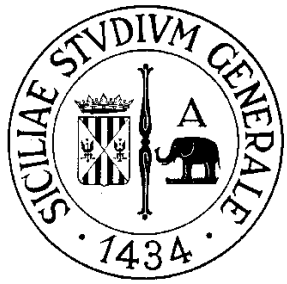

UNIVERSITY OF CATANIA

International Ph.D. Program in Neuroscience

XXXII Cycle

NEUROPHYSIOLOGICAL FEATURES OF DRUG-NAIVE PARKINSON'S DISEASE: FROM DIAGNOSIS TO TREATMENT

PhD Thesis

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Chapter I. General Introduction

1. Parkinson's disease

Parkinson's disease (PD) is a slowly progressive neurodegenerative disorder, due to decreased dopaminergic transmission in the substantia nigra pars compacta of the basal ganglia. The etiopathogenesis results from a combination of genetic and environmental factors, acting on multiple neuroanatomical areas and involving neurotransmitters other than dopamine and regions of the nervous system outside the basal ganglia (*Kalia V et al. 2015*). PD is the second most common progressive neurodegenerative disorder after Alzheimer's disease. Reported standardized incidence rates of PD are 8-18 per 100.000 person-years and its prevalence in industrialized countries is generally estimated at 0.3% of the entire population and about 1% in people over 60 years of age (*de Lau LML et al. Lancet Neurol 2006*).

PD is clinically characterized by a broad range of motor and non-motor symptoms, that begin years before diagnosis can be made (*Kalia V et al. 2015*). According to diagnostic criteria developed by the UK Parkinson's Disease Society Brain Bank, the classical motor symptoms of PD include bradykinesia, rigidity and rest tremor and responsiveness to levodopa is also considered as supportive criteria for diagnosis (*Hughes AJ et al. J Neurol Neurosurg Psychiatry 1992*). Recently new diagnostic criteria have been proposed by the Movement Disorders Society (MDS) (*Postuma RB et al. Mov Disord 2015*). As previous criteria, the MDS criteria use a two-step process for PD diagnosis.

First step is the identification of a parkinsonian syndrome, which is defined as presence of bradykinesia in combination with either rest tremor, rigidity, or both. Once identified, the criteria have to establish whether parkinsonism is attributable to PD or to other forms of parkinsonism, including secondary and atypical parkinsonian syndromes (APS) (*Postuma RB et al. Mov Disord 2015*). Despite APS differ from PD for a more rapid progression, disabling functional prognosis and poor response to dopaminergic treatment, differential diagnosis at the early stage of the disease is difficult.

Available therapies for PD are symptomatic, enhancing intracerebral dopamine concentrations or stimulating dopamine receptors. These drugs include levodopa, dopamine agonists, monoamine oxidase type B inhibitors and catechol-o-methyltransferase inhibitors (*Kalia V et al. 2015*). Levodopa still provides the greatest symptomatic benefit for motor signs in PD, but long-term use is associated with the onset of motor complications (*Kalia V et al. 2015*). Development of disease-modifying drugs, targeting selected dysfunctional molecular pathways and slowing the underlying neurodegenerative process in PD, is worthy. Studies conducted on drug-naive PD patients could show pathogenetic mechanisms of the disorder, avoid potential disease-modifying effect of dopamine replacement therapy on neuronal activities.

2. Diagnosis

Despite PD is characterized by a slowly progressive course and APS presented with more rapid progression and severe prognosis, the differential diagnosis between PD and APS, such as Multiple System Atrophy (MSA), Progressive Supranuclear Palsy (PSP) and Cortico-basal syndrome (CBS), is often difficult because of overlap of common clinical features, which makes APS indistinguishable from PD at the early stage of disease. Therefore, only post-mortem pathology can definitively establish the diagnosis of a parkinsonism (*Espay AJ et al. J Mol Neurosci 2011*). Between 75% and 95% of patients identified as PD by experts have their diagnosis confirmed on autopsy (*Postuma RB et al. Mov Disord 2015*). Diagnostic accuracy varies considerably according to disease duration, age, the expertise of the clinicians and evolution of research in PD. Diagnostic error can be attributable to failure to recognize other pathologies causing neurodegenerative or secondary parkinsonism (i.e., vascular parkinsonism, MSA, PSP) or to the absence of a real progressive parkinsonian disorders (i.e., essential tremor, dystonic tremor) (*Postuma RB et al. Mov Disord 2015*). The MDS-PD Criteria are designed to minimize both of these diagnostic errors, including two distinct levels of diagnostic certainty: clinically established PD (maximizing specificity and reduced sensitivity) and probable PD (balancing sensitivity and specificity) (*Postuma RB et al. Mov Disord 2015*).

Having established the presence of parkinsonism, the MDS-PD criteria will be applied to determine whether the patient meets criteria for PD as the cause of this parkinsonism.

Diagnosis of clinically established PD requires (*Postuma RB et al. Mov Disord 2015*):

1. Absence of absolute exclusion criteria
2. At least two supportive criteria
3. No red flags

Diagnosis of clinically probable PD can be made in (*Postuma RB et al. Mov Disord 2015*):

1. Absence of absolute exclusion criteria
2. Presence of red flags counterbalanced by supportive criteria, ie, if one red flag is present there must also be at least one supportive criterion; if two red flags, at least two supportive criteria are needed. If there are more than two red flags, clinically probable PD cannot be diagnosed.

Despite the new MDS criteria for PD demonstrated higher sensitivity and specificity than United Kingdom Brain Bank criteria (*Postuma RB et al. Mov Disord 2018*), the necessity of definitive validated diagnostic markers of disease continues to be present, especially in differential diagnosis with APS.

3. Treatment

3.1 Therapeutic response to levodopa treatment

Levodopa is considered the gold standard among PD treatment's options (*LeWitt PA, Mov Disord 2015*). The therapeutic response to levodopa is expressed by two different

modalities: the short-duration response (SDR) and the long-duration response (LDR) (*Zappia M et al. Neurology 1999*).

SDR is featured by a clinical improvement lasting 3-5 hours after the administration of a single dose of levodopa, paralleling the plasma concentration of the drug, whereas LDR is a sustained antiparkinsonian benefit derived from prolonged administration of levodopa, persisting for hours to days after discontinuation of treatment, independently on pharmacokinetics of the drug (*Zappia M et al. Neurology 1999; Zappia M et al. J Neurol 2010; Anderson E et al. Parkinsonism Relat Disord 2011*).

LDR represents a great part of clinical benefit induced by levodopa (*Zappia M et al. J Neurol 2010*) and it is not associated with the long-term levodopa treatment complications, such as dyskinesia and wearing-off phenomenon, that usually accompany SDR (*Zappia M et al. Neurology 1999*). LDR can be assessed by evaluating the motor condition of the patient with clinical scales (*Fahn et al. Recent developments in Parkinson's disease. Florham Park, NJ: Macmillan Healthcare Information, 1987*), the movement time recordings (*Quattrone A et al. Ann Neurol 1995*) and the tapping score (*Nutt JG et al. Neurology 1995*). The mechanisms underlying LDR are still unknown. A presynaptic dopaminergic origin has been postulated (*Quattrone A et al. Ann Neurol 1995*). This hypothesis suggests that LDR might be dependent on the presence of a presynaptic storage compartment releasing dopamine and completely saturated with exogenous supplied levodopa; once the dopamine storage capability has been completely saturated, LDR could exhibit a maximal and stable response (*Quattrone A et al. Ann Neurol 1995*). On the other hand, the observations that LDR also occurs with the

dopamine-agonist drugs suggest that LDR could imply a postsynaptic mechanism in origin (*Barbato L et al. Clin Neuropharmacol 1997*). Further studies on animal models for understanding the physiological basis of LDR are worthy.

3.2 Motor learning and levodopa treatment

Motor learning is classically defined as a set of processes associated with practice or experience, leading to transient or permanent changes in the capability to perform a movement (*Nieuwboer A et al. Parkinsonism Relat Disord 2009*). Motor learning involves three different stages:

1. the cognitive stage of learning, featured by receiving instructions and figuring out what to do and how to do it;

2. the associative stage of learning, marked by associating specific environmental cues with the movements required to achieve the goal or the skill;

3. the autonomous stage, in which automaticity is reached (*Nieuwboer A et al. Parkinsonism Relat Disord 2009*).

The striatum is one of the most involved structure in motor learning, predicting an impairment in PD. Animal models of PD suggest that there is a dynamic interplay between degenerative and regenerative mechanisms of these structures, which are mediated by exercise and learning (*Nieuwboer A et al. Parkinsonism Relat Disord 2009*). In particular, LDR to levodopa and adequate levels of dopamine into the striatum have been demonstrated fundamental for acquisition and maintenance of learned skills in rodents. PITx3-deficient mouse line, whose dopamine-levels in the dorsal striatum are

reduced by 90%, does not learn new motor task and restoring dopaminergic activity improves learning (*Beeler JA et al. Ann Neurol 2010*). Therefore, LDR to levodopa has been demonstrated fundamental for acquisition and maintenance of learned skills in rodents, supporting the hypothesis that LDR is implicated in motor learning (*Beeler JA et al. Ann Neurol 2010*). They used the first animal model of LDR, showing not only the rescue effect of levodopa on motor deficit, but also the influence of levodopa on the maintenance of learned abilities (*Beeler JA et al. Ann Neurol 2010*).

Dopamine is also important for learning in humans, as suggested by studies showing that motor learning is impaired in PD patients compared to controls (*Siegert RJ et al. Neuropsychology 2006; Tremblay PL et al. Exp Brain Res 2010*). In a study of movement chunking, the combining of single movements into integrated sequences, motor sequence learning in PD was compromised in the untreated state and restored with levodopa (*Tremblay PL et al. Exp Brain Res 2010*). However, dose of levodopa and severity of the disease are determinants of the effect of the drug on motor learning.

Therefore, LDR to chronic levodopa treatment could be a manifestation of "rescued" motor learning in dopamine depleted rodents as well as in humans with PD. Nevertheless, to our knowledge, there are no studies that have assessed the relationship between LDR and motor learning in humans.

4. Neurophysiological features

Peculiar neurophysiological features characterize PD, helping to understand the underlying pathogenetic processes of the disorder.

4.1 Blink Reflex and R2 Blink Reflex Recovery Cycle

Blink reflex (BR) is the simultaneous and bilateral closure of eyelids following stimuli of various nature. BR could be evoked by stimulating supraorbital (SO) nerve and the response is simultaneously recorded in both orbicularis oculi muscles (*Kimura J Davis 1989*). The afferent limb of the reflex loop is made up of cutaneous myelinated Ab fibers of medium size, which constitute the sensory trigeminal root and the ophthalmic division, whereas the common efferent limb is the facial nerve. The evoked response is represented by two components:

• Component R1: early and stable response; ipsilateral response to the site of stimulation; it is centrally relayed through an oligosynaptic arc.

• Component R2: late and unstable response (subject to adaptation to a repetitive stimulus); ipsilateral and contralateral response to the stimulus; it is centrally relayed through a polysynaptic arc.

R2 Blink Reflex Recovery Cycle (R2BRRC) is a neurophysiological tool, used to measure brainstem excitability, based on the paradigm of the double stimulus. Therefore, two shocks with same characteristics and equal intensity are applied to the SO foramen (*Kimura J et al. Brain 1973*). The first stimulus is defined as conditioning, whereas the

second one is called test stimulus. The response to the test stimulus is modified by the previous conditioning stimulus in relation to the duration of interstimulus interval (ISI) that separates them. R2 polysynaptic component is strongly modified by a double stimulus for short ISIs, resulting abolished for an ISI of 100 ms and recovering from an ISI of 300 ms in healthy subjects. The value of the response to the test stimulus for different ISIs is expressed as percentage of the amplitude of the response to the conditioning stimulus. Using these percentage values (%) as parameters of an ordinate axis and ISI values (ms) as parameters of an abscissa axis, we obtain a graph-curve identifying the so-called recovery cycle.

The R2 component is less inhibited by preceding impulses and R2BRRC is enhanced, being early and rapid, in PD (*Kimura J et al. Brain 1973*). Despite these data, there are no studies which have focused on possible asymmetric brainstem excitability in untreated PD.

Only few studies have examined alterations of excitability in APS, in which R2BRRC has been reported to be normal in CBS and peculiarly enhanced in MSA and PSP, such as in PD (*Valls-Solé J et al. Brain 1997; Sciacca G et al. Eur J Neurol 2018*).

4.2 Cognitive potential P300, motor evoked potentials and Bereitschaftspotential

Several studies have shown that peculiar neurophysiological tests, such as auditory evoked potential P300, motor evoked potentials (MEPs) and Bereitschaftspotential (BP) could be considered as diagnostic tools for an objective quantification of motor learning, also in PD (*Dick JP et al. Electroencephalogr Clin Neurophysiol 1987; Sohn YH et al.*

J Neurol Sci 1998; Gallasch E et al. Eur J Appl Physiol 2009; Mak M et al. Clin Neurophysiol 2013; MacLean SJ et al. Front Hum Neurosci 2015).

Evoked potentials related to P300 events investigate the cognitive status of patients *(Silva Lopes Md et al. Arq Neuropsiquiatr 2014*). Electrodes are positioned in regions A1, A2, and Fz according to the 10-20 international system and a rare target auditory stimulus is presented according to an "oddball stimulus". Drug-naive PD patients showed typically prolonged P300 latency compared with levodopa-treated patients (*Sohn YH et al. J Neurol Sci 1998*).

Transcranial magnetic stimulation (TMS) is a non-invasive tool for stimulation of the cerebral cortex and for evaluation of the excitability of the motor cortex and pathways. One advantage of TMS over the other diagnostic tools such as neuroimaging techniques is its ability to assess both excitatory and inhibitory mechanisms in primary motor cortex (M1) circuits. TMS evaluated excitatory circuits through MEPs recording and inhibitory circuits through cortical silent period (CSP) (*Kobayashi M et al. Lancet Neurol 2003*). In particular, a recent TMS follow-up study revealed longitudinal changes in plasticity and inhibition in PD, that could be considered useful objective markers of early disease progression (*Kojovic M et al. Mov Disord 2015*).

BP is a movement-related potential, appearing prior to a simple voluntary movement (*Shibasaki H et al. Clin Neurophysiol 2006*). BP assessment results from recording of voluntary-movements that are time-locked to averaged fluctuations of electroencephalographic (EEG) activity. Two main components can be distinguished over the course of BP recording. The first part of BP, starting 1-2 s before a movement,

is the so-called “early BP” and it has a more diffuse, yet midline distribution over the cortex (*Shibasaki H et al. Clin Neurophysiol 2006*). The early BP reflects general preparation for the forthcoming movement and it is generated by the pre-supplementary motor area (pre-SMA), supplementary motor area (SMA) and lateral premotor cortex bilaterally (*Shibasaki H et al. Clin Neurophysiol 2006*). The early BP is followed by the “late BP”, starting 400–500 ms before the movement, characterized by a lateralization to the hemisphere contralateral to the side of the movements and generated by M1 (*Brunia CHM et al. The Oxford handbook of event-related potential components 2012; pp.198-207*). The dysfunction of the basal-ganglia-thalamo-cortical circuits, including the SMA, is responsible for BP alterations in PD patients (*Georgiev D et al. Clin Neurophysiol 2016*).

Chapter II. Neurophysiological features for diagnosis of Parkinson's disease

Asymmetry Index of Blink Reflex Recovery Cycle differentiates early Parkinson's disease from Atypical Parkinsonian Syndromes

Abstract

Background: Differential diagnosis between PD and APS, such as MSA and PSP, is often difficult because of overlap of common clinical features. We evaluated R2BRRC in drug-naive PD patients and in MSA and PSP patients to differentiate early PD from APS.

Methods: We investigated 43 patients: 15 drug-naive PD patients, 16 MSA patients and 12 PSP patients. R2BRRC was evaluated bilaterally at ISIs of 100, 150, 200, 300, 400, 500 and 750 ms. An asymmetry index (AI) of R2BRRC for each ISI was computed.

Results: R2BRRC of PD patients showed an increased brainstem excitability for less affected side (LAS) stimulation at ISIs of 100, 150, 200 (p<0.001) and 300 ms (p=0.03) compared to more affected side (MAS) stimulation, whereas no differences between LAS and MAS stimulation were found in APS. AI of 0.87 at ISI of 100 ms differentiated PD from MSA with a sensitivity of 86.7% and a specificity of 100%, whereas AI of 0.78 at ISI of 100 ms permitted to discriminate PD from PSP with a sensitivity of 86.7% and a specificity of 91.7%.

Conclusions: AI of R2BRRC may represent a reliable tool in differentiating PD from APS, especially at the early stage of the disease.

1. Aim of the study

The aim of this study was to evaluate differences of brainstem excitability in drug-naive PD, MSA and PSP patients through a side-to-side comparison. To test this idea, we computed an asymmetry index (AI) of R2BRRC which may help clinicians in differentiating PD from APS, especially at the early stage of the disease.

