Università degli Studi di Cagliari

DOTTORATO DI RICERCA

In Neuroscienze

Ciclo XXXI

TITOLO TESI

Polysomnographic markers of REM sleep behavior disorder in Parkinson's Disease: methodological issues, diagnostic accuracy and progression over time

Settore/i scientifico disciplinari di afferenza

Area 06 – Scienze Mediche MED/26 Neurologia

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Esame finale anno accademico 2017 – 2018

Tesi discussa nella sessione d'esame Gennaio-Febbraio 2019

Université d'Auvergne **Università degli Studi di Cagliari**

Academic year:

N° d'ordre 5-DOC XXXI Ciclo

2017-2018

ECOLE DOCTORALE DES SCIENCES DE LA VIE, SANTÉ, AGRONOMIE, ENVIRONNEMENT

CORSO DI DOTTORATO IN NEUROSCIENZE

INTERNATIONAL CO-TUTORING THESIS

POLYSOMNOGRAPHIC MARKERS OF REM SLEEP BEHAVIOR DISORDER IN PARKINSON'S DISEASE: METHODOLOGICAL ISSUES, DIAGNOSTIC ACCURACY AND PROGRESSION OVER TIME

Presented at the University of Cagliari

In order to obtain the grade of PhD

Specialty: Neurosciences

Disserted by

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SUMMARY

Up to 60% of Parkinson's disease (PD) patients have REM sleep behavior disorder (RBD),[1] a parasomnia characterized by a loss of REM sleep muscle atonia and dream-enacting behaviors, usually associated to vivid dreams.[2,3] REM sleep without atonia (RSWA), characterized by a sustained tonic and/or phasic muscle activity during REM sleep, is the polysomnographic hallmark of RBD. PD patients with RBD (PDRBD+) are more severely impaired in both motor[4–6] and non-motor domains,[7–13] compared to those without RBD,[7,8,14–16] and they have an increased risk of dementia.[12,17] Thus, RBD may be a biomarker of more widespread/malignant phenotype and correct identification of RBD in PD may bear clinical, therapeutic, and prognostic implications. However, RBD diagnostic criteria have been defined and screening tools have been developed mainly based on idiopathic RBD population. Moreover, little is known about the evolution of both clinical and video-polysomnographic (vPSG) measures of RBD in relationships with the progression of motor and non-motor symptoms of PD. Actually, RBD may precede, concurs or follow the onset of PD by many years, but an improvement of RBD symptoms is also occasionally reported in PD patients over time. Longitudinal assessment of RBD performed by questionnaire in PD population has led to controversial results and, so far, only one vPSG study has been performed in patients with PDRBD+. In this thesis, we first aimed to assess the concordance of two visual scoring method for RSWA, namely the Montreal and the SINBAR, and to compare the two methods with an automated scoring method, in a large cohort of patients with PD consecutively seen at Movement Disorder Centers. Then, in a second study, we aimed to ascertain whether current diagnostic criteria for RBD, mainly developed based on idiopathic RBD, are appropriate to diagnose RBD in PD patients and to assess the sensitivity and specificity of the two most used RBD screening questionnaires, namely the RBDSQ and the RBD1Q. Finally, in the third study, we sought to longitudinally evaluate clinical and neurophysiological features of RBD at the end of a 3-years follow-up including RSWA, and to assess

the relationship between the evolution of RSWA and the progression of symptoms in a large cohort of PD patients with RBD, in order to ascertain whether RBD represents a stable marker in PD. Assessing the appropriateness of screening and diagnostic criteria and elucidating the time course of RBD in PD would be crucial to determine the usefulness of this marker in view of future neuroprotective and disease modifying trials.

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ACKNOWLEDGEMENTS

First, I would like to thank Dr. Monica Puligheddu, who allow me both to cultivate my passion for sleep medicine and to become an active actor within her research team. Moreover, I would like to thank her for encouraging me to take up a professional trajectory oriented towards clinical research. Without her there would have been none of the steps that led me to undertake this research project. I would like to thank her for always encouraging and supporting me in the numerous and enriching experiences abroad that I have faced during my training course, among which the precious collaboration with Professor Maria Livia Fantini.

Equally, I would like to thank Professor Maria Livia Fantini, who welcomed me in the research group “EA7280 - Laboratory of Neuropsychopharmacology of Subcortical Dopaminergic Systems” at the University of Clermont-Auvergne leads by Professor Franck Durif, offering me the opportunity and the resources to do several research projects. I would thank her kind willingness and patience she had in these years guiding me in my research training course, teaching me the thoroughness and transmitting me the passion for sleep medicine. Also, I would thank her authentic support, her precious advices and for the countless opportunities of exchanging in discussions and continuous stimulations. Her encouragement and advice are and will always be valuable to me.

I must express my gratitude to Professor Franck Durif, who also welcomed me into his research group, allowing me to do the research projects of the present thesis. I would like to thank him for the sustenance and advice.

And to Dr. Ana Marques who friendly welcomed me in the Neurology Service of the University Hospital of Clermont-Ferrand.

Thanks to all the staff of the Sleep Center of the University of Cagliari, colleagues and technicians, for having accompanied me in this intense adventure.

And to all the staff of the EEG Units of the University Hospital of Clermont-Ferrand, all the secretaries, technicians and colleagues for the teamwork.

Thanks to M.me Tiphaine Vidal e a M.me Laure Delaby for their exceptional work on neuropsychological testing.

An endless thanks to Massimo, my beloved husband, for encouraging me in all my pilgrimages, for supporting me always and in every way, and for encouraging me every day to follow my passions. I will never thank him enough for his patience, his optimism and his practical side, more than once he has helped me to face moments of discouragement or uncertainty.

And to my parents, that have never stopped to teach me how important is curiosity, dedication and passion.

Finally, thanks to all the participant to the study, without whom all this research would have never been possible.

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ABBREVIATIONS

AASM: American Academy of Sleep Medicine

DBS: Deep Brain Stimulation

HADS: Hospital Anxiety and Depression Rating Scale

ICSD: International Classification of Sleep Disorders

LARS: Lille Apathy Rating Scale

N1: Non-REM sleep stage 1

N2: Non-REM sleep stage 2

N3: Non-REM sleep stage 3 and 4

PD: Parkinson's Disease

RBD: REM Sleep Behavior Disorders

RBDSQ: RBD screening questionnaire

RBD1Q: RBD single question screen

REM: Rapid eye movements

SE: sleep efficiency

SL: sleep time

TBT: total bed time

TST: total sleep time

UPDRS: Unified Parkinson's Disease Rating Scale

vPSG: video-polysomnography

INTRODUCTION

Parkinson's Disease

Parkinson's Disease (PD) is a neurogenerative disorder involving the central nervous system, firstly described in the 19th century by James Parkinson and later refined by Jean-Martin Charcot.[18] PD is the second commonest neurodegenerative disease, outreached only by Alzheimer's Disease (AD).[19] Its estimated prevalence in western countries is about 0.3/1000 in general population, but its frequency increases nearly exponentially with age, achieving 9.5/1000 in people over the age of 65. [20] However, up to 10% of patients have young-onset PD in which symptoms begin before the age of 40 years old. Parkinson's disease affects both sexes with only a slight predominance among males.

The core pathological finding of PD is the early prominent death of dopaminergic neurons in the substantia nigra pars compacts (SNpc), resulting in dopaminergic deficiency within the basal ganglia and typical parkinsonian motor symptoms as a consequence. However, PD is also associated with numerous non-motor symptoms, some of which may precede the motor dysfunction by more than a decade, suggesting neurodegeneration in neurotransmitter networks other than dopamine and outside the basal ganglia.[19] Thus, PD is increasingly recognized as a multifaceted slowly progressive neurodegenerative disorder that begins years before the onset of parkinsonian motor symptoms, involving multiple neuroanatomical areas of central, autonomic and peripheral nervous system.

The pathophysiology of PD is still not entirely elucidated. The pathological hallmark of PD is loss of dopaminergic neurons in the SNpc, more precisely in the ventrolateral tier in which there are projections to the dorsal putamen and striatum, but also in the ventral tegmental area (VTA). Thus,

dysfunction in both dopaminergic nigro-striatal pathways from SNpc, as well as in meso-limbic circuits, originating from VTA, leads to an imbalance of cortical and subcortical loops implicated in motor control, and also in modulation of emotion and cognition. Additionally, neuronal loss in PD occurs in many other areas, namely the locus coeruleus, nucleus basalis of Meynert, pedunculopontine nucleus, raphe nucleus, dorsal motor nucleus of the vagus nerve, amygdala and hypothalamus, and involves neurotransmitter networks other than dopaminergic.[21] Another peculiar features of PD pathology is the aggregation of abnormally folded alpha-synuclein within the cell body and processes of neurons, namely the Lewy bodies.[22] Additionally, the accumulation of Lewy bodies is not merely restricted to the brain but Lewy body inclusions can be found in the spinal cord and in the peripheral nervous system, like Vagus nerve, sympathetic ganglia, cardiac plexus, enteric nervous system, salivary glands, adrenal medulla, cutaneous nerves, and sciatic nerve.[23,24]

Neurodegenerative process has been hypothesized to progress in a peculiar manner over the course of PD. Braak and colleagues[25] have suggested six stages of neurodegeneration, beginning in the peripheral nervous system and progressively affecting the central nervous system in a caudal-to-rostral direction within the brain (Table 1). The Braak model attempts to explain spatial and temporal progression of neurodegenerative process and consequentially natural history of the clinical course of Parkinson's Disease. Thus, stage 1 and stage 2 could parallel onset of premotor symptoms, stage 3 could represent onset of motor features suggesting depletion of dopaminergic neurons within nigrostriatal circuits, stage 4 to 6 might represent advanced stage of disease.

Recently Postuma and Berg have identified three stages of PD progression: preclinical, prodromal and clinical, suggesting markers for each stage (Figure 1).[26] In the preclinical phase, the neurodegenerative process has started, but symptoms, both motor and non-motor, are absents. Important to note that the stage of preclinical PD implies neurodegeration and does not refer to

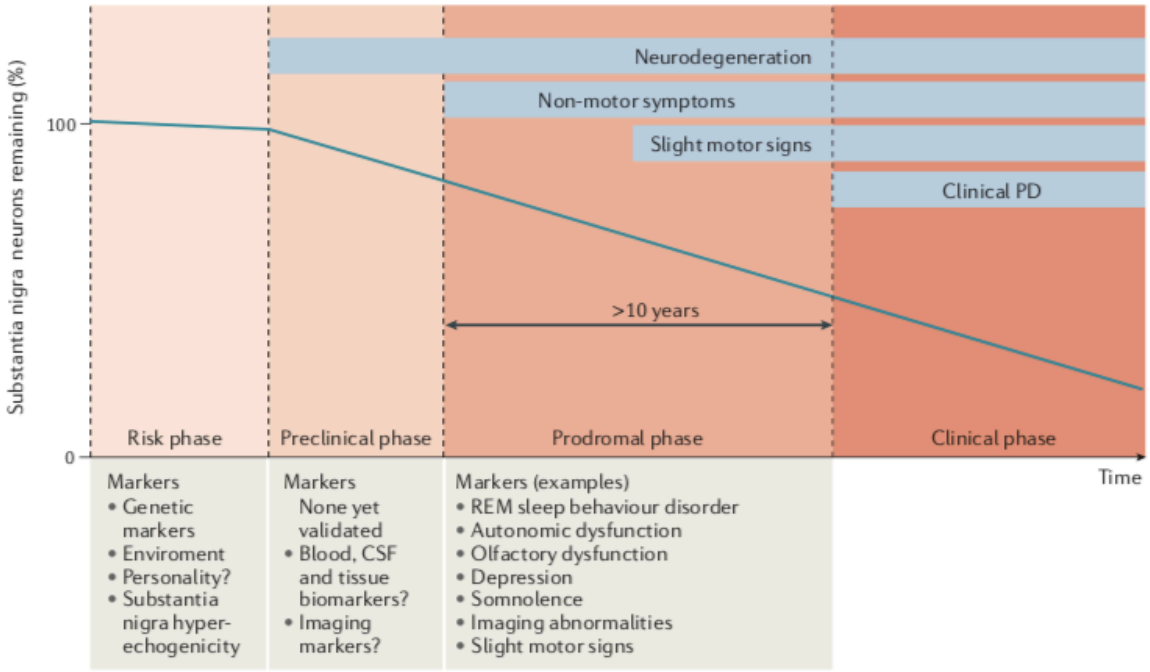
simply being at risk of PD. Diagnosis of preclinical PD remains speculative due to the lack of validated biomarkers, such as blood, cerebrospinal fluid or neuroimaging markers. In prodromal PD, clinical symptoms or signs of neurodegeneration are evident, with predominance of non-motor symptoms and only slight motor signs, but the full-blown diagnosis of PD cannot yet be made. Prodromal biomarkers that have identified as being predictive of PD in at least two prospective studies include REM sleep Behavior Disorder (RBD), autonomic dysfunction (such as constipation, orthostatic hypotension, urinary dysfunction and sexual dysfunction), olfactory loss, depression, somnolence, imaging abnormalities and slight motor signs.

Thus, prodromal stage implies presence of neurodegeneration outside the substantia nigra or event outside the brain at least 10 years before clinical PD could be diagnosed. Conversely, clinical PD implies a reduction by up to 80% of dopaminergic cells in substantia nigra that leads to the presence of full parkinsonism, with progressive bradykinesia, plus either rest tremor and/or rigidity.[26]

Table 1. The Braak Model of Lewy body pathology in Parkinson's Disease.

Brake stage	Anatomy (Lewy Body)	Symptoms
Stage 1	Peripheral nervous system (autonomic neurons)	Autonomic dysfunction
	olfactory system	Hyposmia or anosmia
	medulla (dorsal motor nuclei of vagal and glossopharyngeal nerves)	Dysautonomia, gastrointestinal disturbances, constipation, glossopharyngeal control deficit
Stage 2	Pons (locus ceruleus, magnocellular portions of reticular formation, posterior raphe nuclei, spinal cord grey matter)	Sleep disturbances (including RBD), anxiety
	Hypothalamus (orexin)	Sleep disturbances
	Mesolimbic DA system and nigrostriatal DA system	Depression, anhedonia, apathy, anxiety, hypomimia, impaired movement motivation
Stage 3	Pons (pedunculopontine nucleus), midbrain (SNpc), basal forebrain (magnocellular nuclei), limbic system (central subnucleus of amygdala)	Progressive bradykinesia, akinesia, stiffness, tremor, postural instability Dysfunction in meso-cortico-limbic pathway
Stage 4	Limbic system, Thalamus, temporal cortex (anteromedial temporal mesocortex, CA2 region of hippocampus)	Progressive in motor and non-motor disability
Stage 5 and 6	Multiple cortical regions (insular, associative areas, primary cortical areas)	Cognitive and memory alteration, motivational, sensory and motor deficits

Figure 1. Stage and markers in Parkinson's Disease (From Postuma et al. 2016)



Clinical features

In the past years, Parkinson's disease has been regarded as a mere movement disorder. Currently, it is increasingly recognized as a multidimensional neurodegenerative disease characterized by motor and non-motor symptoms, among which there are sleep disorders, autonomic dysfunction, cognitive and neuropsychiatric features.

a. Motor symptoms

The cardinal motor features of Parkinson's Disease are bradykinesia, in combination with either rest tremor and/or rigidity.[27]

Bradykinesia is defined as slowness of movement together with decreased amplitude and/or speed during motion. Bradykinesia can be assessed by finger tapping, alternating hand movements, pronation-supination movements and foot tapping. Bradykinesia may affect various aspect of movements, like voice, face, axial and gait, but limb bradykinesia alone must be observed to diagnose PD.