2. Materials and methods

2.1 Participants

Patients affected by untreated PD, PSP and MSA were enrolled for 24 months, according to MDS criteria for PD (*Postuma RB et al. Mov Disord 2015*) and PSP (*Höglinger GU et al. Mov Disord 2017*) and Gilman's diagnostic criteria for MSA (*Gilman S et al. Neurology 2008*). Clinical diagnosis of APS was confirmed by subsequent follow-up visits. The study was approved by the Local Ethic Committee and patients were enrolled after signing the written informed consent.

2.2 Clinical assessment

Clinical evaluation was performed through Unified Parkinson's Disease Rating Scale - Motor Examination section (UPDRS-ME) (*Fahn et al. Recent developments in Parkinson's disease. Florham Park, NJ: Macmillan Healthcare Information, 1987*) and Hoehn and Yahr (H&Y) stage (*Hoehn M et al. Neurology 1967*).

2.3 Neurophysiological assessment

Blink Reflex (BR) and R2BRRC (*Kimura J, Brain 1973*) were recorded in all patients by a neurophysiologist unaware of clinical data. Bipolar electrical stimulation was applied to supraorbital nerve (intensity: 15-25 mA; duration: 0.2 ms). Electromyographic responses were recorded in orbicularis oculi muscles with surface silver–silver chloride electrodes (filters: 20 Hz–10 kHz) (*Kimura J, Brain 1973*).

R2BRRC was performed with the technique of paired stimulation at ISIs of 100, 150, 200, 300, 400, 500, 750 ms. For each ISI the R2 amplitude ratio (expressed as percentage ratio between R2 peak-to-peak amplitudes of conditioned and unconditioned responses) was calculated (*Kimura J, Brain 1973*). R2BRRC was evaluated by plotting the R2 amplitude ratio for all the tested ISIs and for both sides.

The absolute value of R2BRRC AI was estimated using the following formula: [(Side1-Side2)/(Side1+Side2)], where the two sides are the percentage values of R2BRRC for each ISI, calculated by stimulating both more and less clinical affected side of each patient. Treated APS patients were evaluated at least after 12 h of withdrawal of anti-parkinsonian medication.

2.4 Statistical analysis

Differences of means and proportions between two selected groups were evaluated by t-test and chi-square test, respectively. Overall differences in R2BRRC and AI between the three groups were assessed by analysis of variance (ANOVA) for each ISIs, using Tukey post-hoc test for further comparisons. Sensitivity and specificity of AI in differentiating PD from MSA and PSP patients together with 95% Confidence Intervals (95%CI) were calculated using the optimal cut-off values determined by ROC (receiver operating characteristic) curve analysis.

3. Results

Forty-three subjects were enrolled: 12 patients with PSP, 16 patients with MSA and 15 patients with untreated PD. Demographics and clinical characteristics are summarized in table 1.

PSP and MSA patients presented with significantly longer disease duration and higher UPDRS-ME and H&Y scores as compared to PD patients.

BR responses to single stimulation were present at a normal latency, similarly in all participants.

R2BRRC curves of the three groups are shown in figure 1. All PSP and MSA patients showed an early R2 recovery starting at ISI of 100 ms on both sides of stimulation, thus no statistically significant differences were found in a side-to-side comparison. R2BRRC of PD patients was increased from ISI of 100 ms stimulating less affected side (LAS),

but it was normal stimulating the more affected side (MAS), revealing a significantly different amplitude of response at ISIs of 100, 150, 200 and 300 ms between LAS and MAS stimulation.

AI of R2BRRC was computed for each ISI. The absolute value of AI ranged between 0 and 1, where 0 represented the absence of asymmetry and 1 represented the maximum asymmetry between the two sides. AI at ISI of 100 ms showed the greatest significant difference between groups as shown in figure 2, being higher in PD compared respectively to PSP and MSA. A cut-off of AI greater than 0.78, estimated using the ROC curve analysis method (accuracy of AI: Area Under the ROC Curve (AUC) = 0.83; $p<0.001$), differentiated PD from PSP patients with a sensitivity of 86.7% (95%CI: 59.5-98.3) and a specificity of 91.7% (95%CI: 61.5–99.8). A cut-off of AI greater than 0.87 (accuracy: AUC = 0.87; $p<0.001$) differentiated PD from MSA patients with a sensitivity of 86.7% (95%CI: 59.5–98.3) and a maximum specificity of 100% (95%CI: 79.4–100). In order to exclude effects related to disease duration or treatment in APS, we conducted a further analysis only on drug-naive patients. Thirteen patients affected by APS had never been treated: 8 patients with PSP (4 men; age 69.1 ± 5.9 years) and 5 patients with MSA (4 men; age 68.2 ± 10.6 years). There were no statistically significant differences in disease duration (PSP: 2 ± 0.8; MSA: 2.4 ± 1.1) and UPDRS-ME score (PSP: 31.2 ± 16.2; MSA: 35.2 ± 10.9) between drug-naive APS and PD patients. AI cut-off greater than 0.78 (accuracy: AUC = 0.83; $p<0.001$), differentiated PD from drug-naive APS patients with a sensitivity of 86.7% (95%CI: 59.5–98.3) and a specificity of 92.3% (95%CI: 64.0–99.8).

4. Tables and Figure legends

Table 1. Demographic and clinical characteristics of participants.

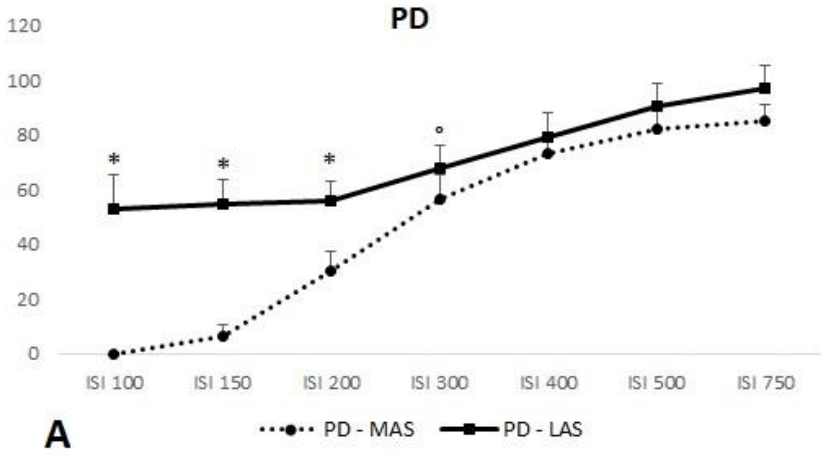
	PSP (12)	**MSA (16)**	**PD (15)**
Age (years)[a]	70.3 ± 6.8	68.0 ± 9.1	64.6 ± 7.3
Sex (M)	6 (50%)	9 (56%)	10 (67%)
Disease duration (years)[a]*	3.6 ± 2.6	4.6 ± 2.1	1.7 ± 1.3
UPDRS-ME (score)[a]°	35.7 ± 15.0	43.2 ± 12.5	25.2 ± 11.7
H&Y (score)[a]*	3.0 ± 0.6	2.7 ± 0.6	1.9 ± 0.3

Notes: [a]Data are shown as mean ± standard deviation (SD). *p<0.001; °p=0.002.
Legend: PSP = Progressive Supranuclear Palsy, MSA = Multiple System Atrophy, PD = Parkinson's Disease, UPDRS-ME = Unified Parkinson's Disease Rating Scale–Motor Examination, H&Y = Hoehn and Yahr stage.

Figure 1. R2 blink reflex recovery cycle graph-curve for PD, MSA and PSP patients.

Ratios of the conditioned R2 component (amplitude) to the unconditioned response are shown as mean + standard error (S.E.). X-axis: interstimulus intervals (ISIs) in milliseconds (ms). Y-axis: ratio of the conditioned to the unconditioned R2 response in percentage (%). **A.** R2 blink reflex recovery cycle graph-curve of PD patients; *p<0.001, °p=0.03 when comparing more affected side (MAS) stimulation vs less affected side (LAS) stimulation. **B.** R2 blink reflex recovery cycle graph-curve of MSA patients; no statistically significant differences were found when comparing MAS stimulation vs LAS stimulation. **C.** R2 blink reflex recovery cycle graph-curve of PSP patients; no statistically significant differences were found when comparing MAS stimulation vs LAS stimulation.

Legend: R2BRRC = R2 blink reflex recovery cycle, PD = Parkinson's Disease, MSA = Multiple System Atrophy, PSP = Progressive Supranuclear Palsy, MAS = more affected side stimulation, LAS = less affected side stimulation.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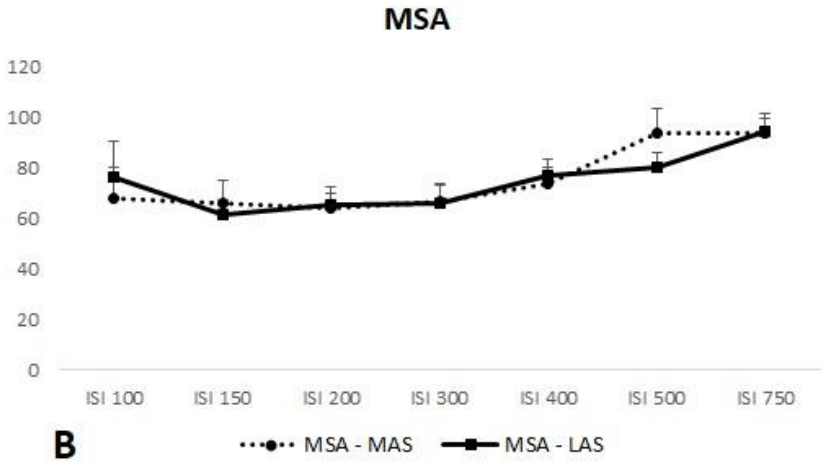
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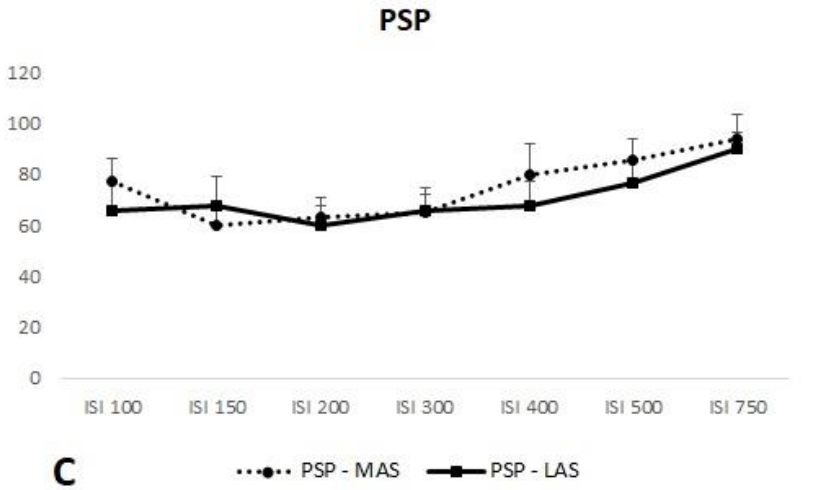
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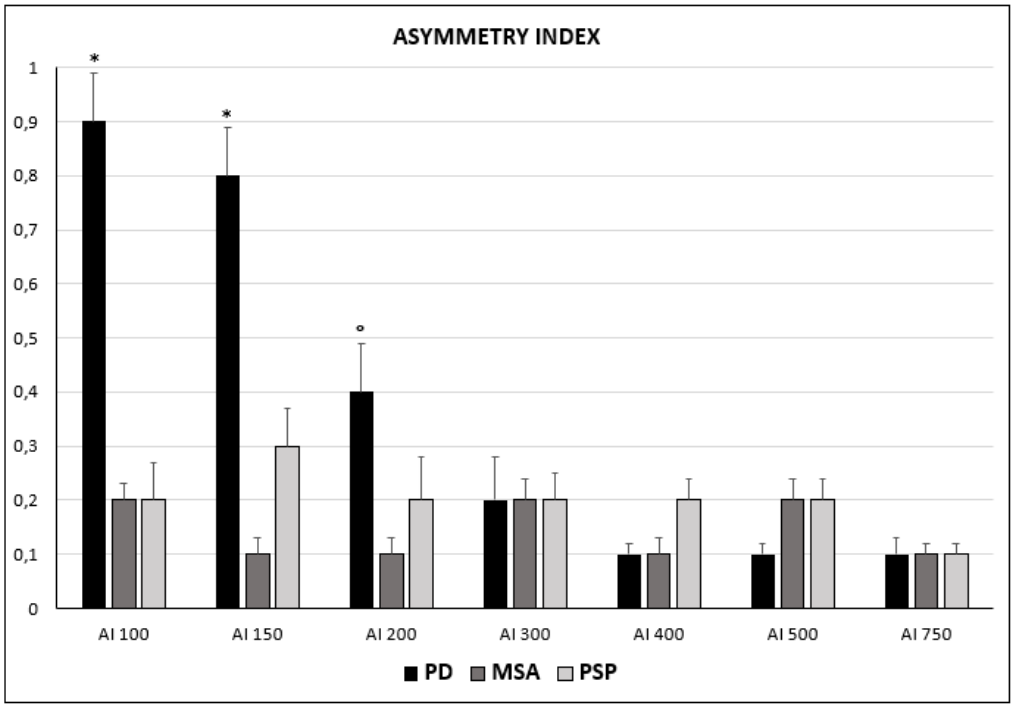
Figure 2. Asymmetry indexes for PD, MSA and PSP patients.

Asymmetry indexes are shown as mean + standard error (S.E.). X-axis: asymmetry indexes at interstimulus intervals (ISIs) of 100, 150, 200, 300, 400, 500, 750 milliseconds (ms). Y-axis: absolute value of asymmetry index; *p<0.001 and °p<0.02 when comparing PD, MSA and PSP.

Legend: AI = asymmetry index, PD = Parkinson's Disease, MSA = Multiple System Atrophy, PSP = Progressive Supranuclear Palsy.



Chapter III. Neurophysiological features for treatment of Parkinson's disease

Motor learning and long-duration response to levodopa in Parkinson's disease

Abstract

Background: LDR derives from prolonged administration of levodopa in PD. Animal models support the hypothesis that LDR is implicated in motor learning. P300, MEPs and BP are neurophysiological tools which evaluate motor learning in humans. We aimed to define the role of LDR in motor learning, evaluating neurophysiological parameters of PD patients with and without LDR and undergone or not motor learning skills.

Methods: Drug-naive PD patients underwent a 15-day treatment of levodopa/carbidopa 250/25 mg at 24 h interdose intervals and randomized to perform or not motor training skills. Achievement of LDR was assessed at the 15th day of treatment. Patients underwent clinical and neurophysiological (P300, MEPs and BP) assessments at baseline (T0) and at the 15th day of treatment (T15).

Results: Forty-one PD patients were enrolled: 11 trained patients with a sustained LDR (Group 1), 10 untrained patients with a sustained LDR (Group 2), 10 trained patients without a stable LDR (Group 3) and 10 untrained patients without a stable LDR (Group 4). Statistically significant improvements of P300 latency, MEPs amplitude and early and late BP latencies were observed in Group 1 from T0 to T15 ($p<0.01$; $p<0.05$; $p<0.01$;

$p<0.01$). Early and late BP latencies significantly improved in Group 2 from T0 to T15 ($p<0.05$; $p<0.05$). No statistically significant differences of neurophysiological parameters were found in Groups 3 and 4.

Conclusions: Our findings support the hypothesis that LDR promotes motor learning in PD patients. This observation might change therapeutic approach for PD.

1. Aim of the study

Despite animal models support the hypothesis that LDR to levodopa treatment is implicated in motor learning, to date there are no studies demonstrating this relationship in humans. In this study, we evaluated changes in neurophysiological parameters assessing motor learning in drug-naive PD patients with and without LDR to levodopa and undergone or not motor learning skills, with the aim to define the role of LDR in motor learning in humans.

2. Materials and methods

2.1 Participants

Drug-naive patients affected by a clinical diagnosis of idiopathic PD, according to MDS clinical diagnostic criteria for PD (*Postuma RB et al. Mov Disord 2015*) and with H&Y score (*Hoehn M et al. Neurology 1967*), ranging from I to III, were consecutively

enrolled from January 2017 to June 2019. Patients with current diagnosis or recent history of stroke, seizures, head injury, neurosurgery or substance abuse, history or current diagnosis of psychosis or depression, cognitive impairment with Mini-Mental State Examination (MMSE) <24 (*Folstein MF et al. J Psychiatr Res 1975*), severe postural hypotension, hearing threshold more than 60 dB at 1 or 2 KHz, orthopedic or arthritic disease that affects thumb and finger movements and history of use of PD medications before the study were excluded. The study was approved by the Local Ethic Committee and patients were enrolled after signing the written informed consent.

2.2 Pharmacological assessment

All enrolled patients underwent levodopa challenge test, consisting in the oral administration of levodopa/carbidopa 250/25 mg. Afterwards, patients underwent a 15-day regimen scheduling full doses of levodopa/carbidopa 250/25 mg at fixed interdose intervals of 24 hours (IDI 24) (*Quattrone A et al. Ann Neurol 1995; Zappia M et al. Neurology 1999*). Administration of a single dose of 250 mg levodopa once a day permits to establish and maintain a sustained LDR in most patients with mild or moderate PD (*Quattrone A et al. Neurology 1993; Zappia M et al. Neurology 2000*). Furthermore, all patients have been randomized through a dedicated computer-software, in order to establish if a patient had to perform or not a motor training skill for 15 days (*Wu T et al. Brain 2005*).

After the 15-day established regimen scheduling of levodopa, an independent operator with special competence in movement disorders assessed the achievement or not of LDR

to levodopa therapy by Movement Time Analyzer (MTA®), a computer-controlled tachystoscope dedicated at recording movement time (*Nicoletti G et al. Aging Clin Exp Res 2005*). The formula [(B-X)×100/(B-P)], where B was the movement-time basal value at absolute baseline, X was the basal value at the 15th day of treatment, and P was the peak value of the levodopa challenge test, was applied (*Quattrone A et al. Ann Neurol 1995; Zappia M et al. Neurology 1999*). LDR >50% was considered sustained and satisfactory (*Zappia M et al. Neurology 1999*).

2.3 Clinical and neurophysiological assessments

All enrolled patients were evaluated for both clinical and neurophysiological assessments at their baseline motor condition (T0), at the 15th day of established regimen scheduling of levodopa before taking the first daily dose of levodopa (T15) and at the 16th day of treatment after two hours from the intake of levodopa (T16).

Clinical evaluation was performed through UPDRS-ME (*Fahn et al. Recent developments in Parkinson's disease. Florham Park, NJ: Macmillan Healthcare Information, 1987*) and H&Y (*Hoehn M et al. Neurology 1967*) scores.

Neurophysiological assessment consisted of P300, TMS and BP.

- P300 was performed by using Deymed - TruTrace® electromyography (EMG). An auditory stimuli presentation according to an oddball stimulus paradigm was presented to patients who had to keep a mentally count of the rare (20%) target tones (65 dB, 2000 Hz) interspersed against a background of more common (80%) non-target tones (65 dB, 1000 Hz). Silver-chloride electrodes were

positioned in regions Fz, Cz and Pz according to the international 10/20 system referenced to the mastoid processes (filter settings: 30 ± 100 Hz) (*Heinze HJ et al. Recommendations for the Practice of Clinical Neurophysiology: Guidelines of the International Federation of Clinical Physiology. International Federation of Clinical Neurophysiology, 1999*). P300 was identified as the major positive point in tracing of the rare stimulus.

- MEPs were recorded from the first dorsal interosseus (FDI) muscles of both hands using silver-chloride electrodes. EMG activity was monitored continuously to ensure the absence of any activity during TMS application (filter settings: 10–1000 Hz) (*Mak M et al. Clin Neurophysiol 2013*). MagPro Compact Magnetic Stimulator (Medtronic®), connected to the stimulating coil (circular type, mean diameter 9 cm), was used to elicit a single pulse stimulation to the hemisphere contralateral to FDI. Resting motor threshold (RMT) was determined as the minimum stimulus intensity required to elicit a MEP in the relaxed FDI of at least 50 μV in amplitude in three out of five consecutive trials. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to elicit a MEP in the FDI muscle of at least 200 μV in amplitude in three out of five consecutive trials during a low-level voluntary index finger abduction. The mean value of MEPs was obtained with the increase of TMS intensity to produce the maximum MEP amplitude, collecting ten trials for the analysis. Duration of the cortical silent period (CSP), examining the intracortical inhibitory mechanisms, was obtained by applying TMS at 130% of RMT with

FDI at 20% of maximum voluntary contraction (*Mak M et al. Clin Neurophysiol 2013*). Ten trials were collected and the mean CSP duration was used for analysis.

- BP was recorded trough Deymed - TruTrace® EMG. EEG and EMG were simultaneously recorded while the patients repeated a voluntary muscle contraction at a self-paced rate every 10 s. EEG electrodes were positioned in regions C4, Cz and C3 according to the international 10/20 system referenced to the mastoids (filter settings: 0.05 ± 500 Hz) (*Shibasaki H et al. Recommendations for the Practice of Clinical Neurophysiology: Guidelines of the International Federation of Clinical Physiology. International Federation of Clinical Neurophysiology 1999*). EMG was recorded with surface electrodes placed over abductor pollicis brevis (ABP) muscle (filter setting of 30 ± 1000 Hz) (*Shibasaki H et al. Recommendations for the Practice of Clinical Neurophysiology: Guidelines of the International Federation of Clinical Physiology. International Federation of Clinical Neurophysiology 1999*). EEG was analyzed off-line by visually marking the EMG onset of ABP muscle. Epochs lasted from -2000 to 2000 ms with respect to EMG onset. Early BP was defined as the mean voltage from 1500 to 500 ms, whereas late BP as the mean voltage from 500 to 0 ms.

2.4 Motor training

Several studies investigated the potential of motor learning in PD rehabilitation (*Nieuwboer A et al. Parkinsonism Relat Disord 2009*). We have chosen to perform in our study the same training procedure illustrated by Wu and colleagues (*Wu T et al. Brain 2005*). Randomized patients for motor training (MT) skills executed two sequences of finger tapping, respectively with right and left hand, referred to as "sequence-4" and "sequence-12", based on the number of movements in each unit of the sequence. "Sequence-4" was "1-3-4-2" and "Sequence-12" was "1-4-3-2-2-4-1-3-4-1-2-3", where 1, 2, 3 and 4 referred to the index, middle, ring and little fingers, respectively. The achievement of automatized motor learning was evaluated by having patients performed a visual letter-counting task simultaneously with these sequential movements, consisting of a random series of the letters A, G, L and O presented on a screen: patients had to identify the number of times they saw a specified target letter. Patients performed these tasks until they could execute sequential movements from memory 10 times in a row without errors, as well as the dual tasks accurately. During practice period, the errors were underlined by the examiner and a feedback was provided to inform subjects whether their finger movements were correct or incorrect. Patients were trained during 5 sessions of 30 min per week, for 15 days. Every session started at the same time of a day.

2.5 Statistical Analysis

Data are expressed as means ± standard deviation (S.D.). The intergroup differences in clinical features and neurophysiological parameters (P300, TMS, BP) between the four groups of patients were assessed by the Kruskal-Wallis test. The intragroup differences between means at T0, T15 and T16 were evaluated by the Wilcoxon-test. A significant difference implies $p<0.05$.

3. Results

Forty-one patients were overall enrolled (23 men; age 64.7 ± 6.9 years).

According to the achievement of LDR value and to the performance of MT skills, all enrolled patients were divided into four groups:

- Group LDR and MT: 11 trained PD patients with a sustained LDR to levodopa treatment;
- Group LDR and not MT: 10 untrained PD patients with a sustained LDR to levodopa treatment;
- Group not LDR and MT: 10 trained PD patients without a stable LDR to levodopa treatment;
- Group not LDR and not MT: 10 untrained PD patients without a stable LDR to levodopa treatment.

Demographics and clinical characteristics are summarized in table 1.

Neurophysiological characteristics of all enrolled patients were similar at baseline conditions (T0), as shown in table 2.

The four groups showed statistically significant differences in MEPs amplitude and in early and late BP latencies at T15, as LDR and MT group and LDR and not MT group presented with higher MEPs amplitude and earlier BP latencies compared to the others. Earlier P300, AMT and BP latencies and higher MEPs and early BP amplitudes were found in LDR groups compared to not LDR groups at T16. Neurophysiological characteristics of patients at T15 and T16 are showed in table 3 and 4.

LDR and MT group showed a statistically significant improvement in almost all neurophysiological parameters comparing baseline conditions to the 15th day of treatment (table 5). Earlier P300, RMT, AMT and BP latencies and higher MEPs and late BP amplitudes were observed at T15 compared to T0. Duration of CSP was similar at T0 and T15. There were no statistically significant differences in neurophysiological parameters between T15 and T16.

LDR and not MT group exhibited a statistically significant improvement in RMT, AMT and BP latencies at T15, whilst no improvement was observed in P300 latency and MEPs and BP amplitudes (table 6) as for LDR and MT group. Duration of CSP was not influenced by the achievement of LDR. As in the previous group, no statically significant differences were found in neurophysiological parameters between T15 and T16.

Not LDR and MT group did not show any statistically significant difference in neurophysiological parameters between T0 and T15, except for a borderline value in late BP left-side recording latency (p=0.05; table 7).

Not LDR and not MT group had similar neurophysiological features of not LDR and MT group, except for the duration of CSP, which was shorter after 15-day-treatment (table 8). The remaining neurophysiological parameters have not changed at T15 compared to T0.

Figures compared the four groups of patients at T0, T15 and T16 for each examined neurophysiological parameter. In details, figure 1 showed an improvement in P300 latency from T0 to T15 only in LDR and MT group. RMT latency was bilaterally earlier in LDR and MT group compared to the others (figure 2). AMT latency became earlier after 15-day treatment in both LDR groups, as shown in figure 3. Figure 4 showed an improvement in MEPs amplitude at T15 only in LDR and MT group. Despite the performance of MT skills, early and late BP latencies were earlier at T15 compared to baseline motor conditions in both LDR groups (figures 5 and 6). Late BP amplitudes became higher at T15 only in LDR and MT group, as shown in figure 7.

4. Table and figures legends

Table 1. Demographic and clinical characteristics of all enrolled patients.

	LDR and MT group (n=11)	LDR and not MT group (n=10)	Not LDR and MT group (n=10)	Not LDR and not MT group (n=10)
Age (years)[a]	63.9 ± 7.8	63.9 ± 8.2	66.5 ± 5.9	64.4 ± 6.3
Men (n, %)	7, 64%	5, 50%	5, 50%	6, 60%
Disease duration (years)[a]	2.1 ± 1.4	1.4 ± 0.5	1.6 ± 0.7	1.8 ± 1.5
Right affected side (n, %)	5, 45%	4, 45%	2, 20%	6, 60%
UPDRS-ME score (T0)[a]	22.9 ± 10.6	30.2 ± 9.1	28.8 ± 7.9	23.9 ± 6.5
UPDRS-ME score (T15)[a]	21.5 ± 10.6	29.6 ± 9.4	28.1 ± 7.1	23.7 ± 6.3
UPDRS-ME score (T16)[a]	18.4 ± 10.4	25.2 ± 9.4	24.9 ± 6.7	21.6 ± 5.8
MTA (T0) right[a]	346.8 ± 107.1	396.4 ± 51.1	368 ± 83.3	328.6 ± 72.9
MTA (T0) left[a]*	346.8 ± 86.5	460.7 ± 123.4	380.4 ± 58.1	321.5 ± 86.1
MTA (T15) right[a]	292.1 ± 92.1	347.8 ± 51.3	342.4 ± 55.5	319.5 ± 70.6
MTA (T15) left[a]*	295.9 ± 77.9	391.1 ± 83.9	359.9 ± 54.3	332 ± 83
LDR right[a]°	193.1 ± 214.9	474.8 ± 99.7	-362.7 ± 922.4	4.1 ± 45.2
LDR left[a]°	224.3 ± 216.2	121.5 ± 64.8	-115.2 ± 453.5	-3.5 ± 260.4

Notes: [a]Data are means ± standard deviations. Kruskal-Wallis test: *$p<0.05$; °$p<0.01$.
Legend: LDR = long duration response, MT = motor training, UPDRS-ME = Unified Parkinson's Disease Rating Scale-Motor Examination section, MTA = movement time analyzer (ms), LDR = long duration response (%), T0 = baseline, T15 = 15th day of treatment before taking the first daily dose of levodopa, T16 = 16th day of treatment after two-hours from intake of levodopa.

Table 2. Neurophysiological characteristics of patients at T0.

	LDR and MT group (n=11)	LDR and not MT group (n=10)	Not LDR and MT group (n=10)	Not LDR and not MT group (n=10)
P300 (ms)	329.7 ± 24.6	318.6 ± 39.2	322.8 ± 29.7	311.8 ± 30
RMT r (ms)	22.5 ± 1.9	22.2 ± 1.3	22.1 ± 1.6	22.7 ± 2.5
RMT l (ms)	22.2 ± 2	22.7 ± 1.4	22.1 ± 1.4	22.9 ± 2.2
AMT r (ms)	20.3 ± 2.1	20.6 ± 1.4	19.9 ± 1.9	20.2 ± 3
AMT l (ms)	20.1 ± 2.2	21.0 ± 0.9	19.5 ± 1.7	21.3 ± 2.7
MEPs r (μV)*	155.5 ± 80.8	282 ± 116.1	204 ± 115.3	139.4 ± 79.3
MEPs l (μV)	157.2 ± 104.4	230.7 ± 102.2	225.2 ± 126.3	142.9 ± 96.6
CSP r (ms)	133.8 ± 54	101.2 ± 33.5	93.3 ± 46.4	115.8 ± 47.8
CSP l (ms)	119.7 ± 40.6	86.4 ± 37.1	100.6 ± 43.4	123 ± 37.5
BP early r (ms)	1901.5 ± 120.9	1974.9 ± 85.8	2037 ± 104.9	1967.8 ± 86.2
BP early l (ms)	1875 ± 155.8	1970.3 ± 54.6	1991.9 ± 79.7	1963.4 ± 89.2
BP late r (ms)	614.1 ± 61.8	563.8 ± 63	589.4 ± 53.5	597.8 ± 54.5
BP late l (ms)	581.6 ± 86.4	560.8 ± 73.3	581.4 ± 43.1	602.5 ± 58.2
BP early r (μV)	4.3 ± 2	4 ± 3	3.2 ± 1.6	2.9 ± 1.4
BP early l (μV)	4.6 ± 2.9	5 ± 3.7	3.1 ± 1.5	3.7 ± 2
BP late r (μV)	4.8 ± 3	6.8 ± 4.3	4.6 ± 2.4	7.6 ± 3.8
BP late l (μV)	4.2 ± 1.8	8.8 ± 6.8	5.7 ± 3	7.5 ± 3

Notes: Data are means ± standard deviations. *Kruskal-Wallis test: $p<0.01$.
Legend: T0 = baseline, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 3. Neurophysiological characteristics of patients at T15.

	LDR and MT group (n=11)	LDR and not MT group (n=10)	Not LDR and MT group (n=10)	Not LDR and not MT group (n=10)
P300 (ms)	299 ± 25.8	311 ± 56.5	337.8 ± 31.7	328.9 ± 35.1
RMT r (ms)	21.1 ± 2.5	21.3 ± 1.7	22.2 ± 1.4	23.1 ± 2.9
RMT l (ms)	21 ± 2.5	21.6 ± 1.8	22.8 ± 1.5	23.4 ± 2.3
AMT r (ms)	18.9 ± 2	19.7 ± 1.3	20.1 ± 1.2	20.9 ± 2.7
AMT l (ms)	18.9 ± 2.4	20 ± 1.8	20.1 ± 2.0	21.3 ± 2.9
MEPs r (µV)*	290 ± 246.3	361 ± 148	183.2 ± 89.5	149.7 ± 85.7
MEPs l (µV)*	329.6 ± 194.3	279.9 ± 123.9	204.4 ± 76	127 ± 71.4
CSP r (ms)	124.4 ± 42.2	95.4 ± 71.1	98.8 ± 46.3	82.9 ± 34.9
CSP l (ms)	115.2 ± 30.1	87.9 ± 36.9	99.2 ± 33.4	88.3 ± 37.8
BP early r (ms)*	1785.3 ± 154.9	1887.5 ± 72.5	2008.7 ± 685	2003.7 ± 145.7
BP early l (ms)*	1739.1 ± 180.7	1874.3 ± 79.1	2002.9 ± 83.2	1979.5 ± 98.3
BP late r (ms)*	469.8 ± 64.9	463.9 ± 49.1	593 ± 37.3	592.8 ± 62.7
BP late l (ms)*	492.5 ± 78.8	496.6 ± 49.2	616 ± 62.9	611.8 ± 45.7
BP early r (µV)	5.2 ± 4.5	5.1 ± 5.1	2.9 ± 1.4	3.2 ± 2.1
BP early l (µV)	5.2 ± 4.2	5.3 ± 3.8	2.2 ± 0.8	3.6 ± 1.6
BP late r (µV)	6.7 ± 4.2	7.5 ± 6	4.1 ± 2.1	5.6 ± 1.7
BP late l (µV)	5.4 ± 2.5	7.6 ± 6.8	4.6 ± 3.1	6.8 ± 2.1

Notes: Data are means ± standard deviations. *Kruskal-Wallis test: $p<0.01$.
Legend: T15 = 15th day of treatment before taking the first daily dose of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 4. Neurophysiological characteristics of patients at T16.