Rigidity in PD is defined as lead-pipe, velocity-independent, resistance to passive movement not only reflecting inability to relax. The cogwheel phenomenon is often present, but alone does not met minimum criteria for parkinsonian rigidity.

Resting tremor is characterized by frequency from 4 to 6 Hz, in fully resting limbs, and is suppressed during movement initiation. A parkinsonian resting tremor may recur after prolonged posture, as re-emergent tremor.

Other motor sign and symptoms are postural and gait disturbances, like camptocormia and festination, speech and deglutition impairment, hypomimia and micrography.

Patients with Parkinson's disease may experience various motor complications as the disease progress and with the chronic use of dopamine replacement therapy (DRT), namely freezing of gait, dyskinesias [28], motor and non-motor fluctuations, wearing-off phenomenon, morning akinesia, on-off phenomenon [29] and dystonia [30].

b. Non-motor symptoms

Several non-motor symptoms that may appear in all stage of disease and may even precede by many years the onset of motor-symptoms.[31] Non-motor symptoms include sensory alterations, autonomic dysfunctions, vision impairment, sleep disorders, cognitive impairment and neuropsychiatric features.

Sensory alterations include hyposmia, pain and paresthesia.[32] Autonomic dysfunction encompasses constipation, orthostatic hypotension, excessive sweating, urinal and sexual problems. Vision impairment includes alteration in contrast sensitivity, color discrimination, visual processing speed.[33] Sleep problems comprise insomnia, sleep fragmentation, excessive daytime sleepiness, obstructive sleep apnea syndrome (OSAS), restless legs syndrome (RLS) and REM sleep behavior Disorders (RBD).[34,35]

Cognitive impairment in PD may interest executive and visuospatial, memory, attention and language functions. [36] Dementia occur late in disease natural history, appearing in almost 83% in patients after 20 years of disease.

Neuropsychiatric features are various and encompass symptoms like apathy, depression, anxiety, hallucination, psychosis and impulse-control disorders.[36,37]

Non-motor symptoms are very common in early stage of PD and they impair health-related quality of life.[38–40] Moreover, non-motor features seems to appear before the onset of motor symptoms identifying a prodromal phase of disease.[41]

Moreover, progression of Parkinson's disease also involves non-motor symptoms which impact severely quality of life of PD patients with an adverse effect on institutionalization rates and burden of disease.[42–44]

c. Sleep disorders in PD

Sleep disorders are among the most common non-motor symptoms in Parkinson's Disease, affecting up to 90% of patients, and may negatively impact the quality of life of patients and their co-sleepers. Usually, sleep problems in PD increase in frequency over the course of PD and disability progression.

The most frequent reported sleep disorders include insomnia, sleep fragmentation, excessive daytime sleepiness, restless legs syndrome (RLS), and REM-sleep behavior disorder (RBD).[34,35]

Patients with Parkinson's Disease may complain of frequent nighttime awakenings and sleep fragmentation, resulting in insomnia, reduction of total sleep time, daytime fatigue and sleepiness.[35,45] Pathophysiology of sleep disturbances in PD patients is multifactorial. Indeed, neurodegenerative process can affect also brain areas involved in sleep-wake regulation, and in the circadian system. Also, other causes of sleep disruptions may be concomitant motor and non-motor symptoms (specially rigidity, difficulties in changing position in bed, mood disorder and autonomic dysfunction), DRT and other pharmacological therapies (e.g. antidepressants), advanced age, and comorbidities.[34,35,44,45,46] All these sleep disturbances may overlap in the same patient, making the management of sleep problems in PD patients someway difficult.

For the purpose of this thesis, RBD will be developed later and will be the object of a separate paragraph.

PD diagnosis: the new diagnostic criteria

Recently the Movement Disorders Society (MDS) published the new diagnostic criteria for Parkinson's disease, in order to update diagnosis process and stress the notion of the disease as multifaceted combination of motor and non-motor features.[27,47]

The previous most widely used diagnostic criteria have been published by the UK Brain Bank about 30 years ago, and they were designed to be used in pathologic series, but have been adapted by the community.[48] The UK brain bank criteria diagnosed parkinsonian syndrome by the presence of bradykinesia, at al least one of the following: muscular rigidity, 4-6Hz rest tremor, postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction. These criteria included a list of 16 exclusion features, all of which rule out the diagnosis, namely history of repeated stroke with stepwise progression of parkinsonian features, history of repeated head injury, history of definite encephalitis, oculogyric crises, neuroleptic treatment at onset of symptoms, more than one relative affected, sustained remission, strictly unilateral features after 3 years, supranuclear gaze palsy, cerebellar signs, early severe autonomic involvement, early severe dementia, Babinski sign, presence of cerebral tumor or communication hydrocephalus on neuroimaging, negative response to large doses of levodopa in absence of malabsorption, MPTP exposure. Moreover, a list of supportive features is listed, like unilateral onset, rest tremor, progressive disorder, persistent asymmetry affecting side of onset most, excellent response to levodopa, severe levodopa-induced dyskinesia, levodopa response for 5 years or more, clinical course of ten years or more. According to the UK brain bank criteria for PD, probable PD can be defined by the absence of exclusion features, while clinically definite PD is determined by

combination of 3 supportive and 0 exclusion features. The UK brain bank criteria has shown high positive predictive value with 99% patients clinically diagnosed that had pathologic confirmation.[49]

According to the newest diagnostic criteria published by MDS in 2015, the first essential feature of PD is the presence of parkinsonism which is defined as bradykinesia, in combination with at least one among rest tremor or rigidity.[27] Once parkinsonism has been diagnosed:

1. Diagnosis of clinically established PD requires (all of the following):
    a. Absence of absolute exclusion criteria
    b. At least two supportive criteria
    c. No red flags
2. Diagnosis of clinically probable PD requires (all of the following):
    a. Absence of absolute exclusion criteria
    b. Presence of red flags counterbalanced by supportive criteria
        i. If one red flag is present, there must also be at least one supportive criterion
        ii. If two red flags, at least two supportive criteria are needed
        iii. No more than two red flags are allowed for this category

Supportive criteria

1. Clear and dramatic beneficial response to dopaminergic therapy. During initial treatment patient returned to normal or near-normal level of function. In the absence of clear documentation of initial response, a dramatic response can be classified as:
    a. Marked improvement with dose increases or marked worsening with dose decreased. Mild changes do not qualify. Document this either objectively (>30% in UPDRS III with change in treatment) or subjectively (clearly documented history of marked changes from a reliable patient or caregiver)

b. Unequivocal and marked on/off fluctuations, which must have at some point included predictable end-of-dose wearing off

2. Presence of levodopa-induced dyskinesia
3. Rest tremor of a limb, documented on clinical examination (in past, or on current exam)
4. The presence of either olfactory loss or cardiac sympathetic denervation on MIBG scintigraphy

<u>Absolute exclusion criteria (the presence of any of these features rules out PD):</u>

1. Unequivocal cerebellar abnormalities, such as cerebellar gait, limb ataxia, or cerebellar oculomotor abnormalities (e.g., sustained gaze-evoked nystagmus, macro square wave jerks, hypermetric saccades)
2. Downward vertical supranuclear gaze palsy or selective slowing of downward vertical saccades
3. Diagnosis of probable behavioral variant frontotemporal dementia or primary progressive aphasia defined according to consensus criteria, within the first 5 years of disease
4. Parkinsonian features restricted to the lower limbs for more than 3 years
5. Treatment with a dopamine receptor blocker or a dopamine-depleting agent in a dose and time-course consistent with drug-induced parkinsonism
6. Absence of observable response to high-dose levodopa despite at least moderate severity of disease
7. Unequivocal cortical sensory loss (e.g., graphesthesia, stereognosis with intact primary sensory modalities), clear limb ideomotor apraxia, or progressive aphasia
8. Normal functional neuroimaging of the presynaptic dopaminergic system

9. Documentation of an alternative condition known to produce parkinsonism and plausible connected to the patient's symptoms, or, the expert evaluating physician, based upon the full diagnostic assessment feels than an alternative syndrome is more likely than PD

RED flags:

1. Rapid progression of gait impairment requiring regular use of wheelchair within 5 years of onset
2. A complete absence of progression of motor symptoms or signs over 5 or more years unless stability is related to treatment
3. Early bulbar dysfunction: severe dysphonia/dysarthria (speech unintelligible most of the time) and/or severe dysphagia (requiring soft food, NG tube or gastronomy feeding) within first 5 years of disease
4. Inspiratory respiratory dysfunction: either diurnal or nocturnal inspiratory stridor and/or frequent inspiratory sighs
5. Severe autonomic failure in the first 5 years of disease, including:
    a. Orthostatic hypotension: orthostatic decrease of blood pressure within 3 minutes of standing by at least 30 mmHg systolic or 15 mmHg diastolic, in the absence of dehydration, medication, or other disease that could plausibly explain autonomic dysfunction, or
    b. Severe urinary retention or urinary incontinence in the first 5 years of disease (excluding long-standing or small amount stress incontinence in women), that is not simply functional incontinence. In men, urinary retention must not be due to prostate disease, and must be associated with erectile dysfunction
6. Recurrent (>1/year) falls due to impaired balance within 3 years of onset

7. Disproportionate anterocollis (dystonic) and/or contractures of hand or feet within the first 10 years
8. Absence of any of the common nonmotor features of disease despite 5 years disease duration. These include sleep dysfunction (sleep-maintenance insomnia, excessive daytime sleepiness, RBD), autonomic dysfunction (constipation, daytime urinary urgency, orthostatic hypotension), hyposmia, or psychiatric symptoms (depression, anxiety, or hallucinations)
9. Otherwise unexplained pyramidal tract signs, defined as pyramidal weakness and/or clear pathologic hyperreflexia (excluding mild reflex asymmetry and isolated extensor plantar response)
10. Bilateral symmetric parkinsonism. The patient or caregiver reports bilateral symptom onset with no side predominance, and no side predominance is observed on objective examination.

These diagnostic criteria are intended for use in clinical research but also, they might be used to guide clinical diagnosis.

Additionally, the MDS have published diagnostic criteria for Prodromal PD, proposed as research criteria only, since identification of prodromal PD is currently of uncertain clinical benefit as neuroprotective therapy is not available yet. [50] However, subjects with prodromal PD might represent ideal candidates for future disease-modifying and neuroprotective therapy trials. The diagnosis of prodromal PD is based upon probability, by means of high likelihood (≥80%) that prodromal PD is present. More precisely, the likelihood ratios (LRs) signifies the strength of a diagnostic test, with positive LRs (LRs+) signifying an increased disease probability and LRs negative (LRs-) a decreased probability.

There are now many markers that indicate prodromal stage and many of these are non-motor marker, according to the hypothesis that neurodegeneration of substantia nigra occur relatively late in disease process.[25] However, most of the prodromal markers are not so specific.[47] Prodromal markers for PD identified by the task force of the MDS are PSG-diagnosed RBD or probable RBD based on expert interview; Dopaminergic PET/SPECT clearly abnormal (e.g., <65% normal, 2 SD below mean); possible subthreshold parkinsonism (UPDRS >3), excluding action tremor, or abnormal quantitative motor testing; olfactory loss; constipation; excessive daytime somnolence; symptomatic hypotension; severe erectile dysfunction; urinary dysfunction; depression and/or anxiety.

Among them, RBD, confirmed by PSG, has been found to be the most promising prodromal markers, with a likelihood ratio of 130 based upon the relative risk as well as the prevalence of the risk factor. However, there are limited number of prospective studies that have analyzed these markers before the onset of PD, and it may be impossible to determine whether markers are really independent. Another caveat is that the duration of prodromal PD is unknown. Some studies in RBD cohorts have shown a prodromal duration of 20 years.[51,52]

Further studies are needed to estimate the precise predictive value of each markers. Moreover, the speed of progression from prodromal to full clinical PD differs among patients and cannot be consistently predicted on the individual level.

REM sleep behavior disorder

REM Sleep Behavior Disorder (RBD) was first identified and described in 1986 by Schenck[3], and it is a parasomnia characterized by partial or complete loss of normal muscle atonia during REM sleep, associated with vivid dreams and dream enactment behaviors.[1] Patients with RBD seem to "act out their dreams"[53] and they often report an altered dream mentation with a dream content involving defensive-aggressive themes. REM sleep without atonia (RSWA), characterized by a sustained tonic and/or phasic muscle activity during REM sleep, is the polysomnographic hallmark of RBD.