	LDR and MT group (n=11)	LDR and not MT group (n=10)	Not LDR and MT group (n=10)	Not LDR and not MT group (n=10)
P300 (ms)*	295 ± 26.5	310.3 ± 50.4	346.2 ± 44.4	327.5 ± 35.5
RMT r (ms)	21.4 ± 1.9	21.6 ± 2.1	22 ± 1.4	22.9 ± 2.9
RMT l (ms)	21.1 ± 2.2	21.8 ± 1.8	22.4 ± 1.4	23.5 ± 2.5
AMT r (ms)	18.9 ± 1.9	19.8 ± 1.4	20.2 ± 1.8	20.7 ± 2.6
AMT l (ms)*	18.2 ± 1.9	19.7 ± 2.2	20.2 ± 1.4	21.2 ± 2.6
MEPs r (μV)*	373.8 ± 212.9	320 ± 227.8	249.6 ± 154.4	162 ± 116.5
MEPs l (μV)*	273.2 ± 132	262.2 ± 138.3	202.3 ± 63.7	129.9 ± 99.9
CSP r (ms)	118.2 ± 36.5	95.5 ± 69.3	118.5 ± 42.4	100.2 ± 52
CSP l (ms)	123.9 ± 30.6	104.5 ± 67.6	101.9 ± 32.3	112.3 ± 72.5
BP early r (ms)°	1771.5 ± 146.9	1868.7 ± 68.9	1974.9 ± 77.1	1952.1 ± 66.2
BP early l (ms)°	1729.2 ± 186.6	1844.8 ± 50.9	1965.6 ± 99.5	1942.2 ± 78.9
BP late r (ms)°	474.1 ± 92.6	485.5 ± 52.8	576.1 ± 73.7	564.7 ± 62.9
BP late l (ms)°	515.7 ± 107	482.6 ± 45.9	574.1 ± 64.9	594.9 ± 53.9
BP early r (μV)*	4.8 ± 3.7	7.9 ± 7.1	3.5 ± 2.6	2.6 ± 2.5
BP early l (μV)	4.6 ± 3.3	5.9 ± 2.9	2.7 ± 1.3	4.7 ± 4.8
BP late r (μV)	5.9 ± 3.3	5.7 ± 4.4	4.9 ± 2.8	6 ± 3.2
BP late l (μV)	5.2 ± 2.2	6.9 ± 5.3	5.2 ± 3.9	6.3 ± 2.9

Notes: Data are means ± standard deviations. *Kruskal-Wallis test: $p<0.05$. °Kruskal-Wallis test: $p<0.01$.
Legend: T16 = 16th day of treatment after two-hours from intake of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 5. LDR and MT group at T0, T15 and T16.

	T0	T15	T16	*p*-value*	*p*-value°	*p*-value[a]
P300 (ms)	329.7 ± 24.6	299 ± 25.8	295 ± 26.5	**0.005**	0.5	**0.02**
RMT r (ms)	22.5 ± 1.9	21.1 ± 2.5	21.4 ± 1.9	**0.01**	0.2	**0.03**
RMT l (ms)	22.2 ± 2	21 ± 2.5	21.1 ± 2.2	**0.01**	0.9	0.07
AMT r (ms)	20.3 ± 2.1	18.9 ± 2	18.9 ± 1.9	**0.03**	0.9	**0.04**
AMT l (ms)	20.1 ± 2.2	18.9 ± 2.4	18.2 ± 1.9	**0.03**	0.1	**0.006**
MEPs r (μV)	155.5 ± 80.8	290 ± 246.3	373.8 ± 212.9	**0.02**	0.2	**0.01**
MEPs l (μV)	157.2 ± 104.4	329.6 ± 194.3	273.2 ± 132	**0.01**	0.3	**0.02**
CSP r (ms)	133.8 ± 54	124.4 ± 42.2	118.2 ± 36.5	0.1	0.3	**0.05**
CSP l (ms)	119.7 ± 40.6	115.2 ± 30.1	123.9 ± 30.6	0.8	0.07	0.7
BP early r (ms)	1901.5 ± 120.9	1785.3 ± 154.9	1771.5 ± 146.9	**0.003**	0.2	**0.004**
BP early l (ms)	1875 ± 155.8	1739.1 ± 180.7	1729.2 ± 186.6	**0.003**	0.8	**0.008**
BP late r (ms)	614.1 ± 61.8	469.8 ± 64.9	474.1 ± 92.6	**0.003**	0.7	**0.003**
BP late l (ms)	581.6 ± 86.4	492.5 ± 78.8	515.7 ± 107	**0.004**	0.2	**0.008**
BP early r (μV)	4.3 ± 2	5.2 ± 4.5	4.8 ± 3.7	0.4	0.7	0.9
BP early l (μV)	4.6 ± 2.9	5.2 ± 4.2	4.6 ± 3.3	0.2	0.9	0.9
BP late r (μV)	4.8 ± 3	6.7 ± 4.2	5.9 ± 3.3	**0.05**	0.9	0.2
BP late l (μV)	4.2 ± 1.8	5.4 ± 2.5	5.2 ± 2.2	**0.01**	0.8	0.1

Notes: Data are means ± standard deviations. Wilcoxon test: *T0 *versus* T15; °T15 *versus* T16; [a]T0 *versus* T16.
Legend: T0 = baseline, T1 5= 15th day of treatment before taking the first daily dose of levodopa, T16 = 16th day of treatment after two-hours from intake of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 6. LDR and not MT group at T0, T15 and T16.

	T0	T15	T16	*p*-value*	*p*-value°	*p*-value[a]
P300 (ms)	318.6 ± 39.2	311 ± 56.5	310.3 ± 50.4	0.9	0.8	0.5
RMT r (ms)	22.2 ± 1.3	21.3 ± 1.7	21.6 ± 2.1	0.08	0.5	0.2
RMT l (ms)	22.7 ± 1.4	21.6 ± 1.8	21.8 ± 1.8	**0.02**	0.6	**0.03**
AMT r (ms)	20.6 ± 1.4	19.7 ± 1.3	19.8 ± 1.4	**0.05**	0.8	0.1
AMT l (ms)	21.0 ± 0.9	20 ± 1.8	19.7 ± 2.2	**0.04**	0.3	**0.02**
MEPs r (μV)	282 ± 116.1	361 ± 148	320 ± 227.8	0.4	0.6	0.6
MEPs l (μV)	230.7 ± 102.2	279.9 ± 123.9	262.2 ± 138.3	0.4	0.4	0.9
CSP r (ms)	101.2 ± 33.5	95.4 ± 71.1	95.5 ± 69.3	0.5	0.4	0.6
CSP l (ms)	86.4 ± 37.1	87.9 ± 36.9	104.5 ± 67.6	0.8	0.5	0.9
BP early r (ms)	1974.9 ± 85.8	1887.5 ± 72.5	1868.7 ± 68.9	**0.01**	0.3	**0.01**
BP early l (ms)	1970.3 ± 54.6	1874.3 ± 79.1	1844.8 ± 50.9	**0.02**	0.1	**0.007**
BP late r (ms)	563.8 ± 63	463.9 ± 49.1	485.5 ± 52.8	**0.007**	0.3	**0.01**
BP late l (ms)	560.8 ± 73.3	496.6 ± 49.2	482.6 ± 45.9	**0.03**	0.4	**0.03**
BP early r (μV)	4 ± 3	5.1 ± 5.1	7.9 ± 7.1	0.3	0.1	**0.02**
BP early l (μV)	5 ± 3.7	5.3 ± 3.8	5.9 ± 2.9	0.9	0.5	0.4
BP late r (μV)	6.8 ± 4.3	7.5 ± 6	5.7 ± 4.4	0.4	0.6	0.07
BP late l (μV)	8.8 ± 6.8	7.6 ± 6.8	6.9 ± 5.3	0.4	0.9	0.3

Notes: Data are means ± standard deviations. Wilcoxon test: *T0 *versus* T15; °T15 *versus* T16; [a]T0 *versus* T16.
Legend: T0 = baseline, T15 = 15th day of treatment before taking the first daily dose of levodopa, T16 = 16th day of treatment after two-hours from intake of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 7. Not LDR and MT group at T0, T15 and T16.

	T0	T15	T16	*p*-value*	*p*-value°	*p*-value[a]
P300 (ms)	322.8 ± 29.7	337.8 ± 31.7	346.2 ± 44.4	0.1	0.3	0.2
RMT r (ms)	22.1 ± 1.6	22.2 ± 1.4	22 ± 1.4	0.9	0.6	0.6
RMT l (ms)	22.1 ± 1.4	22.8 ± 1.5	22.4 ± 1.4	0.3	0.2	0.2
AMT r (ms)	19.9 ± 1.9	20.1 ± 1.2	20.2 ± 1.8	0.7	0.8	0.4
AMT l (ms)	19.5 ± 1.7	20.1 ± 2.0	20.2 ± 1.4	0.1	0.9	0.1
MEPs r (μV)	204 ± 115.3	183.2 ± 89.5	249.6 ± 154.4	0.6	**0.05**	0.2
MEPs l (μV)	225.2 ± 126.3	204.4 ± 76	202.3 ± 63.7	0.4	0.7	0.7
CSP r (ms)	93.3 ± 46.4	98.8 ± 46.3	118.5 ± 42.4	0.7	0.2	0.09
CSP l (ms)	100.6 ± 43.4	99.2 ± 33.4	101.9 ± 32.3	0.7	0.8	0.9
BP early r (ms)	2037 ± 104.9	2008.7 ± 685	1974.9 ± 77.1	0.09	0.3	0.1
BP early l (ms)	1991.9 ± 79.7	2002.9 ± 83.2	1965.6 ± 99.5	0.3	0.3	0.4
BP late r (ms)	589.4 ± 53.5	593 ± 37.3	576.1 ± 73.7	1	0.4	0.9
BP late l (ms)	581.4 ± 43.1	616 ± 62.9	574.1 ± 64.9	**0.05**	0.07	0.6
BP early r (μV)	3.2 ± 1.6	2.9 ± 1.4	3.5 ± 2.6	0.4	0.4	0.6
BP early l (μV)	3.1 ± 1.5	2.2 ± 0.8	2.7 ± 1.3	0.2	0.4	0.2
BP late r (μV)	4.6 ± 2.4	4.1 ± 2.1	4.9 ± 2.8	0.7	0.6	0.3
BP late l (μV)	5.7 ± 3	4.6 ± 3.1	5.2 ± 3.9	0.3	0.6	0.9

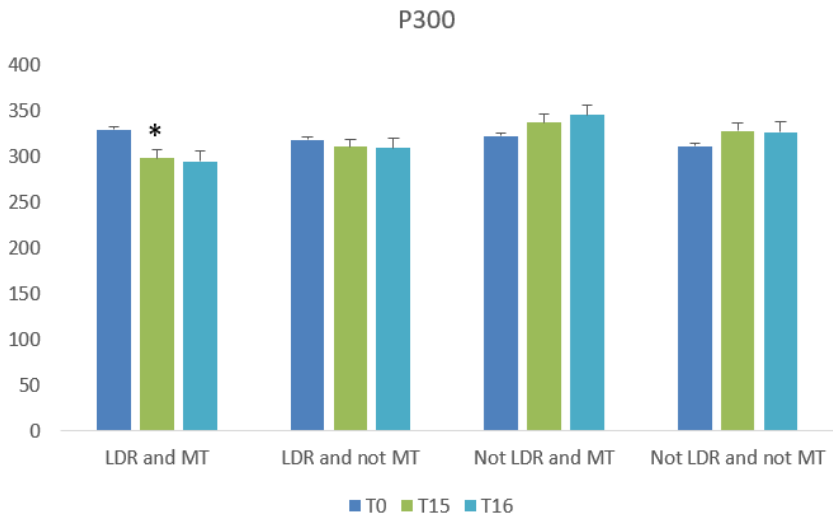
Notes: Data are means ± standard deviations. Wilcoxon test: *T0 *versus* T15; °T15 *versus* T16; [a]T0 *versus* T16.
Legend: T0 = baseline, T15 = 15th day of treatment before taking the first daily dose of levodopa, T16 = 16th day of treatment after two-hours from intake of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Table 8. Not LDR and not MT group at T0, T15 and T16.

	T0	T15	T16	*p*-value*	*p*-value°	*p*-value[a]
P300 (ms)	311.8 ± 30	328.9 ± 35.1	327.5 ± 35.5	0.1	0.7	0.4
RMT r (ms)	22.7 ± 2.5	23.1 ± 2.9	22.9 ± 2.9	0.09	0.5	0.3
RMT l (ms)	22.9 ± 2.2	23.4 ± 2.3	23.5 ± 2.5	0.4	0.9	0.2
AMT r (ms)	20.2 ± 3	20.9 ± 2.7	20.7 ± 2.6	0.2	0.7	0.3
AMT l (ms)	21.3 ± 2.7	21.3 ± 2.9	21.2 ± 2.6	0.8	0.5	0.8
MEPs r (μV)	139.4 ± 79.3	149.7 ± 85.7	162 ± 116.5	0.9	0.8	0.6
MEPs l (μV)	142.9 ± 96.6	127 ± 71.4	129.9 ± 99.9	0.2	0.9	0.5
CSP r (ms)	115.8 ± 47.8	82.9 ± 34.9	100.2 ± 52	**0.03**	0.5	0.07
CSP l (ms)	123 ± 37.5	88.3 ± 37.8	112.3 ± 72.5	**0.03**	**0.03**	0.4
BP early r (ms)	1967.8 ± 86.2	2003.7 ± 145.7	1952.1 ± 66.2	0.3	0.1	0.2
BP early l (ms)	1963.4 ± 89.2	1979.5 ± 98.3	1942.2 ± 78.9	0.2	0.3	0.7
BP late r (ms)	597.8 ± 54.5	592.8 ± 62.7	564.7 ± 62.9	0.8	0.08	0.07
BP late l (ms)	602.5 ± 58.2	611.8 ± 45.7	594.9 ± 53.9	0.4	0.2	0.9
BP early r (μV)	2.9 ± 1.4	3.2 ± 2.1	2.6 ± 2.5	0.7	0.2	0.6
BP early l (μV)	3.7 ± 2	3.6 ± 1.6	4.7 ± 4.8	0.7	0.5	0.8
BP late r (μV)	7.6 ± 3.8	5.6 ± 1.7	6 ± 3.2	0.1	0.8	0.5
BP late l (μV)	7.5 ± 3	6.8 ± 2.1	6.3 ± 2.9	0.6	0.5	0.3

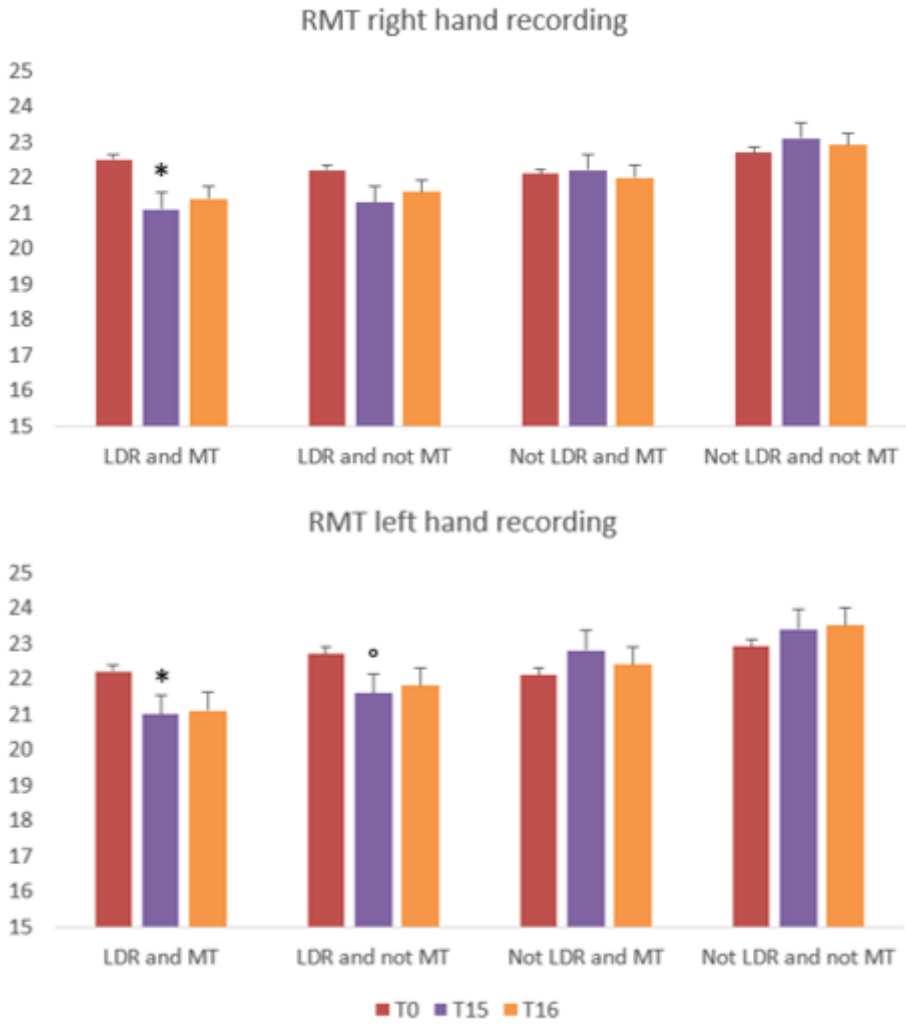
Notes: Data are means ± standard deviations. Wilcoxon test: *T0 *versus* T15; °T15 *versus* T16; [a]T0 *versus* T16.
*Legend:*T0 = baseline, T15 = 15th day of treatment before taking the first daily dose of levodopa, T16 = 16th day of treatment after two-hours from intake of levodopa, LDR = long duration response, MT = motor training, r = right hand recording, l = left hand recording, RMT = resting motor threshold, AMT = active motor threshold, MEPs = motor evoked potentials amplitude, CSP = cortical silent period, BP = Bereitschaftspotential.