The prevalence rate of RBD in the general population is estimated to be between 0,30 and 0,5%[1,54], but it increases in individuals with neurodegenerative disorders, especially synucleinopathies, such as Parkinson's disease (PD), multiple system atrophy (MSA) and dementia with Lewy bodies (DLB)[11,55–58]. Several studies have shown that RBD represent an early marker of alpha-synucleinopathy.[52,55,59,60] In fact, up to 90% of idiopathic RBD (I-RBD), occurring isolated, will develop Parkinson's disease (PD), Multiple System Atrophy (MSA), or Dementia with Lewy Body (DLB) within 14 years from RBD onset.[52]

Pathophysiology

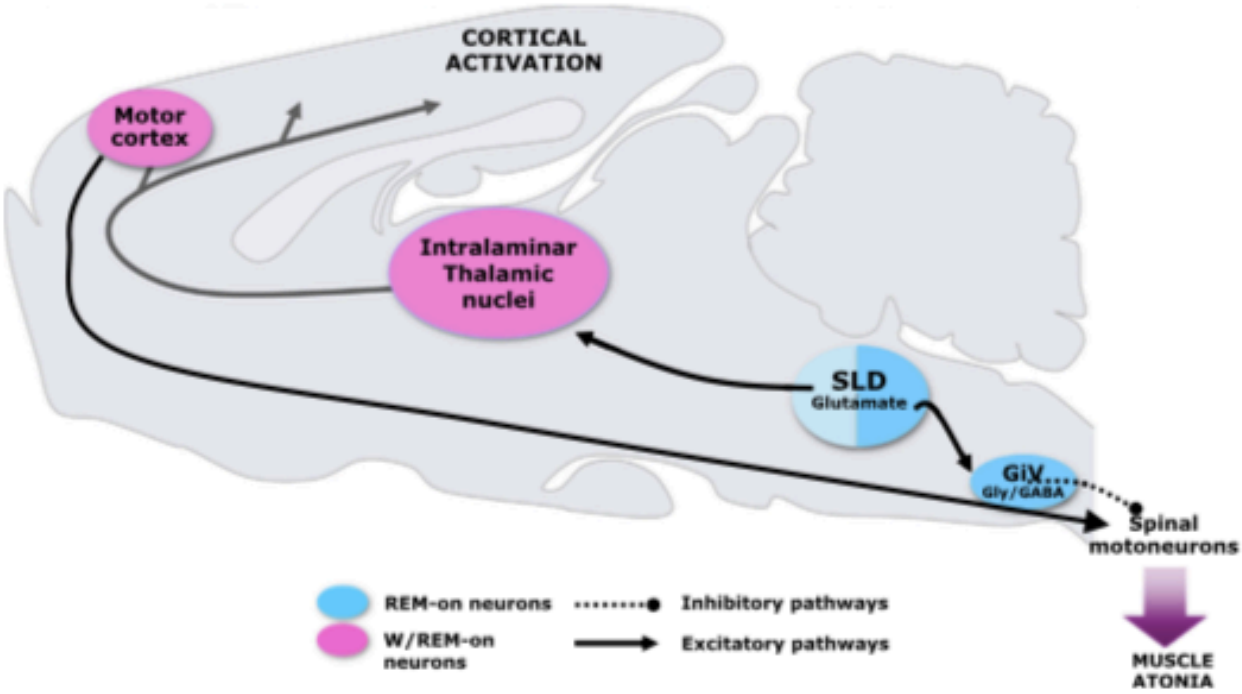
The pathophysiology of RBD is not completely understood but is supposed to be related to a dysfunction within the brainstem neuronal networks involved in modulation of REM sleep and motor control during REM sleep. The neuronal circuits underlying REM sleep physiology include the ventral mesopontine junction, the pedunculopontine nucleus, the laterodorsal tegmental nucleus, the locus coeruleus, and the peri-LC alpha area in the pons, magnocellular, gigantocellular and paramedian nuclei in the medulla.

In rat, during physiological REM sleep, it has been demonstrated that the REM-on glutamatergic neurons of the sublaterodorsal tegmental nucleus (SLD) excite the REM-on gamma-aminobutyric acid (GABA)/glycinergic neurons in the ventral medullary reticular formation, namely the raphe magnus (RMg), the ventral (GiV) and alpha gigantocellular (Gia) reticular nuclei.[61] The latter hyperpolarize the cranial and spinal motor neuron leading to muscle atonia. Moreover, SLD glutamatergic neurons excite the spinal inter-neurons that inhibit directly the motor neurons. [62,63] Concurrently, REM-on glutamatergic neurons of the SLD also send excitatory projections towards intralaminar thalamocortical neurons, which in turn activate the cortex.[61]

In RBD, the loss of muscle atonia is supposed to be due to dysfunction and/or degeneration of the descending SLD glutamatergic pathway and/or the GiV GABA/glycinergic neurons.

Figure 2 summarizes networks responsible for REM sleep in rats and its potential dysfunction during RBD.

Fig.2 REM sleep networks and RBD. (From PH Luppi et al. Sleep Medicine 2013)



Nevertheless, the phasic component of REM motor activity is far from being utterly elucidated. Both animal and human studies have demonstrated phasic activations of peduncolo-pontine tegmental nucleus (PPT) and SLD in synchronization of phasic muscular activity during REM sleep. [64,65] Moreover, phasic EMG activity during normal REM sleep is mediated by glutamatergic neurons of the red nucleus which in turn activate the spinal motor neurons, resulting in phasic muscular twitches.[66] This physiologic phasic muscular activity is mitigated by the inhibition by ventral medial medulla of both red nucleus and spinal motor neurons.[61,66] It has been proposed that phasic EMG activity during is responsible of muscle twitches occurring during REM sleep.[61]

Additionally, motor activity during REM sleep is modulated also by periacqueductal gray nucleus, locus coeruleus, dorsal raphe, SN, lateral hypothalamus, thalamus, and cortex. [67,68]

Thus, the intense motor activity typical of RBD might be related to an imbalance between excitatory glutamatergic projections from motor cortex neurons to spinal and cranial motor neurons and inhibitory brainstem degenerated circuits.[61]

Pathological data on RBD are limited and most derived from RBD associated with neurodegenerative disease studies. However, lesions of brainstem (i.e., vascular, neoplastic, infective, inflammatory etc.) are associated with emergence of RBD.[69] Neuroimaging studies have shown dysfunction within neuronal circuits of brainstem implicated in modulation of REM sleep in RBD patients. [70,71] Recently, functional neuroimaging studies have demonstrated a correlation between neuronal loos into the sub-coeruleus complex and the REM sleep without atonia, either in patients with idiopathic RBD and RBD associated with PD. [70,72] Other studies have shown an impairment of cholinergic networks in patients suffering from PD associated with RBD. [73–75] Moreover, several studies have demonstrated in idiopathic RBD patients abnormalities in basal

ganglia, like decreased expression of dopamine transporter and denervation of striatum, suggesting a neurodegenerative process within these areas. [76–78]

Furthermore, some cases of RBD has been associated with limbic encephalitis consisting of inflammatory lesions into medial temporal lobe and absence of lesions within the brainstem, suggesting that RBD might be related to a dysfunction into limbic system. [79,80] In fact, limbic system appears to be highly activated during REM sleep, perhaps modulating the emotive element of dream mentation.[81] Therefore, a disruption within limbic system might participate to the pathogenesis of RBD, perhaps in term of dream content, together with dysfunction of networks implicated in modulation on muscle tone during REM sleep.

Clinical features of RBD

The clinical hallmark of RBD is the presence of motor behavior frequently accompanied by vivid dreams. Patients seems to "act-out" their dreams in a various phenomenology. [53] Typically, motor behaviors are complex and violent, trying to enact unpleasant, action-filled and violent dreams in which patient is being confronted, attacked or chased. Semiology of motor behaviors are various in complexity, including punching, kicking, gesturing, reaching, grabbing, arm flapping, sitting up and jumping out of bed.[1,82] The more violent the RBD episodes, the more the risk of suffering injuries to the patients and the bed-partner. In fact, injuries are frequently reported, like ecchymosis, lacerations, bone fractures, concussion, and even subdural hematomas.[1]

However, some studies have reported non-violent behaviors during RBD like gesture of daily living, eating and smoking, picking apples, dancing, teaching, selling, thumbs up, kissing, clapping, sorting, acting sexual behaviors, urinating, scoring goal, bicycling, greeting, flying, getting dressed.[82–

[84] Besides violent and complex behavior, patients might experience also simple motor activity like jerks or grimaces.

In addition to motor behaviors, patients might display various vocalization like mumbling, talking, shouting, swearing, laughing, singing, whistling, crying.[1] Generally, patients could speak with appropriate prosody, fluency and syntax.[84]

Usually, patient with RBD seeks medical attention after sleep-related injuries occurred to either him/herself or bed-partner, or because bedpartner sleep disruption, rarely because of self-sleep disruption.

Diagnosis

According to the American Academy of Sleep Medicine diagnostic criteria for RBD published in 2014 in the International classification of sleep disorders-third edition (ICSD-3)[2,85], diagnosis of RBD requires:

1) presence of repeated episodes of sleep-related vocalization and/or complex motor behaviors;[a,b]

2) these behaviors are documented by vPSG to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep;

3) polysomnographic recording has to demonstrate REM sleep without atonia (RSWA);[c]

4) the disturbance is not better explained by another sleep disorder, mental disorder, medication or substance use.

The ICSD-3 has also provided 6 notes:

a. *This criterion can be filled by observation of repetitive episodes during a single night of video polysomnography.*

b. *The observed vocalizations or behaviors often correlate with simultaneously occurring dream mentation, leading to the frequent report of "acting out one's dreams."*

c. *As defined by the guidelines for scoring PSG features of RBD in the most recent version of the American Academy of Sleep Medicine (AASM) Manual for the Scoring of Sleep and Associated Events.*

d. *Upon awakening, the individual is typically awake, alert, coherent, and oriented.*

e. *On occasion, there may be patients with a typical clinical history of RBD with dream-enacting behaviors, who also exhibit typical RBD behaviors during vPSG, but do not demonstrate sufficient RSWA, based on the current evidence-based data, to satisfy the PSG criteria for diagnosing RBD. In such patients, RBD may be provisionally diagnosed, based on clinical judgment. The same rule applies when vPSG is not readily available.*

f. *Polysomnography demonstrates an excessive amount of sustained or intermittent loss of REM atonia and/or excessive phasic muscle twitch activity of the submental and/or limb EMGs during REM sleep. Some patients have exclusively arm and hand behaviors during REM sleep, indicating the need for both upper and lower extremity EMG monitoring in fully evaluating for RBD. Some patients preserve most of their REM atonia but have excessive EMG twitching during REM sleep. The most current evidence-based data for detecting RWA in the evaluation of RBD indicate that <u>any (tonic/phasic) chin EMG activity combined with bilateral phasic activity of the flexor digitorum superficialis muscles in >27% of REM sleep (scored in 30-second epochs)</u> reliably distinguishes RBD patients from controls.*

Thus, the presence of quantified RSWA is required for the diagnosis of RBD and the ICSD-3[2] has suggested for the first time a quantitative cut-off value, based on data published by the SINBAR group.[86–88]

Figure 3 and Figure 4 show respectively a normal REM sleep epoch and a REM sleep epoch with REM sleep without atonia.

Thus, although the clinical suspicion of RBD relies on history and clinical presentation, assessed by an in-deep semi-structured interview, vPSG is mandatory for the diagnosis of RBD, allowing to demonstrate motor behaviors during REM sleep, to assess RSWA, and to rule-out other potential differential diagnosis.

Indeed, other sleep disorders can mimic RBD in adult population, like NREM parasomnia (sleepwalking and sleep terrors), obstructive sleep apnea syndrome (OSAS), nocturnal seizures, rhythmic movement disorders, sleep related dissociative disorders, episodic nocturnal wandering, frightening hypnopompic hallucinations, post-traumatic stress disorders, and malingering.[1,2]

In RBD, during dream-enactment behaviors, patient has their eyes closed and quickly awaken after an episode. Conversely, in sleepwalking and other NREM sleep parasomnia, patients have their eyes opened, the episodes are not followed by rapid alertness, and rarely they are associated with vivid dream mentation. On the other hand, sleep-related seizures are usually characterized by repetitive and stereotyped behaviors. Finally, status dissociatus is characterized by a confusional state in which one is asleep, awake or dreaming, together with various motor behaviors, and by the inability to discern sleep stages on vPSG.

Additionally, a severe and vigorous Periodic Limb Movements during sleep (PLMS) might be followed by arousals associated to abnormal motor behaviors and unpleasant dreams mentation, mimicking RBD symptomatology.[89]

Likewise, severe obstructive sleep apnea might also induce arousals from REM sleep, with dream-related complex and violent behaviors, similar to those seen in RBD. On the other hand, treating OSAS with continuous positive airway pressure (CPAP) reduces those motor behaviors.[1,90] This OSAS-related RBD-like phenomenon has been called “pseudo-RBD”.[1,90]

Moreover, there are patients having a condition named “Parasomnia overlap syndrome” in which vPSG-documented NREM-REM sleep motor behaviors coexist, namely sleepwalking, sleep terrors, and RBD.

Figure 3. Epoch of normal REM sleep with preserved muscle atonia.

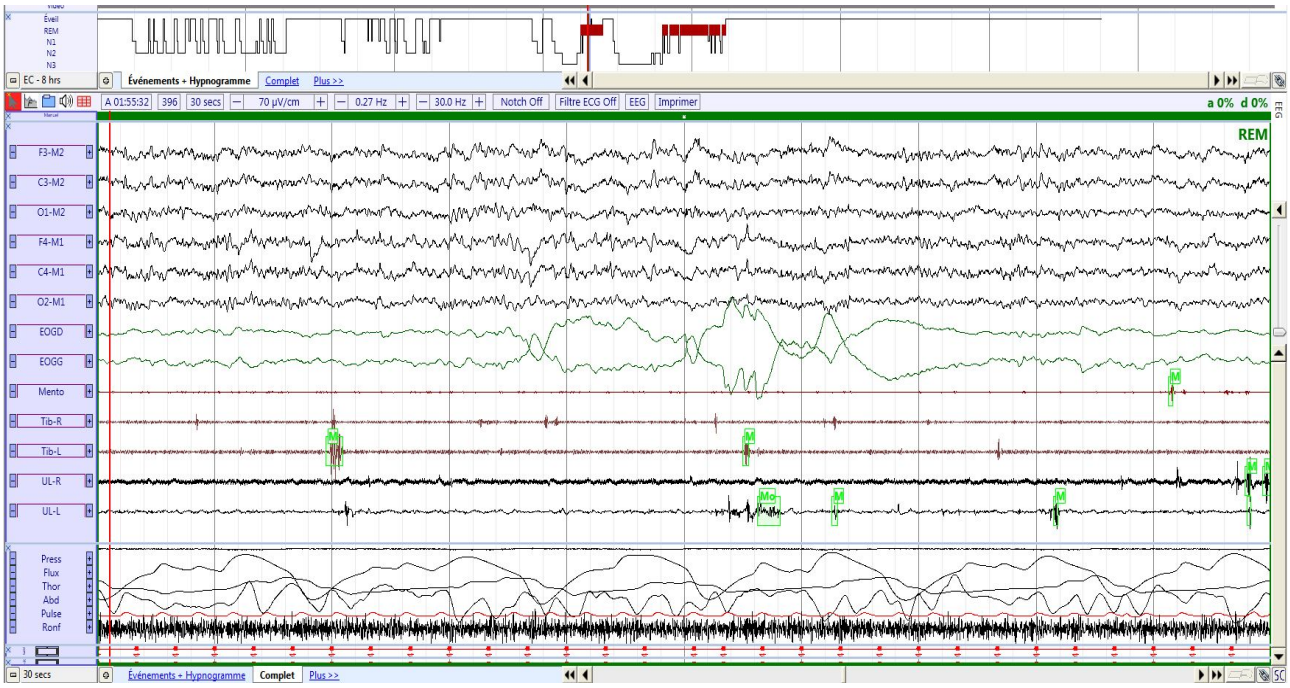
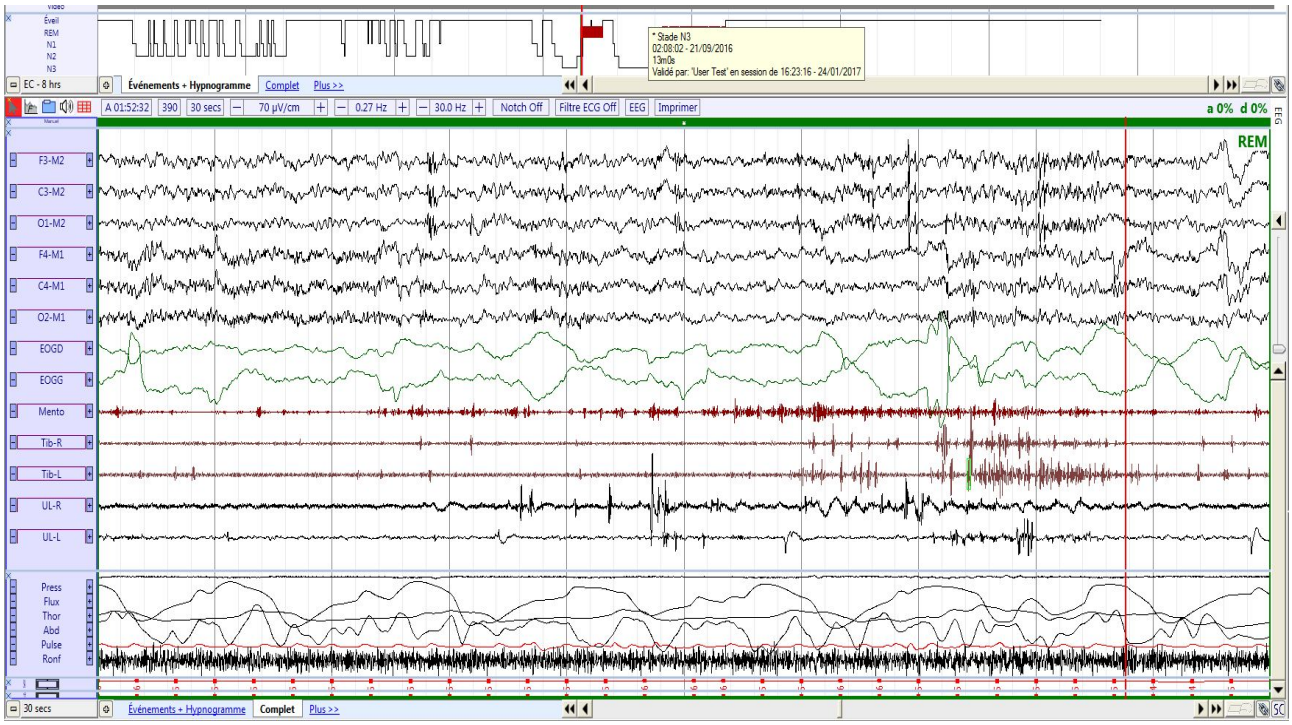