Figure 1. P300 at T0, T15 and T16 in the four groups.



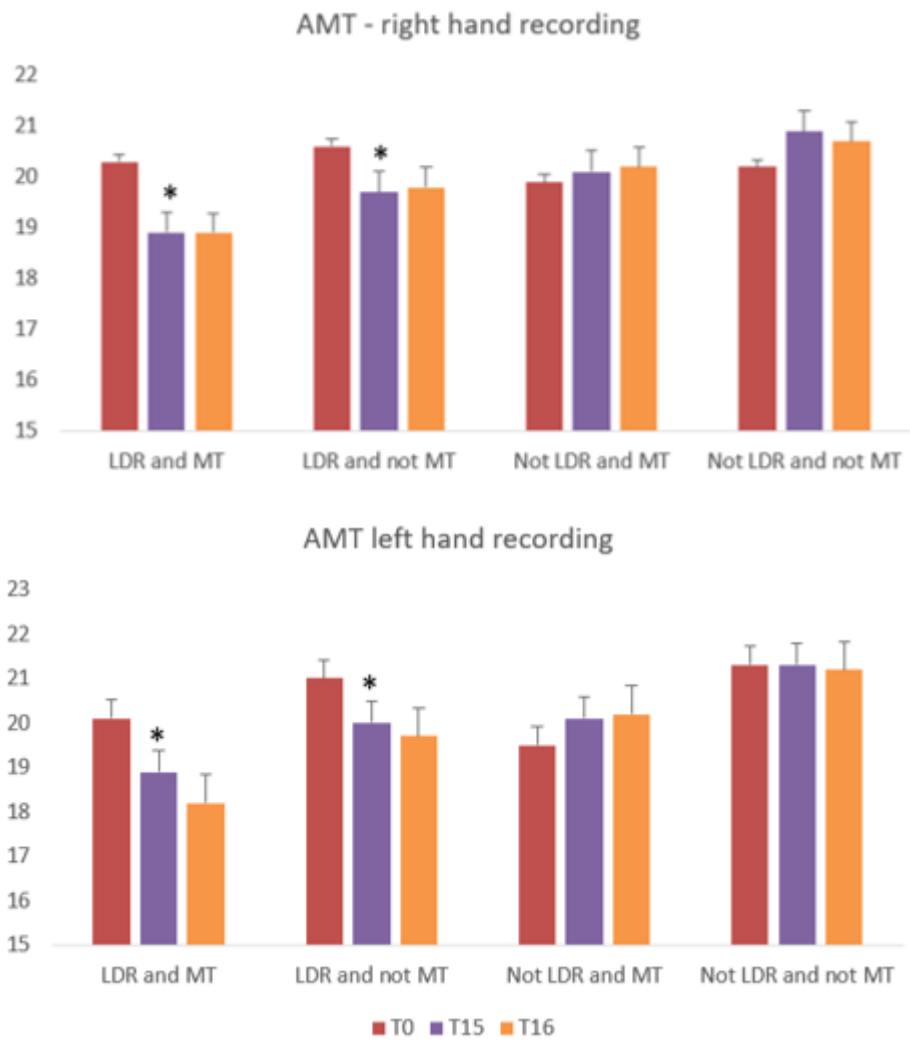
Notes: Data are means ± standard error (S.E.). *T0 *versus* T15: $p<0.01$.
Legend: T0: drug-naive condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug. y-axis: latency in millisecond (ms).

Figure 2. Resting motor threshold at T0, T15 and T16 in the four groups.



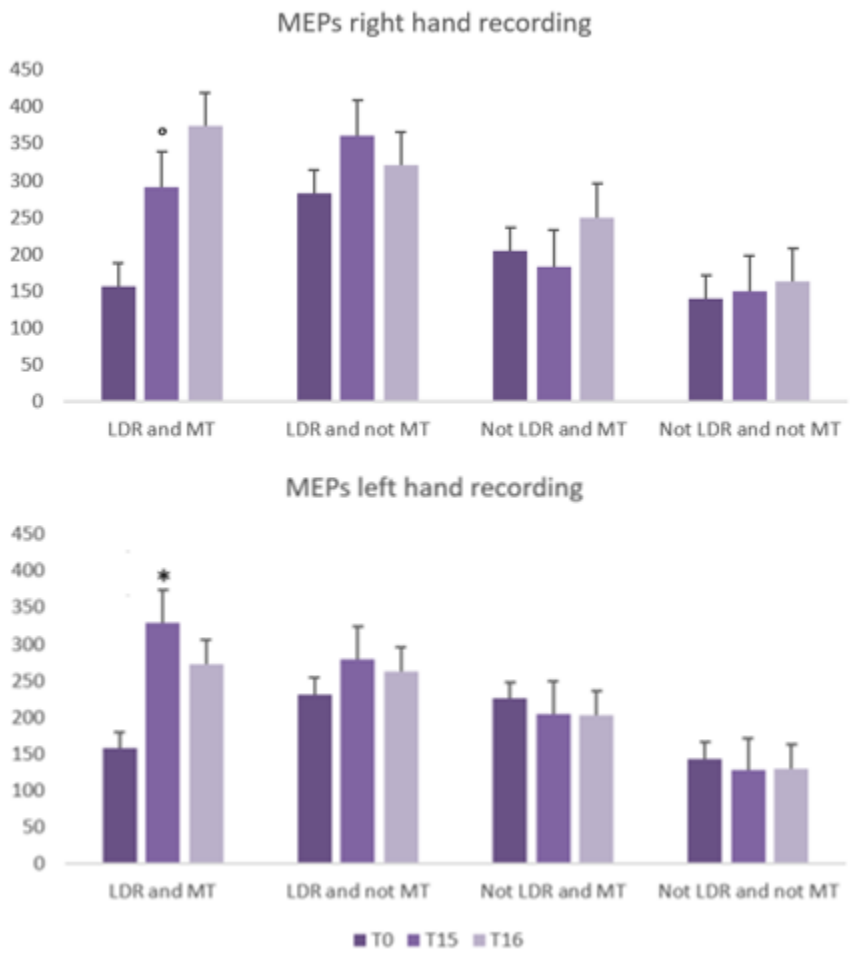
Notes: Data are means ± standard error (S.E.). T0 *versus* T15: *p<0.01; °p=0.02.
Legend: RMT = resting motor threshold; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug.
y-axis: latency in millisecond (ms).

Figure 3. Active motor threshold at T0, T15 and T16 in the four groups.



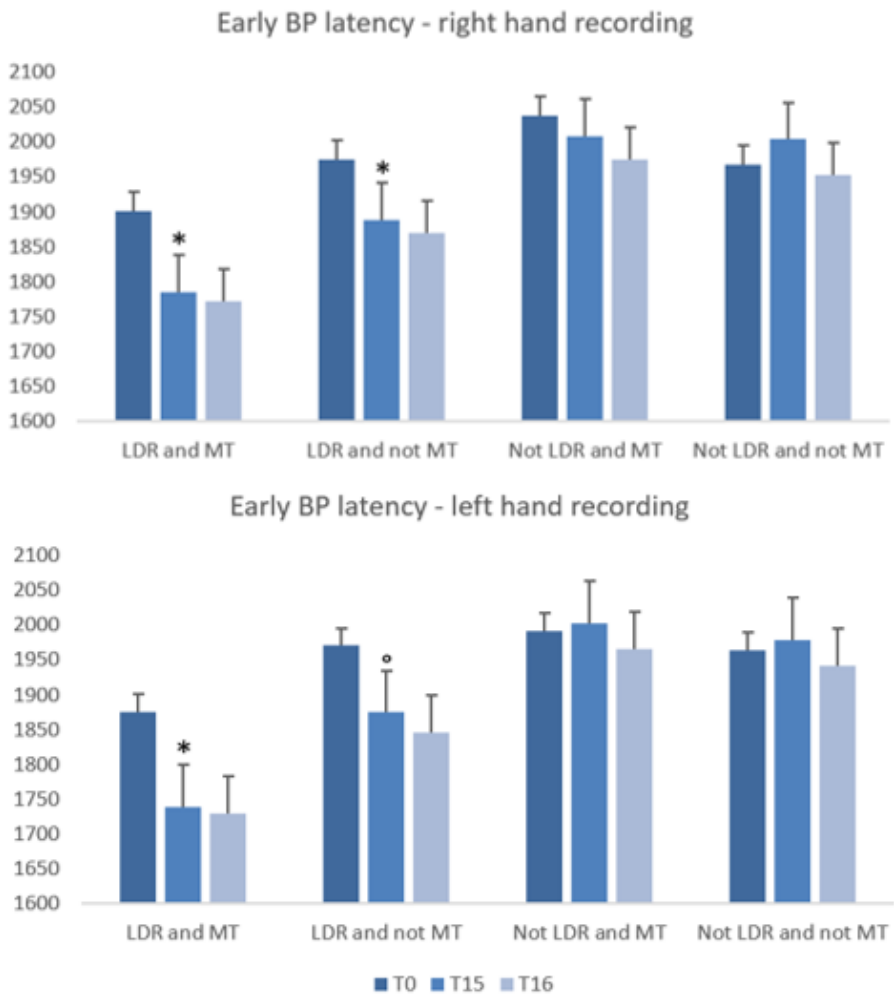
Notes: Data are means ± standard error (S.E.). *T0 *versus* T15: $p<0.05$.
Legend: AMT = active motor threshold; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug.
y-axis: latency in millisecond (ms).

Figure 4. Motor evoked potentials at T0, T15 and T16 in the four groups.



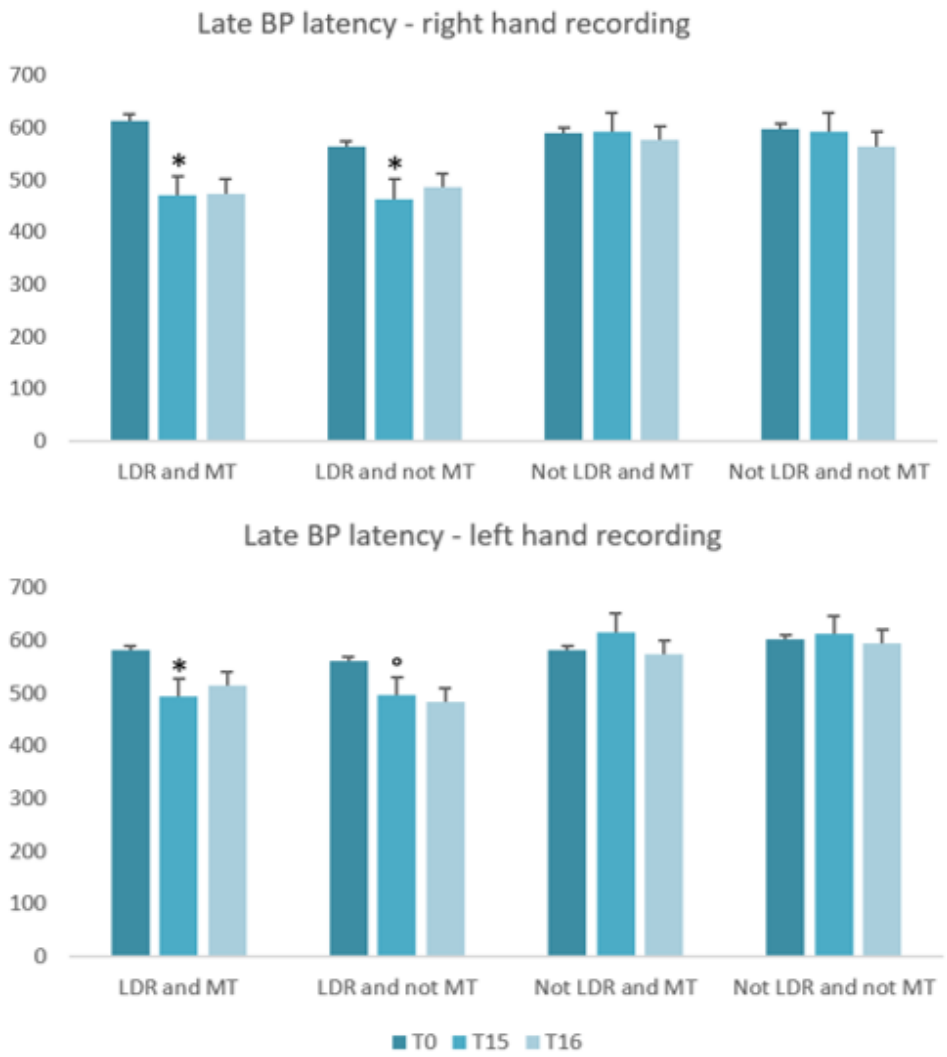
Notes: Data are means ± standard error (S.E.). T0 *versus* T15: °p=0.02; *p<0.01.
Legend: MEPs = motor evoked potentials amplitude; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug. y-axis: amplitude in microvolt (μV).

Figure 5. Early Bereitschaftspotential latency at T0, T15 and T16 in the four groups.



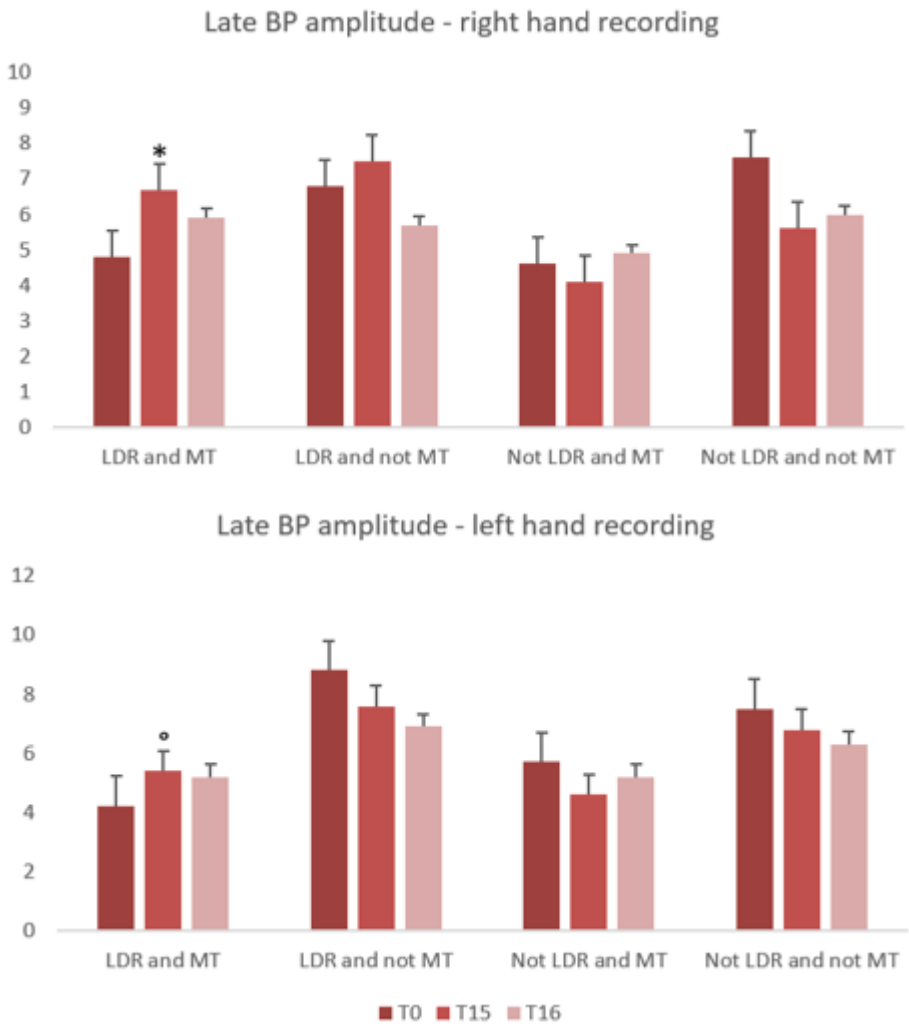
Notes: Data are means ± standard error (S.E.). T0 *versus* T15: *p<0.01; °p=0.02.
Legend: BP = Bereitschaftspotential; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug.
y-axis: latency in millisecond (ms).