Figure 4. Epoch of REM sleep with REM sleep without Atonia.



RBD IN PARKINSON'S DISEASE

Up to 60% of PD patients suffer from RBD, that may precede, follow or being concomitant with the onset of parkinsonism.[1,3] At the present time, it is increasingly known that RBD in PD is a marker of a more widespread neurodegenerative process with a heavier disease burden in term of both motor and non-motor features.[7,8,10,16,57,91]

Indeed, RBD in PD has been associated with more rigid akinetic form, more axial symptoms, and increased risk of falls, more dopa-induced dyskinesia.[92,93,6] Postuma et colleagues have found that patients with PD and RBD showed less tremor dominant phenotype and a trend towards higher proportion of axial symptoms and freezing of gait, associated with a significant more frequency of falls.[6] Also, the same authors have shown that patients with PD and RBD demonstrated a lower amplitude in response to their dopamine-replacement therapy, perhaps due to presence of levodopa resistant symptoms, like axial symptoms and freezing.[6] Thus, the presence of peculiar motor subtype of PD associated with RBD might suggest a different underlying pattern of neurodegeneration in these patients compared to those without RBD. Sixel-Doring et al. have shown that PD associated with RBD is characterized by longer disease duration, higher Hoehn & Yahr stage, a higher frequency of falls and fluctuations, compared to PD without RBD.[93] Moreover, these authors have found that PD patients with RBD required higher doses of Levodopa compared to those without RBD.[93] They did not found a non-tremor predominant subtype of PD perhaps due to a longer disease duration of their population compared to other previously published study.[6,93]

Furthermore, PD patients with probable RBD could have a worse outcome to SNT-DBS, by means of less prominent improvement of overall motor performances and axial symptoms at 3 years follow-up.[4]

These patients could have also increased autonomic dysfunctions[94,95], in terms of orthostatic hypotension (OH) either assessed by questionnaires or quantitative measure, such as tilt tests or Orthostatic hypotension test. Orthostatic hypotension by tilt test was defined by a drop ≥ 20mmHg of systolic blood pressure and/or ≥10 mmHg of diastolic blood pressure during 60° tilt for 10 minutes. On the other hand, orthostatic hypotension might be confirmed comparing blood pressure and heart rate value in the supine position, after 5 minutes of lying, and after 1 minutes, 3 minutes after standing. In particular, OH is defined as a fall in systolic blood pressure of at least 20 mmHg and/or in diastolic blood pressure of at least 10 mmHg between supine and standing position.

PD patients with RBD also show impaired cortical activity. Indeed, Gagnon et al. have found higher theta power in frontal, parietal, temporal and occipital regions during wakefulness in non-demented PD patients with RBD in comparison to those without RBD and control subjects.[9] The authors suggested that RBD-associated EEG slowing might be correlated to an impairment of cortical activation that may lead to cognitive dysfunction in daytime performances.[9] Moreover, the EEG slowing have been related to RBD itself and not to progression of PD, indeed it was found also in idiopathic RBD patients.[96] Thus, the presences of EEG slowing in PD patients with RBD might represent an early sign of an evolution toward dementia, as the same pattern of EEG slowing has been reported in patients with Alzheimer disease, Dementia with Lewy bodies and PD-dementia.[9]

Additionally, PD patients with RBD have also an increased risk to develop cognitive deficits and dementia.[7,8,10,11,51] Neuropsychological abnormalities in Parkinson's Disease are thought to manifest predominantly with a frontal and subcortical cognitive syndrome, including impairment in executive function, working memory, attention, set-shifting, and visuo-spatial difficulties. Vendette et al. have shown that PD patients with RBD performed worse on episodic verbal memory, executive functions, visuospatial and visuo-perceptual functions, when compare to PD patients without RBD and control subjects, even after adjusting for several potentially confounding factors, such as age, educational

level, mental status, depression and sleep-related respiratory disorders.[97] Marques el al. have found an impairment in visual-perception function, in patients with PD and RBD, as previously found in idiopathic RBD population, suggesting an impairment in visual information processing by means dysfunction in ventral visual pathway.[8] The authors have also suggested the involvement of the perirhinal cortex (PRh), a cholinergic structure placed in the medial temporal lobe (MTL), that contributes to both object memory and perception. Thus, the visual-perception impairment found in PD patients with RBD might not be related to perceptual impairment alone but might also suggest dysfunction in retrieval processing of mental images. Interestingly, in this study, the authors did not find any differences between PD with RBD and idiopathic RBD, suggesting a non-dopaminergic pathophysiology for this visual disorder.

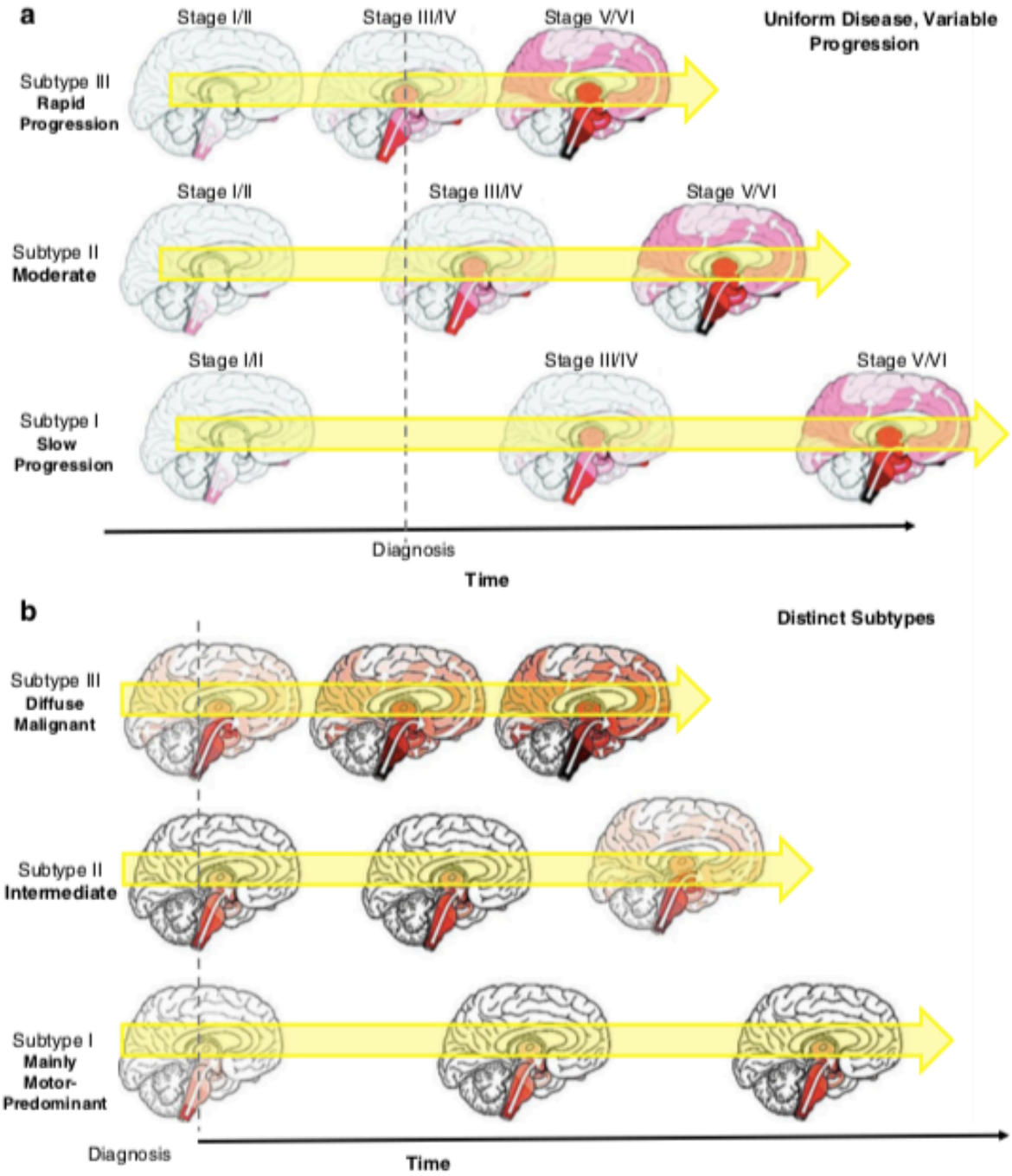
On the other hand, Kotagal et al. have demonstrated neocortical, limbic and thalamic cholinergic denervation in PD patients with probable RBD, assessed by screening questionnaire, suggesting that cholinergic network in the pontine tegmentum and basal forebrain complex might play a key role in the pathogenesis of RBD and may contribute to cognitive dysfunction.[73] Recently, both RBD and cognitive dysfunction have been correlated to thalamic and cortical cholinergic deficits in positron emission tomography studies.[98]

A prospective study has found higher prevalence of mild cognitive impairment (MCI) and dementia in PD patient with RBD, with 15% risk of dementia at 2 years, 29% at 3 years, and 45% at 4 years, compared to 0% risk for PD patients without RBD.[99] Recently, another prospective study have discovered 8 clinical predictors of dementia in PD patients, including age, male sex, baseline RBD, orthostatic hypotension and MCI.[100] In particular, the strongest determinant for dementia development was the co-existence of RBD, MCI and orthostatic hypotension.

Lately, Fantini et al. have demonstrated an increased risk to develop impulse-compulsive disorder in PDRBD+ patients compared to those without RBD, suggesting a dysfunction in the meso-cortico-limbic pathway.[13,101] The latter plays a central role in reward and impulse control and includes the ventral tegmental area (VTA), the ventral striatum, the amygdala, the hippocampus and the ventromedial and the orbito-frontal regions of the prefrontal cortex.[81]

Finally, a prospective cohort study has found that PD associated with RBD identified a diffuse/malignant phenotype, characterized by the presence of more severe motor and non-motor symptoms, namely orthostatic hypotension, multidomain mild cognitive impairment, and RBD at baseline.[17] More precisely, Fereshtehnejad et al. have assessed a comprehensive spectrum of motor and non-motor features, namely motor severity, motor complications, motor subtypes, quantitative motor tests, autonomic manifestation, psychiatric symptoms, olfaction, color vision, sleep parameters and neurocognitive profile, at baseline and after a mean follow-up time of 4.5 years.[17] According to cluster analysis, the authors have defined three subtypes of PD as mainly motor/slow progression, diffuse/malignant, and intermediate. The diffuse/malignant cluster was more likely to have mild cognitive impairment, orthostatic hypotension, and RBD at baseline, and showed a more rapid progression in cognitive dysfunction, in motor and non-motor symptoms worsening and in the global composite outcome. This peculiar phenotype has shown also more prominent non-psychiatric disorders and color discrimination disturbances, and an increased risk for dementia.[11,12,17,102] Figure 5 summarizes the two pathological theories of subtyping PD based on Braak staging and on newest cluster analysis aforementioned.