Figure 6. Late Bereitschaftspotential latency at T0, T15 and T16 in the four groups.



Notes: Data are means ± standard error (S.E.). T0 *versus* T15: *p<0.01; °p=0.03.
Legend: BP = Bereitschaftspotential; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug.
y-axis: latency in millisecond (ms).

Figure 7. Late Bereitschaftspotential amplitude at T0, T15 and T16 in the four groups.



Notes: Data are means ± standard error (S.E.). T0 *versus* T15: **p*=0.05; °*p*<0.01.
Legend: BP = Bereitschaftspotential; T0: drug-naïve condition; T15: 15th day of treatment with levodopa/carbidopa 250/25 mg at 24 h interdose intervals; T16: 16th day of treatment, after two hours from the first daily intake of drug. y-axis: amplitude in micronvolt (μV).

Chapter IV. Neurophysiological features differentiate Atypical Parkinsonian Syndromes

Blink reflex recovery cycle to differentiate Progressive Supranuclear Palsy from Cortico-basal syndrome

Abstract

Background: PSP and CBS may share similar clinical findings and peculiar tests to distinguish between the two disorders could be useful. We evaluated BR and R2BRRC, determining diagnostic sensitivity, specificity, positive and negative predictive value of R2BRRC in differentiating PSP from CBS patients.

Methods: This was a prospective data collection study investigating BR and R2BRRC at ISIs of 100, 150, 200, 300, 400, 500, 750 ms in 12 PSP patients, 8 CBS patients, and 10 controls.

Results: Patients with PSP have earlier recruitment of R2BRRC as compared to patients with CBS (ISI 100: p=0.002; ISI 150: p<0.001; ISI 200: p<0.001; ISI 300: p=0.02) and controls (ISI 100: p<0.001; ISI 150: p<0.001; ISI 200: p<0.001; ISI 300: p=0.004). The presence of an early recovery of the R2 differentiated PSP from CBS with specificity and sensitivity of 87.5% and 91.7% respectively.

Conclusions: R2BRRC curve might be considered a useful tool in differentiating PSP from CBS patients.

1. Aim of the study

PSP and CBS are rapidly progressive neurodegenerative disorders, belonging to the group of tauopathies. Differential diagnosis between PSP and CBS is difficult because of overlap of common clinical features, particularly at the early stage of disease, and only post-mortem pathology can definitively establish the diagnosis (*Espay AJ et al. J Mol Neurosci 2011*).

R2BRRC is a neurophysiological tool, used to measure brainstem excitability and it is abnormal in several movement disorders (*Kimura J, Brain 1973; Berardelli A et al. Brain 1985*). Only few data on R2BRRC are available for atypical parkinsonism (*Valls-Solé J et al. Brain 1997*). We report clinical and electrophysiological findings of R2BRRC evaluation in PSP and CBS patients. The results of this study could contribute to characterize and differentiate such diseases.

2. Materials and methods

2.1 Participants

Patients affected by PSP and CBS were consecutively enrolled for 15 months. The MDS criteria for PSP (*Höglinger GU et al. Mov Disord 2017*) and Armstrong's diagnostic criteria for CBS (*Armstrong MJ et al. Neurology 2013*) were used. A group of healthy age- and sex-matched subjects was enrolled as controls. The study was approved by the

Local Ethic Committee and patients were enrolled after signing the written informed consent.

2.2 Clinical assessment

Clinical evaluation was performed through the H&Y stage (*Hoehn M et al. Neurology 1967*) and the UPDRS-ME section (*Fahn et al. Recent developments in Parkinson's disease. Florham Park, NJ: Macmillan Healthcare Information, 1987*).

2.3 Neurophysiological assessment

BR and R2BRRC (*Kimura J, Brain 1973*) were performed in all enrolled subjects by a neurophysiologist unaware of clinical data. Bipolar electrical stimulation was applied to SO nerve in SO foramen. Stimulus intensity ranged from 15 to 50 mA (duration: 0.2 ms) and electromyography responses were recorded simultaneously in both orbicularis oculi muscles with surface silver-silver chloride electrodes (filters: 20 Hz-10 KHz) (*Kimura J, Brain 1973*).

R2BRRC was performed with paired stimulation at ISIs of 100, 150, 200, 300, 400, 500 e 750 ms. Pairs of stimuli were separated by time intervals of 15 to 30 s to minimize habituation (*Kimura J, Brain 1973*). For each ISI the R2 amplitude ratio (expressed as percentage of R2 peak-to-peak amplitude of the conditioned response, divided by the R2 peak-to-peak amplitude of the unconditioned response) was calculated. R2BRRC was evaluated by plotting the R2 amplitude ratio for all the tested ISIs.

2.4 Statistical analysis

Differences of means and proportions between two selected groups were evaluated by t-test and Chi-square test respectively. Overall difference in R2BRRC between the three groups was assessed by analysis of variance (ANOVA) for each ISIs, using Tukey post-hoc test for further comparisons. For correlation analysis, the Pearson correlation coefficient (r) was used. Sensitivity, specificity, Positive-Predictive-Value (PPV) and Negative-Predictive-Value (NPV) together with 95% Confidence Intervals (95%CI) of R2BRRC in differentiating PSP from CBS patients were evaluated.

3. Results

Thirty subjects were enrolled: 12 patients with PSP, 8 patients with CBS and 10 controls. Demographics and clinical characteristics are summarized in table 1.

All PSP patients presented with postural instability and falls within first year of disease onset. Nine out of 12 PSP patients showed vertical supranuclear palsy and 3 patients presented with slow vertical saccades. All CBS patients showed asymmetric limb rigidity and ideomotor apraxia, whereas 4 out of 8 presented with alien limb phenomena and 2 out of 8 showed cortical sensory deficit. Only one CBS patient presented with limb myoclonus. All patients were in monotherapy with levodopa, except for a PSP patient who was not in treatment with dopaminergic therapy at the time of enrollment.

BR responses to single SO nerve stimulation were present at normal latencies in all participants.

Eleven out of the 12 PSP patients showed an early recovery of the R2 component: 10 patients presented an increased R2BRRC starting at ISI of 100 ms and one patient at ISI of 150 ms. On the other hand, an early R2BRRC was observed in only one CBS patient. R2BRRC curves of the three groups are shown in figure 1. R2BRRC showed a significantly different amplitude of response at ISIs of 100, 150, 200, 300 ms between PSP and CBS patients and also between PSP and control subjects, whereas no significant differences were found between CBS patients and controls.

We tested the relationship between recruitment at ISIs of 150 and 200 ms and clinical-demographic parameters as well as levodopa cumulative daily dose in PSP group, who showed an early recruitment compared to CBS patients. There were significant correlations between UPDRS-ME and recruitment at ISI of 150 ms (r=0.47; p=0.04; n=20) and at ISI of 200 ms (r=0.48; p=0.03; n=20). No other significant correlations were detected.

Presence of an early recovery of the R2 component in detecting PSP *versus* CBS patients showed a sensitivity of 91.7% (95%CI 61.5-99.8) with a specificity of 87.5% (95%CI 47.3-99.7); PPV was 91.7% (95%CI 61.5-99.7) whilst NPV was 87.5% (95%CI 47.4-99.7).

4. Table and figures legends

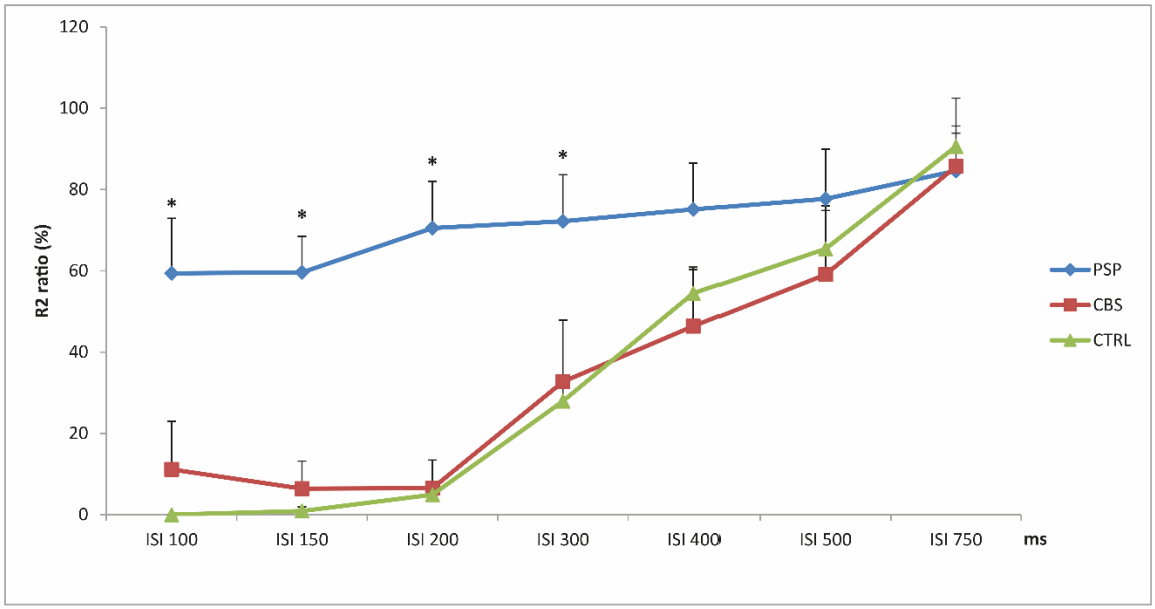
Table 1. Demographic, clinical characteristics and blink reflex results of participants.

	PSP (n=12)	**CBS (n=8)**	**CTRL**	***p*-value**
Age (years)[a]	68 ± 7.3	71 ± 4.7	63.7 ± 15.9	0.3*
Men (n, %)	9, 75%	3, 38%	3, 30%	0.1***
Age at onset (years)[a]	63 ±6.5	67.6 ± 5.4	/	0.08**
Disease duration	5.4 ± 2.7	3.4 ± 1.1	/	0.06**
UPDRS-ME score[a]	41.1 ± 12.9	27.6 ± 13.9	/	**0.04****
H&Y score[a]	3.5 ± 1.0	2.3 ± 1.2	/	**0.02****
Levodopa (daily mg)[a]	329.2 ± 211.3	337.5 ± 40.1	/	0.9**
Blink Reflex				
R1 r (ms)[a]	11.4 ± 0.9	11.2 ± 1.1	10.9 ± 0.83	0.4*
R2i r (ms)[a]	27.7 ± 2.3	28.1 ± 3.6	30.3 ± 4.0	0.2*
R2c r (ms)[a]	28.8 ± 2.4	30.2 ± 4.1	32.4 ± 4.6	0.09*
R1 l (ms)[a]	11.2 ± 0.5	11.0 ± 1.1	10.8 ± 0.8	0.7*
R2i l (ms) [a]	27.5 ± 2.1	26.7 ± 3.8	30.8 ± 4.6	0.9*
R2c l (ms) [a]	28.5 ± 2.2	30.0 ± 4.0	32.8 ± 5.0	0.09*

Notes: [a]Data are means ± standard deviations. *Oneway ANOVA; **unpaired *t*-test; ***PSP *vs* CBS: Chi-square.
Legend: PSP = Progressive Supranuclear Palsy, CBS = Cortico-basal syndrome, CTRL = controls, Unified Parkinson's Disease Rating Scale-Motor-Examination section = UPDRS-ME, H&Y = Hoehn and Yahr stage, r = right stimulation, l = left stimulation, I = ipsilateral, c = contralateral.

Figure 1. R2 blink reflex recovery cycle graph-curve for PSP, CBS and control groups.

Ratios of conditioned R2 component to unconditioned response (amplitude) are shown as mean + standard error. X-axis: interstimulus intervals in milliseconds (ms). Y-axis: ratio of conditioned to unconditioned R2 response in percentage (%). *p<0.05 when comparing PSP *vs* CBS and controls.



Legend: PSP = Progressive Supranuclear Palsy, CBS = Cortico-basal syndrome, CTRL = controls, ISI = interstimulus interval.

General Discussion

1. Neurophysiological features for diagnosis of Parkinson's disease

Brainstem excitability investigated by R2BRRC showed to be enhanced contralaterally to clinically affected side in early PD patients. On the other hand, APS did not show any asymmetric pattern of brainstem excitability compared to PD patients. Despite APS could be clinically symmetric, it is quite common to find an asymmetric presentation as in our sample, which makes APS indistinguishable from PD (*Gilman S et al. Neurology 2008; Höglinger GU et al. Mov Disord 2017*). The AI, a marker of asymmetric brainstem activity, discriminated PD from PSP and MSA with a good accuracy by using a 100 ms ISI stimulation of R2BRRC.

Previous studies explored R2BRRC in PD and APS (*Kimura J, Brain 1973; Valls-Solé J et al. Brain 1997; Sciacca G et al. Eur J Neurol 2018*). Kimura and colleagues first demonstrated the increase of R2BRRC in PD patients (*Kimura J, Brain 1973*). Valls-Solè and colleagues showed that R2BRRC was increased in PSP, MSA and PD patients (*Valls-Solé J et al. Brain 1997*). We found that R2BRRC could differentiate PSP from CBS with a sensitivity of 91.7% and a specificity of 87.5% (*Sciacca G et al. Eur J Neurol 2018*).

To our knowledge, only two studies were conducted on asymmetric BR responses in PD (*Dengler R et al. Electroencephalogr Clin Neurophysiol 1982; Sugiyama T et al. J Clin Neurophysiol 2018*). Dengler and colleagues evaluated BR in treated PD patients with hemiparkinsonism, observing a decreased EMG activity of the BR components on the

clinically affected side (*Dengler R et al. Electroencephalogr Clin Neurophysiol 1982*). Despite they did not evaluate recovery cycle, this observation could be considered in agreement with our results on decreased R2BRRC for MAS stimulation in PD (*Dengler R et al. Electroencephalogr Clin Neurophysiol 1982*). Sugiyama and colleagues assessed R2BRRC in treated PD patients with lateral trunk flexion and with normal posture (*Sugiyama T et al. J Clin Neurophysiol 2018*). An asymmetric disinhibition of R2BRRC was found to be associated with the abnormal posture (*Sugiyama T et al. J Clin Neurophysiol 2018*). Nevertheless, the study did not detect correlations between the side of R2BRRC disinhibition and the side of trunk convexity (*Sugiyama T et al. J Clin Neurophysiol 2018*).

AI of R2BRRC seems to be a promising neurophysiological tool which permits to distinguish early PD from APS. Moreover, AI differentiates drug-naive PD from untreated PSP and MSA patients with similar disease duration, representing a good marker also in early stages.

We hypothesized that an increased neuronal excitability, probably due to a predominant contralateral dysfunction of basal ganglia structures for loss of cortical inhibitory functions, could explain the asymmetric brainstem disinhibition observed in PD. Therefore, early detection of asymmetric brainstem alterations through the use of R2BRRC AI could help clinicians in differential diagnosis between PD and APS, especially at the early onset of the disease.

2. Neurophysiological features for treatment of Parkinson's disease

The achievement of LDR to levodopa and the performance of MT skills improved the neurophysiological parameters which assessed motor learning. Moreover, the evaluation after two hours the intake of levodopa revealed that SDR did not influence the neurophysiological parameters unlike LDR to levodopa treatment. On the other hand, the achievement of LDR without MT exhibited an improvement in TMS and BP latencies but not in the other parameters at T15, revealing a possible direct dopaminergic influence on these neurophysiological features. Neurophysiological parameters were similar at T15 and T16 also in this group, confirming the role played by LDR in motor learning compared to SDR to levodopa treatment. The two groups of patients without a stable LDR did not show any significant difference in neurophysiological parameters comparing baseline conditions to the 15^{th} day of dopaminergic treatment, despite the performance of MT.

LDR to levodopa is the most important component of the therapeutic response to the drug, especially in early PD (*Zappia M et al. Neurology 2000*). Moreover, we previously observed that switching levodopa therapy from "pulsatile" to "pulse" modality through the development and the maintenance of LDR may reduce the severity of wearing-off and dyskinesia in complicated PD (*Mostile G et al. Clin Neuropharmacol 2017*). In addition to the fact that the achievement of LDR can prevent therapy-related motor complications in PD, LDR could be involved in motor learning, as demonstrated by studies conducted on animal models (*Beeler JA et al. Ann Neurol 2010*).