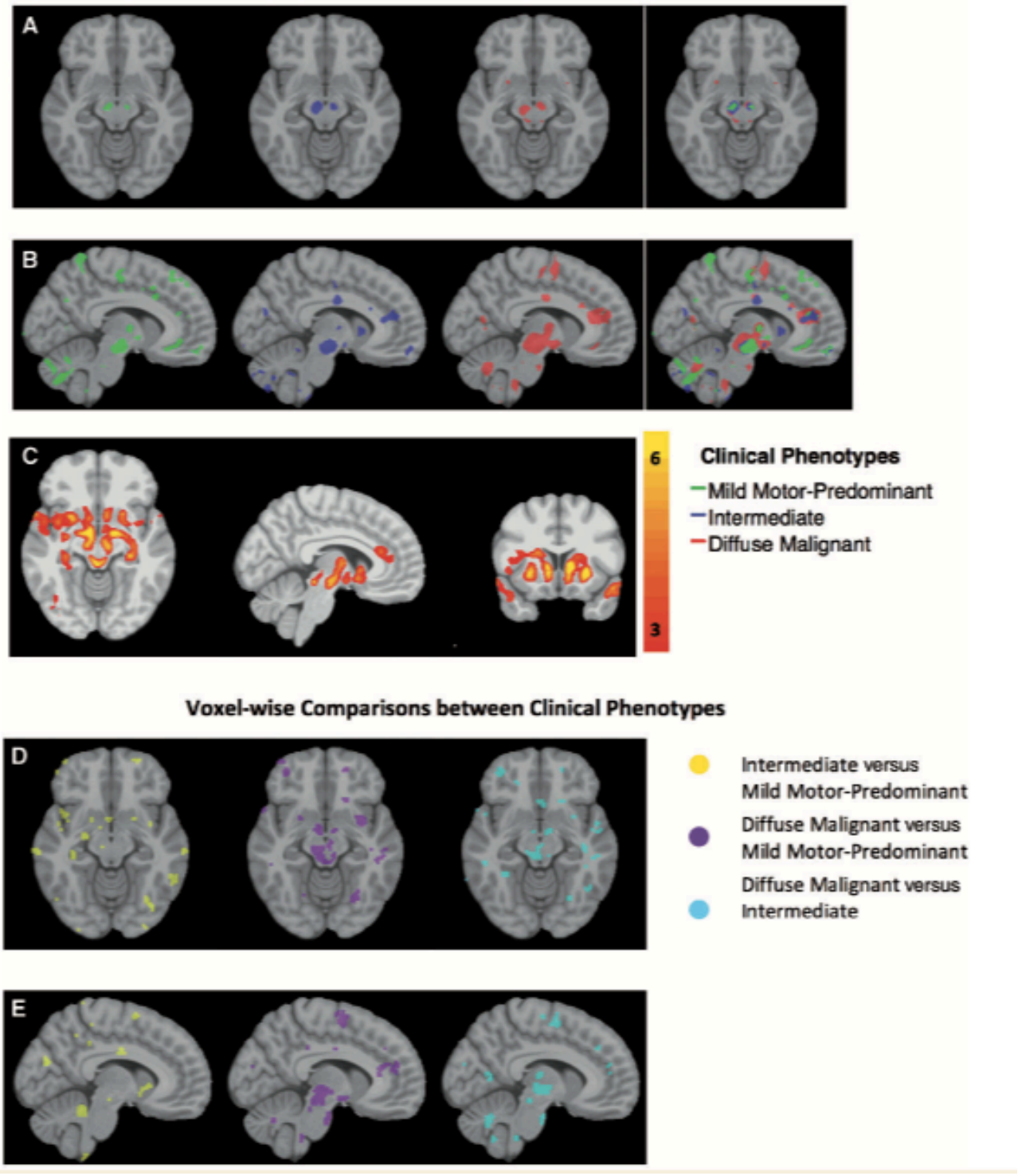
Figure 5. Subtypes and progression of Parkinson's Disease (from Fereshtehnejad SM et al., Curr Neurol Neurosci Rep 2017)



Latterly, a prospective longitudinal multicenter cohort study has shown that PD patients with diffuse/malignant phenotype had the lowest level of CSF amyloid-β and amyloid-β/total tau ratio, like Alzheimer's Disease's CSF profile, compared to other phenotypes.[103] Moreover, the diffuse/malignant phenotype of PD has demonstrated more cortical and basal ganglia atrophy in deformation-based magnetic resonance imaging (MRI) morphometry, while the mild motor-predominant subtype having the least atrophy.[103] Also, on dopaminergic SPECT scanning the diffuse/malignant subtype had the highest level of caudate denervation.[103] Figure 6 shows the structural MRI analysis in different clinical subtypes of PD.

Thus, PDRBD+ phenotype has shown more rapid and malignant progression with worst prognosis compared to the other subtype of PD and should be considered as a biomarker of a more widespread and severe neurodegenerative process. Consequently, it should be recommended to screen patients with PD for mild cognitive impairment, orthostatic hypotension and RBD at baseline. Therefore, RBD in PD may bear clinical, therapeutic and prognostic implications, and an accurate screening and diagnosis would be crucial for managing associated comorbidities. Moreover, PDRBD+ patients might represent ideal candidates for both disease-modifying and neuroprotective therapy trial when they might be hopefully available.

Figure 6. Structural MRI analysis in clinical Parkinson's disease subtypes (from Fereshtehnejad SM et al., Curr Neurol Neurosci Rep 2017)



METHODOLOGICAL ASPECTS

Diagnosing RBD in PD

The diagnosis of RBD in the PD population is more challenging than in the "idiopathic" RBD population, for several reasons.

First, PD patients might have mild RBD episodes, characterized by presence of RSWA with isolated muscle jerking or simple vocalization that may go unnoticed by the patient or the bed-partner or may be considered to be non-pathological and so unreported to medical attention.[1,84,104–106] This is particularly relevant, since patients usually show less complex and violent motor behavior during vPSG in sleep laboratory compared to those observed at home, and the minimum amount and duration of REM-sleep motor behaviors are not defined.[106] Also, PD patients taking dopaminergic treatment may show reduced or absent REM sleep and the minimum duration of REM sleep needed to quantify RSWA is not defined.

Second, RBD polysomnographic features in PD may differ from those of I-RBD, since an increased tonic, rather than phasic, EMG activity was observed in this population, suggesting the existence of a peculiar neurophysiological RBD phenotype.[107]

However, a referral bias has also been suggested, since more complex and violent behaviors in idiopathic RBD patients may lead to medical attention, while more simple and milder motor manifestations may not.

Indeed, other causes of sleep disruption can confound the clinical picture and patients' and/or bed-partner's perceptions of night-time occurrences, like other sleep disorders, other medical

comorbidities, motor and non-motor PD symptoms, and that might lead them to underestimate their concomitant RBD.

On the other hand, some patients might have a "provisional" RBD according to the ICDS-3, with typical history and/or vPSG-documented motor behavior but without RSWA.[2] It is far to being completely understood whether these provisional status represent a prodromal full-blown RBD. However, a recent study on de-novo PD patients, longitudinally assessed by vPSG, have found that subjects with "provisional RBD" at baseline developed a full-blown RBD after two-years follow-up.[108] Thus, REM sleep behavioral events not associated with RSWA might be precursors to RBD, and it should be named "prodromal" RBD.

Finally, ICDS-3 diagnostic criteria for RBD have been mainly established based on clinical features of I-RBD. This is especially true for the RSWA diagnostic cut-off, that has been established based on norms including only small number (n=15) of PD patients.[86]

a. REM sleep without atonia scoring methods

REM sleep without atonia (RSWA) is the polysomnographic hallmark of RBD, characterized by sustained (tonic) loss of normal muscle atonia and/or increased intermittent (phasic) muscle activity during REM sleep. A reliable quantification of RSWA is crucial in order to diagnose RBD, and various scoring methods have been developed.[88,109–111]

The first and widely accepted visual scoring method to quantify RSWA was originally developed by Lapierre and Montplaisir[109,112] (here referred to as the Montréal method) and subsequently validated in 2010 in a study in idiopathic RBD patients.[112] According to this method, each REM sleep

epoch was scored as “tonic” if more the 50% of epoch duration presents increased sustained EMG activity, with an amplitude at least twice the background EMG muscle tone, or more than 10 μV.[112,113] Moreover, phasic chin EMG density was represented by the percentage of 2-s mini-epochs containing EMG events lasting 0.1 to 10 sec, with amplitude beyond four times the amplitude of background activity.[109,112,113] Thus, RSWA is defined by presence of >30% of 20-sec epochs containing tonic chin EMG activity and/or presence of >15% of 2-sec mini-epoch containing phasic chin EMG activity.[109] The same method showed that most PD patients with RBD have >20% of 20-sec epochs containing tonic EMG activity.[114] The Montréal method has also been assessed using 30-seco epochs performing similarly.[113]

Moreover, the Barcelona and Innsbruck groups, known together as SINBAR group, have compared RSWA manually assessed in eleven different body muscles, and in different combinations, in a group of 30 RBD patients including 15 PDRBD+ patients.[86] The SINBAR method assess RSWA evaluating chin EMG activity, as tonic, phasic or “any” (either tonic or phasic), and phasic EMG activity at bilateral Flexor Digitorum Superficialis (FDS) muscle. More precisely, this method scored each 30-s REM sleep epoch as “tonic” referring to the Montreal method, although phasic activity was scored into 3-s mini-epochs and was defined as any burst of EMG activity lasting 0.1 to 5-s with an amplitude exceeding twice the background activity.[86–88] Moreover, phasic chin EMG activity superimposed on a background of tonic activity, during a 3-s mini-epoch, must show at least twice the amplitude of the background activity within the same 3-s mini-epoch. Furthermore, each 3-s mini-epochs was scored as having or not “any” chin EMG activity, when containing either tonic and/or phasic EMG activity within the same mini-epoch, in order to include also EMG activity lasting from 5 to 15 s. Tonic EMG activity was scored only in the chin muscle, while phasic activity was assessed both in the chin muscle and in bilateral FDS muscle. The authors found that a montage

including flexor digitorum superficialis (FDS) muscle of upper limb combined with chin EMG derivations better differentiated RBD patients from control subjects than chin alone.[86] Specifically, among other measures, a cut-off of >32% of 3-sec REM sleep epochs containing the combination of any (either tonic or phasic) chin EMG activity and bilateral Flexor Digitorum Superficialis (FDS) phasic EMG activity brought the best discriminative power.[86] More recently, based on data published by the SINBAR group,[86–88,115] the cut-off value of 27% of 30-sec epochs of REM sleep containing any (either tonic or phasic) chin EMG activity combined with bilateral FDS EMG phasic activity, was indicated to be the most current evidence-based data for detecting RSWA in the diagnosis of RBD by the American Academy of Sleep Medicine, as mentioned in the International Classification of Sleep Disorders third edition (ICSD-3).[54]

However, manual-visual scoring of RSWA is time consuming and requires specialized expertise, making it not always suitable in the clinical practice. Additionally, these methods have been validated only in small cohorts of PD patients.

Recently, an automatic scoring algorithm, also known as the REM sleep Atonia Index (RAI), has been developed in order to overcome these limits.[116,117] RAI showed a good sensitivity, specificity, and correct classification, with general agreement between methods and Cohen's kappa values in the “good” range when compared with the Montréal method in a recent study including seventy-four idiopathic RBD patients.[113] According to this automatic scoring method, each REM sleep epoch included in the analysis is divided into 1-s mini-epochs, and the average amplitude of the rectified chin EMG signal is obtained for each mini-epoch. After a noise reduction procedure,[116] the values of the chin EMG signal amplitude in each 1-s mini-epoch are used to compute the percentage of values in the following 20 amplitude (amp) classes, expressed in μV: amp ≤1, 1<amp≤2, …,

18<amp≤19, amp>19. Muscle atonia is revealed by high values of the first class (amp≤1) whereas phasic and tonic activations are expected to increase the value of the other classes.[116,117] To summarize the degree of predominance of the first class we used the index RAI= amp≤1/(100-1<amp≤2). RAI can vary from 0, suggesting absence of mini-epochs with amp ≤1 that means complete absence of EMG atonia, to 1, indicating that all mini-epochs have an amp ≤1 or stable EMG atonia in the epoch. RAI values <0.8 are strongly indicative of RSWA; while values of RAI between 0.8 and 0.9 indicate a less evident alteration of muscle atonia, and values above 0.9 are characteristic of normal recordings.[116]

All these scoring methods, either manual and automatic, have been validated in cohort of idiopathic RBD patients or in a very limited PD population. Moreover, the validity and the agreement of these different scoring methods has never been assessed in PD patients.

b. Screening questionnaires

The suspicion of RBD can rely on history and clinical presentation, assessed by an in-deep semi-structured interview, but vPSG is mandatory for its diagnosis, allowing to document REM sleep related motor behavior and to assess RSWA. However, vPSG might be not always available, and requires specific expertise, for these reasons several screening questionnaires for RBD have been proposed, for epidemiological studies and in clinical practice.

The first and widely used screening questionnaire is the RBD screening questionnaire (RBDSQ) created by Stiasny-Kolster et al..[118] It is a 10-item patient self-rating questionnaire, with “yes” or

"no" questions, with a maximum score of 13 points, covering the clinical features of RBD. In particular, items 1 to 4 explore frequency and content of dreams and their relationship to nocturnal movement and behavior; item 5 refers to any self-injuries or bed partner injuries; item 6 is addressed to the dream enactment behavior, including four sub-items assessing nocturnal motor behavior as vocalization, sudden limb movements, complex motor behavior, bedding items that fell down; items 7 and 8 are about nocturnal awakening; item 9 is about disturbed sleep in general and item 10 concerns the presence of any neurological disorders, such as Parkinson's disease. The suggested cut-off value for the diagnosis of RBD in general population is 5.[118] The RBDSQ has been validated in idiopathic RBD population and control subjects showing 96% of sensitivity and 56%, of specificity and good internal consistency (Cronbach's 0.885).[118] Nomura et al. have evaluated the usefulness of the RBDSQ in patients with PD finding a fair internal consistency (Cronbach's 0.73) and suggesting that the best cut-off value for detecting RBD in PD patients should be 6, because item 10 scores always 1 in these patients.[119] Recently, Stiasny-Kolster et al. have assessed the diagnostic value of the RBDSQ in two independent sample of PD patients, the first underwent a sleep-focused interview prior to administration of the RBDS, while the second group fulfilled the RBDSQ without prior interview on possible RBD.[120] Considering a cut-off score ≥5, the first group showed 90% of sensitivity and 87% of sensitivity, while the second group showed 68% of sensitivity and 63% of specificity. Using the optimal cut-off value ≥6 for PD, the sensitivity decreased to 64% and the specificity increased to 68% for the second group. These results suggest that the diagnostic value of RBDSQ strongly depends on clinical setting and might be influence by patient's awareness of RBD condition. In fact, PD patients are frequently unaware of their RBD and therefore RBDSQ alone may be of limited utility in this population. Recently, Halsband et al. have assessed the validity of RBDSQ in de novo PD, finding sensitivity/specificity of 0.44/0.84 with area under the curve (AUC) of 0.68 (95% CI, 0.56-0.79), using the cut-off score of 6 for PD patients, suggesting that RBDSQ is not a

reliable screening questionnaire in this population .[121] Interestingly, a sub-analysis of question 6 (subitem 4 exploring dream-enacting behaviors) at a cut-off score of 1 shied a sensitivity pf 0.74 and a specificity of 0.70 for these de novo PD patients, with AUC of 0..74 (95% CI, 0.63-0.84).[121]

The international RBD study group (I-RBDSG) has proposed a single question screening tool, namely the RBD single question (RBD-1Q).[122] It is a single "yes-no" question, self-administered with participation of bedpartner, concerns the dream-enactment behavior of RBD: *"Have you ever been told, or suspected yourself, that you seem to 'act out your dreams' while asleep (for example, punching, flailing your arms in the air, making running movements etc.)?"*. RBD-1Q has been validated in idiopathic RBD patients showing sensitivity of 93.8% and specificity of 87.2%, but it has not been properly assessed in patients with PD.[122]

In the past decade other RBD screening questionnaire have been proposed, among them there are the RBD questionnaire (RBD-HK) and the Innsbruck RBD Inventory (RBD-I).[123,124]

The RBDQ-HK is a self-administered questionnaire, either by patient or bed-partner.[123] RBD-HK includes 13 items covering various clinical features of RBD, moreover, each item is assessed on two scales: lifetime occurrence and last year frequency.[123] The total score of the RBD-HK is calculated by the sum of the scores of all lifetime items and last year frequency items, ranging from 0 to 100.[123] Li et colleagues have validated the RBDQ-HK showing moderate sensitivity of 82.2%, specificity of 86.9%, and finding the best cut-off for total score at 18/19.[123] However, this screening tools has been validated in heterogeneous RBD population but including only 11 PD patients.[123]

Similarly, the RBD-I is a self-rated questionnaire encompassing clinical characteristics and frequency (for the last year) of RBD.[124] More precisely, it comprises 5 items exploring clinical features specific for RBD and 2 items probing alternative sleep disorders (e.g. NREM parasomnia and sleep apnea syndrome).[124] The RBD symptoms score was calculated by the number of positively

answered symptoms-related items divided by the number of answered questions, ranging from 0 to 1 and a cut-off score ≥0.25 was proposed.[124] On the other hand, the RBD frequency score was rated as the sum of all answered frequency-related items divided by the number of questions answered, ranging from 0 to 4.[124] Frauscher et colleagues have validated the RBD-I in a heterogeneous cohort of RBD, comprising only 22 PD patients, showing sensitivity of 91.4% and specificity of 85.7%, using the cut-off score of ≥0.25.[124] Once again, this screening tools has been validated in heterogeneous RBD population, aware about their REM parasomnia, including only 22 PD patients.

AIMS

Several lines of evidence indicate that PD associated with RBD represents a malignant phenotype underlying a more aggressive and widespread neurodegenerative process. In fact, PD patients with RBD has been shown to have a heavier burden of disease, in term of motor and non-motor symptoms, with an increased risk for dementia. Therefore, a correct identification of RBD in PD may bear clinical, therapeutic, and prognostic implications; for instance, a diagnosis of RBD in PD may orient the therapeutic choices of clinicians in order to prevent the increased risk of DRT-associated complications, such as dyskinesia or impulse control disorders. Prognostic implications of having RBD may be important in view of eventual disease-modifying or neuroprotective trials. Despite the prognostic implication of RBD in PD, both screening tools and diagnostic criteria of RBD have been mainly validated in idiopathic RBD population.

Moreover, little is known about the evolution of both clinical and vPSG measures of RBD in PD, in relationships with the progression of motor and non-motor symptoms. Actually, RBD may precede, co-occurs or follow PD onset by many years. Very few longitudinal data are available on clinical symptoms, as well as on vPSG markers of RBD in PD.

On the other hand, In idiopathic RBD (i.e. RBD without evidence of other neurological disease, that may precede by several years the clinical onset of PD), longitudinal studies have found that RSWA measures, including both phasic and tonic components, increase over time as a dynamic marker.[125,126] Indeed, idiopathic RBD patients who developed neurodegenerative disease, namely PD, MSA and dementia, showed an increase in tonic chin EMG activity during REM sleep after 6.7 years of follow-up, compared to those who had not converted.[125] Interestingly, in one of those study, there was no difference in percentage of phasic chin EMG activity between the RBD patients who developed neurodegenerative disease and those who did not.[125] Thus, the percentage of tonic

chin EMG activity during REM sleep might predicts conversion in PD, suggesting a progressive degenerative process of neuronal circuits involved in REM sleep atonia modulation.

On the other hand, an improvement of RBD symptoms is occasionally reported in PD patients, over time. Longitudinal studies on PD patients with RBD, assessed by questionnaires, led to controversial results, reporting both improvement and worsening or no changes in RBD symptoms in PD patients over time.[5,16,91] So far, only one longitudinal vPSG study has assessed RSWA evolution in de-novo PD after 2 years of follow-up, finding that RSWA increased significantly after 2 years and RBD does not resolve over time or with dopaminergic treatment.[108]

The present thesis is articulated into three studies.

In the first, we first aimed to assess the concordance of two visual scoring methods for assessing RSWA, namely the Montreal and the SINBAR, and to compare them with the RAI automated method, in patients with Parkinson's disease, in order to assess their correct classification accuracy and reciprocal agreement, as well as their role in the clinical diagnosis of RBD in PD.

Subsequently, in the second study, we have aimed to assess, on one hand, the sensitivity and specificity of the two most used RBD screening questionnaires, namely the RBDSQ and the RBD1Q, and to other hand, to ascertain whether current diagnostic criteria for RBD established by the ICSD3 are appropriate to diagnose RBD in PD patients.

Finally, in the third study, we aimed to longitudinally evaluate clinical and neurophysiological features of RBD after 3-years follow-up, and to assess the relationship between the evolution of RSWA and the progression of symptoms in a cohort of PD patients with RBD, in order to ascertain whether RBD represents a reliable and stable marker in PD.

RESULTS

STUDY 1: COMPARISON BETWEEN AUTOMATIC AND VISUAL SCORINGS OF REM SLEEP WITHOUT ATONIA FOR THE DIAGNOSIS OF REM SLEEP BEHAVIOR DISORDER IN PARKINSON DISEASE.

Article published on Sleep. 2017 Feb 1;40(2). doi: 10.1093/sleep/zsw060.

pii: zsw060 http://dx.doi.org/10.1093/sleep/zsw060

ORIGINAL ARTICLE

Comparison Between Automatic and Visual Scorings of REM Sleep Without Atonia for the Diagnosis of REM Sleep Behavior Disorder in Parkinson Disease

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Study Objectives: To compare three different methods, two visual and one automatic, for the quantification of rapid eye movement (REM) sleep without atonia (RSWA) in the diagnosis of REM sleep behavior disorder (RBD) in Parkinson's disease (PD) patients.

Methods: Sixty-two consecutive patients with idiopathic PD underwent video-polysomnographic recording and showed more than 5 minutes of REM sleep. The electromyogram during REM sleep was analyzed by means of two visual methods (Montréal and SINBAR) and one automatic analysis (REM Atonia Index or RAI). RBD was diagnosed according to standard criteria and a series of diagnostic accuracy measures were calculated for each method, as well as the agreement between them.

Results: RBD was diagnosed in 59.7% of patients. The accuracy (85.5%), receiver operating characteristic (ROC) area (0.833) and Cohen's K coefficient (0.688) obtained with RAI were similar to those of the visual parameters. Visual tonic parameters, alone or in combination with phasic activity, showed high values of accuracy (93.5–95.2%), ROC area (0.92–0.94), and Cohen's K (0.862–0.933). Similarly, the agreement between the two visual methods was very high, and the agreement between each visual methods and RAI was substantial. Visual phasic measures alone performed worse than all the other measures.

Conclusion: The diagnostic accuracy of RSWA obtained with both visual and automatic methods was high and there was a general agreement between methods. RAI may be used as the first line method to detect RSWA in the diagnosis of RBD in PD, together with the visual inspection of video-recorded behaviors, while the visual analysis of RSWA might be used in doubtful cases.

Keywords: REM Sleep without Atonia, REM Sleep Behavior Disorder, Parkinson Disease, REM sleep atonia Index, Montréal method, SINBAR method.

Statement of Significance

The diagnosis of RBD in Parkinson's disease is often challenging, because of subclinical forms, but it may bring prognostic and therapeutic implications. A reliable quantification of REM sleep without atonia (RSWA) is critical in order to diagnose RBD, and various methods, either visual or automatic, have been developed. Visual methods are time-consuming and require specialized expertise. We compared the diagnostic accuracy of two widely used visual methods and one automatic, in the diagnosis of RBD in PD, finding a substantial agreement. The automatic method may be used as first line to detect RSWA in diagnosing RBD in PD, together with the inspection of video-recorded behaviors, while the visual analysis of RSWA might be used in doubtful cases.

INTRODUCTION

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by partial or complete loss of normal muscle atonia during REM sleep, associated with vivid dreams and dream-enacting behavior.[1,2] RBD is very common in patients affected by neurodegenerative diseases, belonging to the group of alpha-synucleinopathies, namely Parkinson's disease (PD), Multiple System Atrophy, and Dementia with Lewy bodies.[3–7] Several lines of evidence indicate that RBD in PD is a marker of a more widespread neurodegenerative process, particularly associated to an increased risk for cognitive decline.[8] Therefore, the correct identification of RBD in PD may bear important prognostic implications for patients and it might become critical when neuroprotective and disease modifying therapies will hopefully be available. REM sleep without atonia (RSWA) is the polysomnographic (PSG) hallmark for the diagnosis of RBD, and consists of sustained (tonic) loss of normal muscle atonia during REM sleep, and/or intermittent (phasic) excessive electromyogram (EMG) activity during REM sleep.

A reliable quantification of RSWA is critical in order to diagnose RBD, and various methods to assess motor activity during REM sleep have been developed. The first and widely accepted visual scoring method to quantify RSWA was originally developed by Lapierre and Montplaisir[9,10] (here referred to as the Montréal method) and subsequently validated in 2010 in a study investigating a sample of eighty idiopathic RBD patients.[10] Authors showed that the presence of >30% of 20-second epochs containing tonic EMG activity led to a correct classification of 82% of patients, while >15% of 2-second mini-epoch containing phasic EMG activity led to a correct classification of 84% of them.[9] The same method showed that most PD patients with RBD have >20% of 20-second epochs containing tonic EMG activity.[11] The Montréal method has also been shown to perform similarly if 30-second epochs are used.[12]

Moreover, the Barcelona and Innsbruck groups, known as SINBAR group, performed a study comparing RSWA assessed in 11 different body muscles, and in different combinations, in a group of 30 RBD patients including 15 PD.[13] Authors found that a montage including upper limb plus chin EMG derivations better differentiated RBD patients from control subjects than chin alone.[13] Specifically, among other measures, a cut-off of >32% of 3-second REM sleep epochs containing the combination of any (either tonic or phasic) chin EMG activity and bilateral Flexor Digitorum Superficialis (FDS) phasic EMG activity brought the best discriminative power.[13]

More recently, based on data published by the SINBAR group,[13–16] a cut-off value of 27% of 30-second epochs of REM sleep containing any (either tonic or phasic) chin EMG activity combined with bilateral FDS EMG phasic activity, was indicated to be the most current evidence-based data for detecting

RSWA in the diagnosis of RBD by the American Academy of Sleep Medicine (AASM), as mentioned in the International Classification of Sleep Disorders third edition (ICSD-3).[17] However, manual-visual scoring is time consuming and requires specialized expertise, making it little convenient in the clinical practice. Additionally, these methods have been validated only in small cohorts of PD patients.

Recently, an automatic scoring algorithm, also known as the REM sleep Atonia Index (RAI), has been developed in order to overcome these limits.[18,19] RAI showed a good sensitivity, specificity, and correct classification, with general agreement between methods and Cohen's kappa values in the "good" range when compared with the Montréal method in a recent study including seventy-four idiopathic RBD patients.[12] So far, no study has compared the accuracy, sensitivity, and specificity of RSWA measures obtained with the three methods, namely the automated and the manual-visual ones, in patients with PD.

Thus, the aims of this present study were: (1) to assess the concordance of the two visual scoring methods for RSWA, namely the Montréal[10] and the SINBAR[13] approaches, in patients with PD and (2) to compare the RAI automated method[18] with the two visual scoring methods, in order to assess their correct classification accuracy and reciprocal agreement, as well as their role in the clinical diagnosis of RBD in PD.

SUBJECTS AND METHODS

Subjects

Seventy-three (44 male, 29 female, mean age 64.10 ± 8.47 years) non-demented PD patients, consecutively seen at two Movement Disorder Centers, namely the University Hospital in Clermont-Ferrand, France (n = 63), and the Le Molinette University Hospital in Turin, Italy (n = 10), for their routine evaluation, were recruited. The inclusion criterion was the diagnosis of idiopathic PD based on the United Kingdom PD Society Brain Bank Criteria.[20] Exclusion criteria were the presence of alternative causes of parkinsonism, a concomitant dementia (defined by a score <26 in the Mini Mental State Examination, MMSE), the presence of a psychiatric disease according to the Diagnostic Statistical Manual (DSM-V), the use of device aided therapy, such as subcutaneous Apomorphine infusion, intra-duodenal gel infusion or deep brain stimulation. RDB was either diagnosed or ruled out according to the ICSD-3 criteria.[17] Patients were examined by a neurologist expert in Sleep Medicine (MLF, MZ) who conducted an in-depth interview, focused on RBD history and features. PD history and symptoms, as well as treatment data were collected by neurologist expert in movement disorders (AM, FD, MZ). The Total Levodopa Equivalent Daily Dose (LEDD), together with the Dopamine Agonist (DA) Levodopa Equivalent Daily Dose (DA-LEDD) were calculated according to Tomlinson et al.[21] The Ethical committee of each center (Clermont-Ferrand, France; Turin, Italy) approved the study and all patients gave written informed consent, according to the Declaration of Helsinki.

PSG Recordings

All patients underwent one full-night attended video-polysomnography (video-PSG) recording in sleep laboratory with digital polysomnography according to the AASM recommendations.[22] Video-PSG was performed with digitally synchronized videography and the following montage was employed: electroencephalographic leads (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), left and right electrooculography (EOG) channels, bilateral surface EMG channels (submentalis, FDS on upper limbs, and tibialis anterior on lower limbs), and electrocardiography. The respiratory analysis included nasal airflow, which was recorded by both thermistor and nasal pressure sensor, thoracic, and abdominal respiratory effort, oxygen saturation recording by cutaneous finger pulse-oxymeter and microphone. Patients were asked to sleep uncovered in order to improve the detection of motor activity, but a light sheet could be allowed for their comfort.

Sleep stages were scored according to AASM criteria,[22] with allowance to chin EMG muscle tone during REM sleep. The following sleep data were collected for descriptive purpose: total bed time, total sleep time, sleep efficiency, sleep latency, wake after sleep onset (W), number of REM sleep episode, percentage of time in each sleep stage (*N*1, *N*2, *N*3, *R*), arousal index, periodic limb movements index, Apnea-hypopnea index, oxygen-desaturation index, arousal index.

Diagnosis of RBD

The diagnosis of RBD was made according to the ICSD-3,[17] including a quantitative measure of RSWA, namely "any chin EMG activity, tonic and/or phasic, combined with bilateral phasic activity of the flexorum digitorum superficialis (FSD) muscle" in ≥27% of REM sleep scored in 30-second epochs. The rational to choose this cut-off, based on the SINBAR method,[16] as reference standard, relies on the fact that the latter has been included in the ICSD-3 "as the most current evidence-based data for detecting RSWA in the evaluation of RBD, reliably distinguishing RBD patients from controls." Patients were excluded from the analysis if they had spent less than 5 minutes in REM sleep, since this REM duration was believed to be insufficient for a reliable assessment of RSWA. Each video-recorded REM sleep period was carefully analyzed in order to detect any motor behaviors or sleep vocalizations referable to RBD, such as violent and non-violent motor complex activity.