Animal studies have indeed demonstrated how dopamine is important for acquisition and maintenance of learned skills in rodents (*Beeler JA et al. Ann Neurol 2010*). Dopamine-dependent motor learning was observed in a mouse model of PD reproducing LDR, in which both dopamine and task-training influenced learning process (*Beeler JA et al. Ann Neurol 2010*). In the absence of dopamine, MT developed aberrant learning in mice and loss of performance capability was observed after a few days from discontinuation of treatment (*Beeler JA et al. Ann Neurol 2010*).

Despite LDR to chronic levodopa treatment is considered strictly associated with motor learning in animal models, there are no systematic studies demonstrating this association in humans. Kang and colleagues examined the effects of activity and dopaminergic treatment in PD patients, comparing motor task performance in dominant and nondominant hands through finger-tapping score and considering the activities of the dominant hand as active training (*Kang UJ et al. Neurology 2012*). The authors observed that the active use of dominant hand associated with levodopa treatment produced an improvement in motor performance compared to nondominant hand, supporting the hypothesis that activity and dopamine enhances LDR synergycally (*Kang UJ et al. Neurology 2012*). However, the lack of specific tasks for motor learning and the use of finger-tapping score to evaluate learning represented the main limits of this study (*Kang UJ et al. Neurology 2012*). In our study, we used neurophysiological tests related to dopaminergic treatment and corticomotor plasticity for evaluating how LDR could influence motor learning in drug-naive parkinsonian patients, who underwent specific MT tasks. According to the study conducted by Kang and colleagues, we observed a

significant improvement of motor learning in trained PD patients with a sustained LDR as shown in neurophysiological assessment, whereas untrained PD patients with a stable LDR presented with an improvement of only some neurophysiological parameters as TMS and BP latencies. Despite dopaminergic treatment could influence neurophysiological assessment in PD, the achievement of LDR facilitates motor learning during the execution of specific motor tasks and MT could enhance the maintenance of LDR to treatment.

Several studies have examined dopaminergic effects on neurophysiological features in PD (*Simpson JA et al. J Neurol Neurosurg Psychiatry 1987; Dick JP et al. Brain 1989; Stanzione P et al. Electroencephalogr Clin Neurophysiol 1991; Prasher D et al. J Neurol Neurosurg Psychiatry 1991; Feve AP et al. Clin Neuropharmacol 1992; Defebvre L et al. J Neurol Neurosurg Psychiatry 1996; Fattapposta F et al. Clin Neurophysiol 2002; Shibasaki H et al. Clin Neurophysiol 2006; Cirillo J et al. Eur J Neurosci 2011; Brunia CHM et al. The Oxford handbook of event-related potential components 2012, p.198-207; Bagce HF et al. J Neurophysiol 2013; Silva Lopes M et al. Arq Neuropsiquiatr 2014; Hirano M et al. Brain Stimul 2015; Kojovic M et al. Mov Disord 2015; Georgiev D et al. Clin Neurophysiol 2016*).

P300 reflects the time required for auditory processing of external stimuli, which physiologically increases with aging and with reduction of cognitive capacity in attention and memory (*Silva Lopes M et al. Arq Neuropsiquiatr 2014*). P300 is also considered a useful tool to investigate the cognitive status of PD patients (*Silva Lopes M et al. Arq Neuropsiquiatr 2014*). Increased blood flow in frontal lobes was observed

during the P300 recording and a significant correlation between P300 latency and performances on frontal-lobe-related cognitive tasks was demonstrated in PD (*Sohn YH et al. J Neurol Sci 1998*). Previous studies have examined the influence of dopaminergic treatment on P300 (*Stanzione P et al. Electroencephalogr Clin Neurophysiol 1991; Prasher D et al. J Neurol Neurosurg Psychiatry 1991; Sohn YH et al. J Neurol Sci 1998*). Drug-naive PD patients showed prolonged P300 latency compared with levodopa-treated patients (*Sohn YH et al. J Neurol Sci 1998*). In particular, Stanzione and colleagues found a shortening of P300 latency in PD after one/two weeks of dopaminergic treatment (*Stanzione P et al. Electroencephalogr Clin Neurophysiol 1991*). On the other hand, the study conducted by Prasher and colleagues showed discordant results compared to the previous one, as a prolongation of P300 latency was observed after long-term levodopa therapy in drug-naive PD patients (*Prasher D et al. J Neurol Neurosurg Psychiatry 1991*). Therefore, data about dopaminergic treatment on cognitive potential are not univocal in the literature. We observed an improvement of P300 latency in PD patients after performing MT tasks and the achievement of LDR, without significant differences between acute and chronic administration of levodopa. The contribution of specific motor tasks on P300 latency could be explained by the role played by P300 in perceptual-motor learning tasks as it was demonstrated during rehabilitation of visuo-spatial neglect through prism adaptation (*MacLean SJ et al. Front Hum Neurosci 2015*). P300 amplitude was largest during the early phase of adaptation for both hit and miss trials, suggesting to reflect a system for context updating and learning (*MacLean SJ et al. Front Hum Neurosci 2015*). These observations could be

considered in agreement with our results, showing the double effect of treatment and MT in the amelioration of motor learning skills.

It is known that the acquisition of skilled movements is associated with characteristic adaptive changes in cortical networks, leading to typical alterations in MEPs (*Gallasch E et al. Eur J Appl Physiol 2009; Cirillo J et al. Eur J Neurosci 2011; Bagce HF et al. J Neurophysiol 2013; Hirano M et al. Brain Stimul 2015*). Intracortical microstimulation demonstrated enlarged motor representations in primary motor cortex following practice of fine motor skills in non-human primates as well as studies with PET, fMRI and TMS showed enlarged post-exercise representations in M1 during acquisition of complex motor skills in humans (*Gallasch E et al. Eur J Appl Physiol 2009*). It has been shown that a training session, albeit lasting a short time period, induces such MEP-facilitation; thereafter, this effect may persist several minutes after a training session (*Gallasch E et al. Eur J Appl Physiol 2009*). TMS could assess also inhibitory GABA-B mediated circuits by CSP recording (*Mak M et al. Clin Neurophysiol 2013*). Mak and colleagues observed the effect of pinch-grip training with visual cues on both MEPs and CSP in PD patients, reporting enhanced motor cortical excitability and intracortical inhibition, expressed as increased MEP amplitude and prolonged CSP respectively (*Mak M et al. Clin Neurophysiol 2013*). In our study, we observed an increase of MEP amplitude only in LDR and MT group, reflecting a synergic effect of chronic administration of levodopa and specific training tasks on motor learning. CSP duration was similar in all four groups, despite dopaminergic treatment and MT. The different results on inhibition circuits between the study conducted by Mak and colleagues (*Mak M et al. Clin*

Neurophysiol 2013) and our study could be related to the patient's selection, as their patients were treated for one year or more with different anti-parkinsonian medication at the time of enrollment, whereas our patients were drug-naive PD, subsequently treated only with levodopa to achieve LDR. We could hypothesize a different adaption of dopaminergic circuits in PD patients who have been treated for several years with dopaminergic drugs compared to drug-naive patients. Kojovic and colleagues conducted a TMS follow-up study in early PD to evaluate disease progression (*Kojovic M et al. Mov Disord 2015*). For all TMS parameters, the authors did not find differences between treated and untreated patients or between early and late treated patients. The authors observed an initially shorter CSP in early PD and a subsequent prolongation of CSP in the follow-up period, which could not be attributed to dopaminergic treatment, as the same changes occurred in both treated and untreated patients (*Kojovic M et al. Mov Disord 2015*). Contrariwise, previous studies found a shortening in CSP latency which was correlated to the advanced stage of the disease (*Kojovic M et al. Mov Disord 2015*). In agreement with Kojovic and colleagues, our results confirmed that inhibitory circuits are not influenced by LDR and MT and GABA-B circuits are probably not implicated in motor learning process.

BP recording results from averaged amplitude fluctuations of the ongoing EEG activity that are time-locked to processes of voluntary movement preparation, initiation and execution (*Shibasaki H et al. Clin Neurophysiol 2006*). Dick and colleagues found lower amplitude of the early BP and higher amplitude of the late BP in PD patients off dopaminergic medication, indicating reduced SMA activity and compensatory M1

activity, compared to controls (*Dick JP et al. Brain 1989*). In an earlier study from the same group, the amplitude of the early BP was found to be smaller in the off-phase of levodopa treatment, returning to normal in the on-phase, without effects on the late BP (*Dick JP et al. Electroencephalogr Clin Neurophysiol 1987*). On the other hand, Fattaposta and colleagues observed that PD patients, who underwent acute administration of levodopa after a pharmacological wash-out, scored a higher percentage of correct performance tasks correlated to a decreased BP amplitude (*Fattapposta F et al. Clin Neurophysiol 2002*). Chronic administration of levodopa in drug-naive PD patients increased the amplitude of the late BP component, but not of the early BP (*Feve AP et al. Clin Neuropharmacol 1992*). In our study, we observed an improvement of late BP amplitude only in LDR and MT group. Therefore, according to the results by Feve and colleagues (*Feve AP et al. Clin Neuropharmacol 1992*), chronic administration of levodopa and performance of MT skills seem to influence M1 activity for the achievement of motor learning. Only few studies have examined alterations of BP latency in PD and its modification after levodopa administration (*Shibasaki H et al. J Neurol 1978; Simpson JA et al. J Neurol Neurosurg Psychiatry 1987; Defebvre L et al. J Neurol Neurosurg Psychiatry 1996*). Simpson and colleagues observed a shorter duration of BP in PD patients (*Simpson JA et al. J Neurol Neurosurg Psychiatry 1987*). Defebvre and colleagues evaluated movement related desynchronization (MRD) pattern in untreated PD patients, showing a delay in the onset of MRD in terms of time required to reach a threshold level of cortical activity for execution of movement (*Defebvre L et al. J Neurol Neurosurg Psychiatry 1996*). On the other hand, Shibasaki and colleagues

reported a marked increase in the time interval from the onset of the negative deflection to the recorded EMG activity in PD patients (*Shibasaki H et al. J Neurol 1978*). In particular, Shibasaki hypothesized that a prolonged BP could be expression of the delay in initiating movement which is a typical clinical feature of PD (*Shibasaki H et al. J Neurol 1978*). Our findings are in agreement with the study conducted by Shibasaki and colleagues, showing earlier latencies for both BP components after the achievement of LDR to levodopa compared to prolonged latencies recorded at baseline conditions. Therefore, dopaminergic treatment could restore the delay between intention and movement in PD, reducing the time for initiating movement. We could hypothesize that striato-thalamo-cortical projection could modulate SMA and M1 activity through dopaminergic circuits, affecting the onset of event-related cortical potentials.

Striatal plasticity is lost after dopamine denervation and restored with levodopa treatment (*Pisani A et al. Mov Disord 2005*). Recent studies highlighted the role of motor cortex in motor learning, suggesting that alterations of neural circuits due to dopamine depletion could cause an impairment in motor learning in PD (*Xu T et al. Mov Disord 2017*). MT modifies movement representations within trained limb areas in M1 of rodents, primates and humans (*Xu T et al. Mov Disord 2017*), inducing both functional and structural synaptic plasticity. Primary motor cortex receives dopaminergic projections from the pars compacta of the substantia nigra via the mesocortical pathways and dopamine depletion determines the aberrant plasticity observed in M1 of animal models (*Xu T et al. Mov Disord 2017*). Therefore, the depletion of dopamine in PD causes dysfunctional synaptic plasticity in motor cortex with subsequent impairment of motor

learning processes. According to our results, we could hypothesize that LDR to levodopa treatment could restore synaptic plasticity of primary motor cortex and MT synergycally reinforces the stability of new connections.

3. Neurophysiological features differentiate Atypical Parkinsonian Syndromes

PSP patients exhibited an increased excitability of brainstem circuits, as measured by R2BRRC. R2BRRC showed a specificity of 87.5% and a sensitivity of 91.7% in detecting PSP *versus* CBS patients.

Previous studies have established the utility of R2BRRC in confirming the diagnosis of several neurological diseases. BRRC is an objective instrument in the differential diagnosis between essential and psychogenic blepharospasm, showing a significant brainstem disinhibition in essential form (*Schwingenschuh P et al. Neurology 2011*). R2BRRC at ISI of 100 ms distinguishes between essential tremor associated with resting tremor and PD de novo (*Nisticò R et al. Parkinsonism Relat Disord 2014*).

To our knowledge, only few studies were conducted on BR and BRRC in APS (*Valls-Solé J et al. Brain 1997; Kızıltan ME et al. J Neurol Sci 2014; Szmidt-Salkowska E et al. Neurol Neurochir Pol 2016*). Kiziltan and colleagues evaluated BR and trigemino-cervical-reflex in patients with primary progressive freezing of gait (PPFOG) and PSP with FOG, observing an increased excitability of trigemino-cervical circuits in PPFOG (*Kızıltan ME et al. J Neurol Sci 2014*). Szmidt-Salkowska and colleagues noted

significant differences in R2 latencies of BR in PD compared to MSA and PSP (*Szmidt-Salkowska E et al. Neurol Neurochir Pol 2016*). Valls-Solè and colleagues evaluated facial reflex responses, BR and R2BRRC in PD, MSA, PSP and CBS (*Valls-Solé J et al. Brain 1997*). The percentage of excitability recovery of BR to paired stimuli at ISI of 200 ms was higher in all patients, except in those with CBS when compared with controls (*Valls-Solé J et al. Brain 1997*).

We hypothesize that the predominant brainstem 4-repeat tau aggregates distribution in PSP, in contrast with the major involvement of neocortex in CBS, could explain the significant brainstem disinhibition observed in PSP patients. Moreover, MRI studies showed a correlation between blinking abnormalities and gray matter loss of basal ganglia nuclei and white matter volume loss of the brainstem in PSP patients, confirming that blinking generators lie in subcortical structures, including brainstem (*Bologna M et al. Mov Disord 2016*). Therefore, R2BRRC assessing brainstem excitability, could reveal blinking abnormalities due to brainstem dysfunctions. According to our results, R2BRRC curve might be considered a useful tool in differentiating PSP from CBS patients.

The main limits of our study are related to the lack of pathological confirmation of the diagnosis, despite worldwide accepted diagnostic criteria have been adopted (*Armstrong MJ et al. Neurology 2013; Höglinger G et al. Mov Disord 2017*), and to the small sample, due to the low frequency of APS in the general population. Multicentric studies on larger population of patients are necessary to support our findings.

Conclusions

Neurophysiological features of drug-naive PD patients have been investigated in order to identify markers of the disease at the early clinical manifestation and to improve and optimize conventional pharmacological treatment for PD.

The computation of AI of R2BRRC showed an asymmetric brainstem excitability in early PD but not in APS, permitting to differentiate the disorders. The use of this new neurophysiological tool could be helpful in daily clinical practice but also in research field, especially for understanding the pathophysiology of PD and for the selection of patients for innovative therapeutic clinical trials. Furthermore, neurophysiological tests assessing motor learning have showed that LDR to levodopa treatment facilities motor learning in PD, whereas SDR does not influence this process. Therefore, a therapeutic approach aims to start an early dopaminergic treatment and to establish and maintain LDR to levodopa through switching therapy from a "pulsatile" administration, consisting in intermittent multiple daily small doses of the drug, to a "pulse" administration, consisting in standard oral doses given at specific interdose intervals, could enhance beneficial plasticity in PD. Moreover, the neurophysiological approach to demonstrate the association between LDR and motor learning could be helpful in clarifying the still unknown origin of LDR, leading to new strategies for increasing the effect of levodopa and thereby improve motor function in PD. Further studies with larger sample size are necessary to confirm our hypothesis.

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Annexes

Publications (2019-2016)

1. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Asymmetry Index of Blink Reflex Recovery Cycle differentiates Parkinson's disease from Atypical parkinsonian syndromes.* [Submitted in Parkinsonism and Related Disorders].
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Abstracts (2016-2019)

1. Bonomo R, Mostile G, Raciti L, Contrafatto D, Dibilio V, Luca A, **Sciacca G**, Cicero C, Vasta R, Nicoletti A, Zappia M. *Quantitative estimation of motor fluctuations in Parkinson's disease*. In: Neurological Sciences 2016. Società Italiana di Neurologia (SIN), Venezia, Italia, 22-25 October 2016; Supplement Volume 37, pp. S284-285.
2. Mostile G, Nicoletti A, Giuliano L, Dibilio V, Luca A, Raciti L, **Sciacca G**, Cicero C, Vasta R, Sofia V, Zappia M. *Complexity of fronto-temporal electrocortical activity in untreated Parkinson's disease. Evidences of a topographical neuronal organization*. In: Neurological Sciences 2016. Società Italiana di Neurologia (SIN), Venezia, Italia, 22-25 October 2016; Supplement Volume 37, pp. S293.