RSWA Visual Scoring Methods

The manual-visual scoring of RSWA was performed according to two previously published methods, the Montréal,[9,10] adapted to 30-second epochs,[12] and the SINBAR method.[13,14,16] The EMG activity of the chin and bilateral FDS were analyzed. REM sleep epochs were carefully examined for artifacts, and increases in EMG tone caused by respiratory arousal were excluded. The minimum amplitude of EMG activity during non-REM (NREM) sleep was considered as the background EMG activity for each patient. The EMG signal was analyzed with a notch filter at 50 Hz and rectified. Visual scoring was performed by a single sleep-specialist scorer (MF), who was blinded to RBD history.

The Montréal Method

According to the method described elsewhere,[9,10] adapted to 30-second epochs, each epochs was scored as "tonic" when

the increased sustained EMG activity was present in more than 50% of the 30-second epoch duration, with an amplitude a least twice the background EMG muscle tone, or more than 10 μV; otherwise epochs were scored as atonic. Tonic EMG density represented the percentage of 30-second epoch scored as tonic. Phasic chin EMG activity was scored dividing each 30-second epoch into 2-second mini-epochs; the phasic EMG activity can be scored both in atonic and tonic epochs. Phasic chin EMG density represented the percentage of 2-second mini-epochs containing EMG events lasting 0.1–10 seconds, with amplitude exceeding four times the amplitude of background EMG activity. According to previous findings, REM sleep chin EMG activity was considered to be abnormal when tonic chin EMG density was ≥30% and/or phasic chin EMG density was ≥15%.[10]

The SINBAR Method

The analysis was made according to previous published data by the SINBAR group,[13,14,16] evaluating chin EMG activity, as tonic, phasic or "any" (either tonic or phasic), and phasic EMG activity at bilateral FDS muscle. Each epoch was scored as "tonic" when the increased sustained EMG activity was present in more than 50% of the 30-second epoch duration with an amplitude at least twice the background EMG muscle tone, or more than 10 μV. Phasic EMG activity was scored into 3-second mini-epochs, and was defined as any burst of EMG activity lasting 0.1 to 5 seconds with amplitude exceeding twice the background EMG activity. Phasic chin EMG burst superimposed on a background of tonic activity, during a 3-second mini-epoch, was required to have at least twice the amplitude of the background tonic EMG activity within the same 3-second mini-epoch. Each 3-second mini-epoch was scored having or not "any" EMG activity, when containing either tonic and/or phasic EMG activity within the same mini-epoch, in order to include EMG activity lasting from 5 to 15 seconds, that was not measured in previous method. The percentages of 3-second mini-epochs containing phasic chin EMG activity as well as "any" chin EMG activity, out of the total REM sleep mini-epochs, was calculated. The percentage of 3-second mini-epochs with "any chin EMG activity combined with bilateral phasic FDS EMG activity," out of the total REM sleep 3-second mini-epochs, was also calculated. The percentage of 30-second epochs containing five or more 3-second mini-epochs with "any chin EMG activity combined with bilateral phasic FDS EMG activity" out of the total REM sleep epochs was calculated. The SINBAR group found the best specificity and sensitivity with the following cut-off values: >16.3% of 3-second mini-epochs with phasic chin EMG activity, >18% of 3-second mini-epochs with any chin EMG activity, >32% of 3-second mini-epochs with any chin EMG activity combined with bilateral phasic EMG activity in the FDS, and >27% of 30-second epochs with any chin EMG activity combined with bilateral phasic EMG activity in the FDS.

RSWA Automatic Scoring (RAI)

The automatic quantification of chin EMG activity was made according to an established automatic scoring algorithm,[18,19,23] by means of the HypnoLab software (SWS-Soft, Italy). The chin EMG signal was digitally band-pass filtered at 10–100 Hz, with a notch filter at 50 Hz and rectified. Each sleep epoch included in the analysis was divided into 1-second mini-epochs, and the average amplitude of the rectified chin EMG signal was obtained for each mini-epoch. After a noise reduction procedure,[18] the values of the chin EMG signal amplitude in each 1-second mini-epoch were used to compute the percentage of values in the following 20 amplitude (amp) classes, expressed in μV: amp ≤ 1, 1 < amp ≤ 2, …, 18 < amp ≤ 19, amp > 19. Muscle atonia is revealed by high values of the first class (amp ≤ 1) whereas phasic and tonic activations are expected to increase the value of the other classes.[18,19] An index summarizing in a single value the degree of preponderance of the first class was used in REM sleep: RAI = amp ≤ 1/(100–1 < amp ≤ 2). RAI can vary from 0 (absence of mini-epochs with amp ≤ 1 that is complete absence of EMG atonia) to 1 (all mini-epochs with amp ≤ 1 or stable EMG atonia in the epoch). RAI values < 0.8 are strongly indicative of altered (reduced) chin EMG atonia during REM sleep; while values of RAI between 0.8 and 0.9 indicate a less evident alteration of atonia, and values above 0.9 are characteristic of normal recordings.[18] RAI was computed completely blinded to the results of the manual scoring methods and to the RBD status of the patients.

Statistical Analysis

Between-group differences on clinical, demographic, and video-PSG features were assessed with the Student's *t* test. Specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and correct classification of RBD were assessed for the following parameters: RAI < 0.8, tonic chin EMG density ≥ 30%, phasic chin EMG density ≥ 15% (scored in 2-second mini-epoch) and ≥ 16.3% (scored in 3-second mini-epoch), any chin EMG activity scored in 3-second mini-epoch ≥ 18%, any 3-second mini-epoch chin EMG combined with bilateral phasic FDS EMG activity ≥ 32% and any 30-second epoch chin EMG combined with bilateral phasic FDS EMG activity ≥ 27%. The accuracy of the different parameters to discriminate RBD from no-RBD patients was evaluated using receiver operating characteristic (ROC) curve analysis and the calculation of the area under the curve (AUC). Additionally, the weighted comparison (WC) measure[24] was calculated for any chin EMG combined with bilateral phasic FDS EMG activity ≥ 27% in 30-second epoch versus all other methods; WC is an index weighting the difference in sensitivity and difference in specificity of two tests, taking into account the relative clinical cost (misclassification costs) of a false positive compared with a false negative diagnosis and disease prevalence. WC was then converted into an equivalent increase in true positive patients per 1000 (if all the benefit is focused into true positive patients) by calculating WC × prevalence × 1000. Finally, the extent of the agreement of the different methods was quantified by means of Cohen's K coefficient.

RESULTS

Subjects

Of the original 73 patients, four did not have any REM sleep during video-PSG and seven had REM sleep duration shorter than 5 minutes, therefore they were excluded from the study.

The comparison of the three methods was then possible in 62 PD patients (35 male, 27 female, mean age 64.7 ± 8.72 years). RBD was diagnosed in 37 out of 62 of our PD patients (PD-RBD; 59.7%), according to the ICSD-3 criteria,[17] including the presence of ≥27% of 30-second epochs of REM sleep containing any chin EMG activity or bilateral FDS phasic EMG activity. The remaining 25 patients constituted the PD-noRBD group. The clinical and demographic features of our patients are shown in Table 1. There were no significant between-group differences, in age, gender, duration, and severity of PD (assessed by Hoehn & Yahr stage and Unified Parkinson Disease Rating Scale). All patients were taking dopamine replacement therapy ($n = 61$ levodopa, $n = 34$ DA), and no difference in LEDD and DA-LEDD was found between the two groups. A total of nine patients were taking drugs known to potentially increase RSWA. More specifically five patients were taking antidepressants (selective serotonin re-uptake inhibitor, SSRI). Among them, three had RBD (two of them developed RBD prior to starting antidepressant therapy) while two didn't have RBD. Four patients (three PD-RBD) were taking beta-blockers, and RBD preceded the initiation of this treatment in two cases. On the other hand, five (three PD-RBD) out of 62 patients were receiving clonazepam, and none was taking melatonin.

PSG Results

The PSG features are reported in Table 2. There were no significant differences between PD patients with or without RBD for sleep architecture, periodic leg movements index, and apnea/hypopnea index. Only the amount of REM sleep was significantly lower in the group of PD-RBD patients compared to PD-noRBD.

Comparison of the Different RSWA Scoring Methods

For the visual scoring, a total of 4777 30-second epochs of REM sleep have been obtained, leading to 47 770 3-second mini-epochs and 71 655 2-second mini-epochs, respectively. Of these, 178 (0.37%) 3-second mini-epochs and 275 (0.38%) 2-second mini-epochs containing arousal-related both EMG activity or movement artifacts were excluded from the analysis. For the automated scoring, a total of 64 (1.34%) 30-second epochs of REM sleep containing artifacts were excluded. Data about EMG tone parameters obtained in PD patients with or without RBD are shown in Table 3. Table 4 summarizes the analysis of the performance of the three methods, one automatic (RAI) and two visual (Montréal, SINBAR), to evaluate RSWA versus the clinical diagnosis of RBD in our patients with PD. The accuracy of both visual methods was high and very similar for those parameters including measures of tonic activities (alone or in combination with phasic activities) that we will call here "tonic" for simplicity. The same was not true for parameters taking into consideration only phasic activities. In particular, the 30-second tonic chin EMG density showed an accuracy of 95.2, an AUC of 0.940, and Cohen's K coefficient of 0.897, as well as the percentage of "any chin EMG activity combined with bilateral phasic EMG activity at FDS," scored in 30-second epoch. Both of these parameters showed the highest PPV (92.5), NPV (100), sensitivity (100%), and specificity (88%). The percentage of 3-second mini-epochs with "any chin EMG activity" showed an accuracy of 93.5, an AUC of 0.920, a Cohen's K coefficient of 0.862, a sensitivity of 100%, and a specificity of 84%. The percentage of 3-second mini-epochs with "any chin EMG activity

Table 1—Clinical and Demographic Features of PD Patients With and Without RBD.

	PD-RBD ($n = 37$)	PD-noRBD ($n = 25$)	*p*
Males	24 (64.9)	11 (44.0)	NS[a]
Age, y	66.0 ± 7.5	62.7 ± 10.1	NS
Bed partner	17 (45.9)	11 (44.0)	NS[a]
PD duration, y	8.2 ± 4.3	8.0 ± 5.0	NS
H&Y stage	2.2 ± 0.5	2.1 ± 0.6	NS
UPDRS III	18.1 ± 11.1	16.2 ± 9.5	NS
UPDRS-tot	35.5 ± 18.3	31.4 ± 19.4	NS
LEDD, mg	796.2 ± 486.0	704.4 ± 421.9	NS
DA-LEDD, mg	106.9 ± 125.9	123.9 ± 139.3	NS
SSRI	3 (8.1)	2 (8.0)	NS[a]
Clonazepam	2 (5.4)	3 (12.0)	NS[a]

DA-EDD = Dopamine-agonist equivalent daily dose; H&Y = Hoehn and Yahr; LEDD = Levodopa equivalent daily dose; PD = Parkinson's disease; RBD = REM sleep behavior disorder; SSRI = selective serotonin re-uptake inhibitor; UPDRS III = Unified Parkinson's disease rating scale III. Data are expressed as mean ± standard deviation or number(percentage of total).
[a]Fisher-test.[45]

Table 2—Polysomnographic Features of PD patients with and without RBD.

	PD-RBD ($n = 37$)	PD-noRBD ($n = 25$)	*p*
Total sleep time, min	321.5 ± 82.9	326.7 ± 81.0	NS
Sleep efficiency, %	72.8 ± 17.3	72.1 ± 18.3	NS
W, min	90.5 ± 79.5	96.5 ± 77.6	NS
*N*1, %	10.4 ± 8.4	8.5 ± 6.2	NS
*N*2, %	58.0 ± 12.3	58.6 ± 15.7	NS
*N*3, %	21.04 ± 13.0	19.1 ± 11.7	NS
R, %	10.5 ± 5.5	13.7 ± 8.2	NS
R, min	34.1 ± 21.4	45.0 ± 29.8	.01
PLMS, number	123.5 ± 143.8	113.0 ± 183.0	NS
PLMS index	23.8 ± 25.7	24.5 ± 44.5	NS
Apnea/hypopnea index	5.5 ± 9.2	2.9 ± 3.9	NS

PD = Parkinson's disease; PLMS = Periodic leg movements during sleep; RBD = REM sleep behavior disorder. Data are expressed as mean ± standard deviation.

combined with bilateral phasic EMG activity at FDS" showed an accuracy of 93.5, a ROC area of 0.933, a Cohen's K coefficient of 0.866, a sensitivity of 94.6%, and a specificity of 92%. Finally, the percentage of phasic chin EMG activity scored in 2-second mini-epoch and 3-second mini-epoch showed, respectively, an accuracy of 61.3 and 56.5, a ROC area of 0.669 and 0.635, a Cohen's K coefficient of 0.296 and 0.230, a sensitivity of 37.8% and 27%, and specificity respectively of 96% and 100%. The PPV and the NPV values for the phasic chin EMG activity scored in 2-second mini-epoch were 93.3 and 51.1 respectively, while for the phasic chin EMG activity scored in 3-second mini-epoch was 100 and 48.1 respectively. RAI, with a cut-off value < 0.8, showed an accuracy of 85.5, a ROC area of 0.833, a Cohen's K coefficient of 0.688, high sensitivity (94.6%), and good specificity (72%), with a PPV of 83.3 and NPV of 90.

Table 3—EMG Tone Parameters in PD Patients With or Without RBD.

	PD-RBD (*n* = 37)	PD-noRBD (*n* = 25)	*p*
Tonic EMG chin 30 s, %	58.5 ± 20.1	10.0 ± 7.9	.00001
Phasic EMG 2 s, %	8.9 ± 6.3	2.5 ± 1.5	.00001
Phasic EMG chin 3 s, %	11.8 ± 8.1	3.6 ± 2.3	.00001
Any EMG Chin 3 s, %	50.6 ± 18.1	12.2 ± 5.9	.00001
Any EMG chin + FSD 3 s, %	53.5 ± 16.6	15.0 ± 6.1	.00001
Any EMG chin + FSD 30 s, %	60.4 ± 19.6	11.1 ± 7.2	.00001
REM atonia index	0.442 ± 0.2	0.830 ± 0.2	.00001
Tonic EMG chin 30 s, %	58.5 ± 20.1	10.0 ± 7.9	.00001
Phasic EMG 2 s, %	8.9 ± 6.3	2.5 ± 1.5	.00001
Phasic EMG chin 3 s, %	11.8 ± 8.1	3.6 ± 2.3	.00001
Any EMG Chin 3 s, %	50.6 ± 18.1	12.2 ± 5.9	.00001

EMG = electromyography; FSD = flexorum digitorum superficialis; PD = Parkinson's disease; RBD = REM Sleep Behavior Disorder; REM = Rapid Eye Movements; 30-s = 30 seconds epoch; 2-s = 2 seconds mini-epochs; 3-s = 3 seconds mini-epochs. Data are expressed as mean ± standard deviation.