3. **Sciacca G**, Nicoletti A, Rampello L, Noto L, Soto Parra HJ, Zappia M. *Benign form of Myasthenia Gravis after Nivolumab treatment*. In: Neurological Sciences 2016. Società Italiana di Neurologia (SIN), Venezia, Italia, 22-25 October 2016; Supplement Volume 37, pp. S98-99.
4. **Sciacca G**, Lo Fermo S, Aprile G, Nicoletti A, Mostile G, Zappia M. *Wernicke encephalopathy and Systemic sclerosis: rare association of rare conditions*. In: Neurological Sciences 2016. Società Italiana di Neurologia (SIN), Venezia, Italia, 22-25 October 2016; Supplement Volume 37, pp. S364-365.
5. **Sciacca G**, Reggio E, Nicoletti A, Zappia M. *Evaluation of Neostigmine responsiveness with Concentric-Needle Single Fiber Electromyography in Myasthenia Gravis: a comparative study*. In: Neurological Sciences 2016. Società Italiana di Neurologia (SIN), Venezia, Italia, 22-25 October 2016; Supplement Volume 37, pp. S70-71 (*Oral communication*).
6. **Sciacca G**, Nicoletti A, Mostile G, Luca A, Raciti L, Dibilio V, Le Pira F, Zappia M. *Blink reflex recovery cycle to differentiate Progressive supranuclear palsy from corticobasal degeneration: a pilot study*. In: 3° Congresso Accademia LIMPE-DISMOV 2017, Verona, Italia, 17-19 May 2017.
7. Mostile G, Nicoletti A, Giuliano L, Dibilio V, Luca A, Raciti L, **Sciacca G**, Cicero CE, Vasta R, Sofia V, Zappia M. *Complexity of frontotemporal electrocortical activity in untreated Parkinson's disease. Evidences of a topographical neuronal organization*. In: 3° Congresso Accademia LIMPE-DISMOV 2017, Verona, Italia, 17-19 May 2017.

8. **Sciacca G**, Nicoletti A, Mostile G, Luca A, Raciti L, Dibilio V, Le Pira F, Zappia M. *Blink reflex recovery cycle to differentiate Progressive Supranuclear Palsy from Corticobasal Degeneration.* In: 21st International Congress - Movement Disorders Society (MDS) 2017, Vancouver, British Columbia, Canada, 4-8 June 2017, Movement Disorders, vol. 32 (supplement 2), p. S363.
9. **Sciacca G**, Reggio E, Donzuso G, Nicoletti A, Zappia M. *Forehead Tremor: a clinical presentation of ocular Myasthenia Gravis?*. In: 21st International Congress - Movement Disorders Society (MDS) 2017, Vancouver, British Columbia, Canada, 4-8 June 2017, Movement Disorders, vol. 32 (supplement 2), p. S302.
10. **Sciacca G**, Nicoletti A, Mostile G, Luca A, Raciti L, Dibilio V, Le Pira F, Zappia M. *Blink reflex recovery cycle to differentiate progressive supranuclear palsy from corticobasal degeneration: a pilot study*. In: 3rd Congress of the European Academy of Neurology (EAN) 2017, Amsterdam, The Netherlands, 24-27 June 2017. European Journal of Neurology, Wiley-Blackwell, vol. 24 (supplement 1), p. 638. *(E-Presentation).*
11. **Sciacca G**, Reggio E, Donzuso G, Nicoletti A, Zappia M. *Forehead Tremor: a clinical presentation of ocular Myasthenia Gravis?*. In: 3rd Congress of the European Academy of Neurology (EAN) 2017, Amsterdam, The Netherlands, 24-27 June 2017. European Journal of Neurology, Wiley-Blackwell, vol. 24 (supplement 1), p. 687.

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14. **Sciacca G**, Nicoletti A, Mostile G, Luca A, Raciti L, Dibilio V, Le Pira F, Zappia M. *R2 recovery cycle for neurophysiological differential diagnosis of cortico-basal degeneration from progressive supranuclear palsy*. In: XXIII World Congress of Neurology (WCN) 2017, Kyoto, Japan, 16-21 September 2017. Journal of the Neurological Sciences 2017;381:54-180. *(Oral communication).*
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18. **Sciacca G**, Reggio E, Giardina F, Chisari C, Patti F, Sortino G, Zappia M. *Subacute combined degeneration due to vitamin B12 deficiency in the absence of macrocytic anemia*. In: Neurological Sciences 2017. Società Italiana di Neurologia (SIN), Napoli, Italia, 14-17 October 2017; Supplement Volume 38, pp. S123-124.
19. Donzuso G, Monastero R, Luca A, Cicero E, Mostile G, Baschi R, Angileri L, **Sciacca G**, Fierro B, Zappia M, Nicoletti A. *Grey matter atrophy in Parkinson's disease with mild cognitive impairment.* In: Neurological Sciences 2017. Società Italiana di Neurologia (SIN), Napoli, Italia, 14-17 October 2017; Supplement Volume 38, p. S179.
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22. Donzuso G, Monastero R, Luca A, Cicero CE, Mostile G, Baschi R, Angileri L, **Sciacca G**, Fierro B, Zappia M, Nicoletti A. *Cortical atrophy in Parkinson's disease with Mild Cognitive Impairment: a Voxel-based Morphometry study*. In: 4° Congresso Accademia LIMPE-DISMOV 2018, Roma, Italia, 24-26 May 2018.
23. Mostile G, Giuliano L, Monastero R, Luca A, Cicero CE, Donzuso G, Dibilio V, Baschi R, Terranova R, **Sciacca G**, Sofia V, Fierro B, Zappia M, Nicoletti A. *Electrocortical connectivity and non-linear quantitative analysis of EEG signal in PD-MCI*. In: 4° Congresso Accademia LIMPE-DISMOV 2018, Roma, Italia, 24-26 May 2018.
24. Dibilio V, Mostile G, Nicoletti A, Luca A, Raciti L, **Sciacca G**, Cicero CE, Donzuso G, Zappia M. C*luster analysis of selected kinematic parameters during Sit-to-Stand task in Parkinson's disease. Association with baseline motor characteristics*. In: 4° Congresso Accademia LIMPE-DISMOV 2018, Roma, Italia, 24-26 May 2018.

25. Raciti L, Nicoletti A, Mostile G, Bonomo R, Dibilio V, Donzuso G, **Sciacca G**, Cicero CE, Luca A, Zappia M. *Accuracy of MDS-UPDRS section IV for detecting motor fluctuations in Parkinsn's disease*. In: 4° Congresso Accademia LIMPE-DISMOV 2018, Roma, Italia, 24-26 May 2018.
26. Bonomo R, Mostile G, Raciti L, Dibilio V, Luca A, **Sciacca G**, Donzuso G, Nicoletti A, Zappia M. *Minimally clinically important change in levodopa-response detecting motor fluctuations in Parkinson's diease patients: usefulness of base-peak evaluation in clinical practice*. In: 4° Congresso Accademia LIMPE-DISMOV 2018, Roma, Italia, 24-26 May 2018.
27. **Sciacca G**, Reggio E, Giardina F, Chisari C, Patti F, Sortino G, Zappia M. *Subacute combined degeneration due to vitamin B12 deficiency in the absence of macrocytic anemia*. In: 4th Congress of the European Academy of Neurology (EAN) 2018, Lisbon, Portugal, 16-19 June 2018. European Journal of Neurology, Wiley-Blackwell, vol. 25 (supplement 2), p. 625.
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29. Donzuso G, Monastero R, Luca A, Cicero CE, Mostile G, Baschi R, Angileri L, **Sciacca G**, Fierro B, Zappia M, Nicoletti A. *Cortical atrophy in Parkinson's*

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32. Raciti L, Nicoletti A, Mostile G, Bonomo R, Dibilio V, Donzuso G, **Sciacca G**, Cicero CE, Luca A, Zappia M. *Accuracy of MDS-UPDRS section IV for detecting motor fluctuations in PD*. In: Neurological Sciences 2018. Società Italiana di Neurologia (SIN), Roma, Italia, 27-30 October 2018; Supplement Volume 39, pp. S226.
33. Luca A, Nicoletti A, Mostile G, **Sciacca G**, Dibilio V, Cicero CE, Raciti L, Donzuso G, Zappia M. *Temperament traits and executive functions in Parkinson's disease*. In: Neurological Sciences 2018. Società Italiana di

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35. **Sciacca G**, Lo Fermo S, Manna R, Nicoletti A, Zappia M. *Superficial Siderosis of the Central Nervous System from clinical presentation to diagnosis: a case series*. In: Neurological Sciences 2018. Società Italiana di Neurologia (SIN), Roma, Italia, 27-30 October 2018; Supplement Volume 39, pp. 324.
36. Dibilio V, Mostile G, Nicoletti A, Luca A, Raciti L, **Sciacca G**, Cicero CE, Donzuso G, Zappia M. *Cluster analysis of selected kinematic parameters during sit-to-stand task in Parkinson's disease. Association with baseline motor characteristics.* In: Neurological Sciences 2018. Società Italiana di Neurologia (SIN), Roma, Italia, 27-30 October 2018; Supplement Volume 39, pp. 371.
37. Chisari C, Reggio E, **Sciacca G**, Arena S, Patti F, Zappia M. *Neostigmine test and eye-movements in ocular myasthenia gravis.* In: Neurological Sciences 2018. Società Italiana di Neurologia (SIN), Roma, Italia, 27-30 October 2018; Supplement Volume 39, pp. 411.
38. Donzuso G, Sciacca G, Mostile G, Nicoletti A, Zappia M. *Grey matter atrophy in Parkinson's disease with long-duration response.* In: 5th Congress of the European Academy of Neurology (EAN) 2019, Oslo, Norway, June 29 – July 2

2019. European Journal of Neurology, Wiley-Blackwell, vol. 26 (supplement 1), p. 153.

39. **Sciacca G**, Nicoletti A, Mostile G, Raciti L, Contrafatto D, Bonomo R, Salomone S, Drago F, Zappia M. *Blink reflex recovery cycle in atypical parkinsonian syndromes: cut-off scores to differentiate corticobasal syndrome from progressive supranuclear palsy and multiple system atrophy.* In: 5th Congress of the European Academy of Neurology (EAN) 2019, Oslo, Norway, June 29 – July 2 2019. European Journal of Neurology, Wiley-Blackwell, vol. 26 (supplement 1), p. 286. *(E-Presentation).*
40. Cicero CE, Raciti L, Monastero R, Mostile G, **Sciacca G**, Luca A, Terravecchia C, Giuliano L, Baschi R, Davi M, Zappia M, Nicoletti A. *Cardiovascular autonomic function and MCI in Parkinson's disease.* In: 5th Congress of the European Academy of Neurology (EAN) 2019, Oslo, Norway, June 29 – July 2 2019. European Journal of Neurology, Wiley-Blackwell, vol. 26 (supplement 1), p. 457.
41. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Motor learning and long-duration response to levodopa in Parkinson's disease.*. In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.
42. Chisari CG, Mostile G, Luca A, Donzuso G, Sciacca G, Bonomo R, Rascunà C, Portaro G, Patti F, Nicoletti A, Zappia M. *Effetti della somministrazione in acuto di levodopa sulla motilità oculare nei pazienti affetti da malattia di Parkinson.*

In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.

43. Donzuso G, **Sciacca G**, Mostile G, Nicoletti A, Zappia M. *Correlati neuroanatomici della risposta di lunga durata nei pazienti con malattia di Parkinson de novo*. In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.
44. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Unilateral increase of blink reflex recovery cycle in drug-naive hemiparkinson syndrome*. In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.
45. Chisari CG, Mostile G, Donzuso G, **Sciacca G**, Portaro G, Rascunà C, Patti F, Nicoletti A, Zappia M. *Effetti della somministrazione acuta di levodopa sulla motilità oculare in pazienti affetti da parkinsonismi atipici*. In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.
46. Cicero CE, Raciti L, Monastero R, Mostile G, **Sciacca G**, Luca A, Terravecchia C, Giuliano L, Baschi R, Davì M, Zappia M, Nicoletti A. *Funzione autonomica cardiovascolare e MCI nella malattia di Parkinson.* In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.
47. Luca A, Nicoletti A, Mostile G, Rascunà C, Terravecchia C, D'Agate C, Donzuso G, Cicero CE, Portaro G, **Sciacca G**, Zappia M. *Il disturbo ossessivo-compulsivo di personalità come fattore di rischio di disfunzione esecutiva nella malattia di*

Parkinson. In: 5° Congresso Accademia LIMPE-DISMOV 2019, Catania, Italia, 22-24 May 2019.

48. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Asymmetry index of Blink Reflex Recovery Cycle differentiates early Parkinson's disease from Atypical Parkinsonian Syndromes.* In: Società Italiana di Neurologia (SIN) 2019, Bologna, Italy, 12-15 October 2019. *(Oral communication).*
49. Chisari C, Mostile G, Mirabella M, Luca A, Contrafatto D, **Sciacca G**, Donzuso G, Bonomo R, Rascunà C, Portaro G, Patti F, Nicoletti A, Zappia M. *Effect of acute l-dopa administration on eye movement parameters in Parkinson's disease.* In: Società Italiana di Neurologia (SIN) 2019, Bologna, Italy, 12-15 October 2019.
50. Donzuso G, **Sciacca G**, Mostile G, Nicoletti A, Zappia M. *Neuroanatomical changes in Parkinson's disease patients with long-duration response*. In: Società Italiana di Neurologia (SIN) 2019, Bologna, Italy, 12-15 October 2019.
51. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Motor learning and long duration response to levodopa in Parkinson's disease*. In: International Congress - Movement Disorders Society (MDS) 2019, Nice, France, 22-26 September 2019.
52. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Unilateral increase of blink*

reflex recovery cycle in drug-naive hemiparkinson syndrome. In: International Congress - Movement Disorders Society (MDS) 2019, Nice, France, 22-26 September 2019.

53. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Asymmetry index of Blink Reflex Recovery Cycle differentiates early Parkinson's disease from Atypical Parkinsonian Syndromes.* In: XXIV World Congress of Neurology (WCN) 2019, Dubai, Emirates, 27-31 October 2019. *(Oral communication).*
54. **Sciacca G**, Mostile G, Disilvestro I, Donzuso G, Manna R, Portaro G, Rascunà C, Salomone S, Drago F, Nicoletti A, Zappia M. *Motor learning and long duration response to levodopa in Parkinson's disease*. In: XXIV World Congress of Neurology (WCN) 2019, Dubai, Emirates, 27-31 October 2019. *(Oral communication).*

Awards (2016-2019)

1. Achievement of the **European Board of Neurology** – awarded as **Fellow of the European Board of Neurology**, 2018.
2. Research Grant "Premio progetto giovani e ricerca" for the scientific contribution "Motor learning and long-duration response to levodopa in Parkinson's disease", LIMPE-DISMOV 2019.
3. Travel Grant for 3rd Congress of the European Academy of Neurology (EAN), Amsterdam, The Netherlands, 2017.

4. Travel Grant for Autumn School for young neurologists of the European Academy of Neurology (EAN), Loutraki, Greece, 2018.
5. Travel Grant for 21st International Congress of Movement Disorders Society (MDS), Vancouver, British Columbia, Canada, 2017.
6. Travel Grant for the International Congress of Movement Disorders Society (MDS), Hong Kong, 2018.
7. Travel Grant for 2nd School for Neuromodulation of Movement Disorders Society (MDS), Wurzburg, Germany, 2018.
8. Attendance Grant for XXIII World Congress of Neurology (WCN), Kyoto, Japan, 2017.
9. Premio Progetto Giovani, Società Italiana di Neurologia (SIN) 2016, Venezia, Italy.
10. Premio Progetto Giovani, Società Italiana di Neurologia (SIN) 2017, Napoli, Italy.
11. Premio Progetto Giovani, Società Italiana di Neurologia (SIN) 2019, Bologna, Italy.

Scientific collaborations (2016-2019)

1. **Scientific Panel of Neurophysiology Residents and Research fellows (RRFS) Representative** of European Academy of Neurology (EAN), September 2019 – to date.

2. Member of the European Academy of Neurology (EAN) Scientific Panel Individual Membership in **Clinical Neurophysiology**, January 2019 – to date.
3. Member of the European Academy of Neurology (EAN) Scientific Panel Individual Membership in **Movement disorders**, January 2019 – to date.
4. Belonged to the **Editorial Board for Abstracts** of the 5th Congress of the European Academy of Neurology (EAN), Oslo, Norway, 2019.
5. Clinical and research training on Deep Brain Stimulation, Ospedale Molinette (Prof. L. Lopiano and Prof. M.G. Rizzone), Torino, Italy, from October 2017 to March 2018.