Table 4 also reports the WC between the results obtained by the reference method (ie, SINBAR 30-second epochs of REM sleep containing any chin EMG activity or bilateral FDS phasic EMG activity ≥ 27%) and all the other methods. A very good agreement with the above measures was found, indicating a substantial equivalence between the reference and the Montréal tonic chin EMG density ≥ 30%, as well as the SINBAR any chin EMG activity scored in 3-second mini-epochs ≥ 18%. Surprisingly, the latter seemed to perform slightly better than the reference method using WC, translating into a benefit equivalent of 2 additional true positives × 1000 cases. Moreover, the reference method showed only a relatively small advantage compared to the RAI, which could be translated into a benefit equivalent of 19 true positives × 1000 cases.

Table 5 illustrates the agreement (Cohen's K coefficient) between all possible pairs of measures of RSWA used in this study. The agreement between tonic chin EMG density and the visual parameter "any chin EMG activity combined with bilateral phasic FDS EMG activity in 30-second" was perfect (K = 1.000), while the agreement between tonic chin EMG density and the visual parameters "any chin EMG activity, scored in 3-second" and "any chin EMG activity combined with bilateral phasic FDS EMG activity in 3-second" was almost perfect[25] (respectively, K = 0.964 and K = 0.897). The agreement between RAI < 0.8 and all visual parameters was substantial (K = 0.784 with tonic chin EMG density, K = 0.745 with any chin EMG activity, scored in 3-second, K = 0.688 any chin EMG activity combined with bilateral phasic FDS EMG activity in 3-second, K = 0.784 any chin EMG activity combined with bilateral phasic FDS EMG activity in 30-second), except

Table 4—Accuracy of Measures of RSWA, Based on Their Suggested cut-offs, for the Clinical Diagnosis of RBD in PD Patients.

	Tonic chin EMG 30 s (≥30%)	Phasic chin EMG, 2 s (≥15%)	Phasic chin EMG, 3 s (≥16%)	Any chin EMG, 3 s (≥18%)	Any chin EMG + FSD, 3 s (≥32%)	Any chin EMG + FSD, 30 s (≥27%)	REM Atonia Index 30 s (<0.8)
Sensitivity	100.0	37.8	27.0	100.0	94.6	100.0	94.6
Specificity	88.0	96.0	100.0	84.0	92.0	88.0	72.0
PPV	92.5	93.3	100.0	90.2	94.6	92.5	83.3
NPV	100.0	51.1	48.1	100.0	92.0	100.0	90.0
Accuracy	95.2	61.3	56.5	93.5	93.5	95.2	85.5
ROC area	0.940	0.669	0.635	0.920	0.933	0.940	0.833
Cohen's K	0.897	0.296	0.230	0.862	0.866	0.897	0.688
Weighted comparison	0.000	0.625	0.730	−0.003	0.056	Ref.	0.032
Benefit equivalent (×1000 cases)	0	373	436	−2	33	Ref.	19

EMG = electromyography; FSD = flexorum digitorum superficialis; NPV = negative predictive value; PD = Parkinson's disease; PPV = positive predictive value; RBD = REM Sleep Behavior Disorder; Ref. = Reference method; REM = Rapid Eye Movements; ROC = receiver operating characteristic; RSWA = REM sleep Without Atonia.

Table 5—Cohen's K (agreement) Between All Possible Pairs of Measures of RSWA.

Phasic chin EMG, %2 s	Phasic chin EMG, %3 s	Any chin EMG, %3 s	Any chin EMG + FSD, %3 s	Any chin EMG + FSD, %30 s	REM atonia index <0.8	
0.299	0.191	**0.964****	**0.897****	**1.000*****	**0.784***	Tonic chin EMG, % 30 s
	0.752*	0.281	0.355	0.299	0.264	Phasic chin EMG, % 2 s
		0.179	0.230	0.191	0.168	Phasic chin EMG, % 3 s
			0.862**	**0.964****	**0.745***	Any chin EMG, % 3 s
				0.897**	**0.688***	Any chin EMG + FSD, % 3 s
					0.784*	Any chin EMG + FSD, % 30 s

EMG = electromyography; FSD = flexorum digitorum superficialis; REM = Rapid Eye Movements; RSWA = REM sleep Without Atonia.
Agreement: *substantial, **almost perfect, ***perfect.

for phasic parameters. The percentages of 3-second or 2-second mini-epochs containing phasic EMG activity performed worse than the other parameters, showing lowest sensitivity, accuracy, AUC area, and the Cohen's K coefficient, whereas they showed good specificity and good positive predictive value. Also WC between the reference method and the phasic parameters was greatly in favor of the reference method.

DISCUSSION

The diagnosis of RBD relies on the presence of an excessive muscle tone during REM sleep but the definition of RSWA is still mostly qualitative, based on the scorer's subjective impression, rather than on a clear cut-off value. Recently published ICSD-3 criteria have specified to quantify RSWA "as defined by the guidelines for scoring PSG features of RBD in the most recent version of the AASM Manual for the Scoring of Sleep and Associated Events,"[22] but the latter does not indicate an univocal way to quantify RSWA.[17] However, several methods have been developed to measure EMG activity during REM sleep and detect RSWA, showing good sensitivity and specificity to discriminate RBD from no-RBD patients.[9,10,13,23,26–32] Among them, the ICSD-3[17] indicates the SINBAR[13] method (>27% of 30-second epochs containing any chin EMG activity combined with bilateral phasic EMG activity in the FDS) as one of the most current evidence-based approaches for detecting RSWA in the evaluation of RBD and, for this reason, we used as the reference method for the subsequent comparison with other methods.

In this study, all three scoring methods assessing RSWA in PD, two visual and one automatic, showed high sensitivity, specificity and accuracy, especially "tonic" or "any EMG activity" parameters, while visual parameters considering only "phasic" EMG activities were associated to lower sensitivity and accuracy. First, this study found perfect or almost perfect agreement between the two visual scoring methods, Montréal and SINBAR, when they consider tonic EMG activities alone or in combination with phasic activities, but not when they measure only phasic activities. Moreover, we found a substantial agreement between the automatic scoring method, for example, the RAI, and the Montréal and SINBAR visual scoring methods, when they consider tonic EMG activities alone or in combination with phasic activities, but not when they measure only phasic activities. These findings confirm previous published data suggesting a good correlation between Montréal method and RAI in patients with idiopathic RBD,[12,19] multiple system atrophy,[19] or narcolepsy.[23]

Visual and automated assessment may differ in some technical aspects, namely the standard of rejection of periods containing artifacts. Indeed, in visual assessment, only mini-epochs containing arousal-related EMG activity are eliminated, while in RAI, 30-second epochs containing major artifacts are excluded, leading to a potential increase in artifact time rejection when assessing RAI that may represent a limitation. However, it has to be pointed out that, in this study, the percentage of rejection was very narrow for both visual and automatic methods (0.4% and 1.3% respectively), making unlikely that this difference would have a significant impact on the results.

It should be pointed out that the diagnosis of RBD was performed according to the ICSD-3 criteria that encompass one of the measures derived from the SINBAR method (namely the percentage of 30-second epochs with any chin EMG activity combined with bilateral phasic FDS EMG activity, with a cut-off value of 27%). Thus, the sensitivity of this particular parameter is necessarily equal to 100% and its performance in accuracy is maximal by definition because of this choice; conversely all the other parameters may be penalized to some extent.

Diagnosing RBD in PD is not a simple task, because of many reasons. First, PD patients with RBD may often have PSG abnormalities either alone (RSWA) or with mild non-clinical behaviors in sleep, such as limb twitching or jerking or simple vocalizations that may go unnoticed by the patient himself, particularly if sleeping alone, or by bed-partners (subclinical RBD[2,33,34]). Moreover, video-behavioral episodes recorded in the sleep lab are often less elaborated and violent compared to those occurring at home, and the minimum amount or duration of video-recorded REM sleep motor behavior required to diagnose RBD is not currently defined.

However, since RBD in PD appears to be associated to a more widespread degenerative process,[35] with a particular increased risk for cognitive decline,[36] the diagnosis of RBD in PD may bear important prognostic and perhaps therapeutic implications in the next future, when disease modifying therapies would hopefully be available. Indeed, at that point, costs and benefits

should be weighted, especially in case of potential severe side effects, and the presence of RBD would represent a strong argument in favor of an eventual disease-modifying strategy.

It has been suggested that the chin EMG alone does not discriminate sufficiently patients from controls. Indeed, in a study on idiopathic RBD, no phasic chin EMG activation was found in 35.5% of the behavioral events observed by video-monitoring, while the simultaneous recording of the mentalis, FDS and extensor digitorum brevis EMG activity was able to detect the highest rates of REM sleep phasic EMG activity, as well as the majority (94.4%) of the motor and vocal manifestations occurring in RBD.[16] The authors thus recommended a montage including both chin and bilateral FDS muscles for the detection of RBD. Following this study, the ICSD-3 indicates a percentage ≥32% of 30-second epochs containing any tonic or phasic chin EMG activity and/or bilateral phasic FDS activity as a reliable way to define RSWA in RBD.

The addition of FDS metrics, in the present study, did not seem to provide an enhanced diagnostic power compared to the assessment of the chin EMG activity alone. Including FSD channels within the routine full PSG montage in PD patients may be time-consuming and add discomfort to the patient. Unless a clear diagnostic benefit is demonstrated from further studies performed by different groups,[13,15] the quantification of FDS activity in the clinical work-up may be questionable, as our findings in patients with PD seem to indicate. On the other hand, recording FDS appears to be of great help in identifying video behavioral episodes when increased phasic EMG activity is observed in these leads on PSG recording.

Our data confirm that the automatic detection of RSWA is highly correlated with manual-visual measures in PD patients. This result is consistent with previous study comparing the RAI with the Montréal visual scoring method.[12] Other studies showed an excellent comparability of the RAI to one visual chin analysis similar to the SINBAR method, assessing directly phasic burst, in PD patients with RBD,[27] or RBD patients with depression,[37] and normal aging.[38] Quantification of RSWA is time-consuming and often unavailable in the clinical practice, while automatic analysis is fast and highly replicable. Furthermore, a limitation of both Montreal and SINBAR visual methods is that they rely on binary measures (ie, positive or negative), while the RAI method, as well as other visual scoring approach,[27] rely on more continuous measures, being more suitable for assessing biological activity like RSWA. On the other hand, the automatic analysis may have some disadvantages, such as incomplete sensitivity in detecting large artifacts, and is not included in most commercial sleep analysis software packages. However, in light of these results, it can be reasonably recommended that, in the clinical practice, automatic assessment of RSWA might be used first, with visual analysis employed when the automatic analysis cannot be applied for technical reasons, or in doubtful cases, together with the visual inspection of video recorded behaviors.

In the present study we found that PD-RBD patients have more "tonic" rather than phasic EMG activity alteration during REM sleep, suggesting a peculiar RBD phenotype in PD. The latter appears to be different from the idiopathic phenotype and from RBD associated with narcolepsy,[23,39] and it seems to be more similar to that found in patients with multiple system atrophy,[19,40] but perhaps with a lower degree of tonic alteration. Indeed, PD patients with RBD may have milder motor behaviors according to previous findings.[40–43] This may be related to the neurodegenerative process itself, perhaps leading to an impairment of brain structures involved in muscle phasic activity generation. On the other hand, idiopathic RBD patients seeking medical attention are likely to be those with the most violent motor behaviors, and the prevalence of subclinical RBD in the general population is largely unknown. Further studies are warranted to ascertain whether PD patients have a reduced phasic EMG activity or an increased tonic EMG activity, or both, compared to idiopathic RBD.

Our study has some potential limitations. As in a previous paper,[12] we adapted the original "Montreal method" from 20-second to 30-second epochs, according to the current American Sleep Disorders Association (ASDA) recommendations for scoring sleep stages, but we choose to maintain the 2-second mini-epoch approach to score phasic activity. First, one must bear in mind that the choice of epochs length (30-second vs. 20-second) may impact on the tonic metrics, since more than 15 seconds rather than 10 seconds of tonic activity are required to score the whole epoch as "tonic," potentially leading to lower scores in the tonic activity using 30-second epoch windows compared to 20-second epochs. This has been shown by the works of the SINBAR groups.[13] Second, phasic activity consists in the ratio between the number of phasic mini-epochs and the total number of REM sleep mini-epochs and would not be affected by the epoch length. However, it may be argued than the total amount of 2-second mini-epochs, using 30-second epochs window, may be slightly higher than the one found using 20-second epoch window (because of the possible inclusion of NREM mini-epochs within REM sleep mini-epochs), leading to possible small differences in the 2-sec mini-epochs phasic metrics. Nevertheless, the difference was shown to be negligible and not to affect the correct classification of patients and controls in a previous study.[12] On the other hand, it is known that the two different visual methods implying the use of 2-second mini-epochs rather than 3-sec, may potentially lead to differences in phasic EMG activity assessment, for example when the same burst of EMG activity overlaps two consecutive mini-epochs in one case and falls within one only mini-epoch in the other case. Indeed, in our study, the percentage of phasic EMG chin activity assessed in 3-second mini-epochs was slightly higher than that of 2-second, as it is illustrated in Table 3. The same can be evicted from past works,[10,13,39,44] although no genuine comparisons can be made between the two methods because of the heterogeneity of the RBD populations included in these studies.

In conclusion, we found a substantial agreement between the automatic method (RAI) and the "tonic" parameters of the two visual methods (Montréal, SINBAR). Therefore, the automatic evaluation of EMG activity during REM sleep, together with visual inspection of video recorded behaviors, may be the first-line method to detect RSWA in PD patients, while visual scoring of RSWA may be useful in doubtful cases. Moreover, a peculiar pattern of REM sleep muscle tone alteration, mainly characterized by an increased tonic, rather than phasic, activity, seems to characterize RBD in PD, in contrast to what observed in both idiopathic and narcolepsy-related RBD.

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FUNDING

The study had no financial support.

ACKNOWLEDGMENTS

This work was performed at the CHU Clermont-Ferrand, Clermont-Ferrand, France, the Sleep Center, Neurophysiology Unit, University of Cagliari, Monserrato (CA), Italy, the Department of Neurology, Oasi Institute for Research on Mental Retardation and Brain Aging (IRCCS), Troina, Italy, and the Department of Neuroscience, University of Turin, Turin.

SUBMISSION & CORRESPONDENCE INFORMATION

Submitted for publication January, 2016
Submitted in final revised form November, 2016
Accepted for publication November, 2016
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DISCLOSURE STATEMENT

MF, PB, MP, AC, FD, AM, and MLF have indicated no financial conflicts of interest. LL and MZ received honoraria from Medtronics, Abbvie and Lundbeck, not related to the present article. RF consulted for EB Neuro, with potential relationship with the present paper.

STUDY 2: DIAGNOSING REM SLEEP BEHAVIOR DISORDER IN PARKINSON DISEASE WITHOUT A GOLD STANDARD: A LATENT CLASSES MODELS' STUDY.

Abstract published on Sleep Medicine 40 (2017) e3-e185, abstracts for the World Sleep 2017, 9th October 2017. Winner of the Young Investigator Sleep Research in Neurodegeneration award.

Article to be submitted to Sleep.

Diagnosing REM sleep behaviour disorder in Parkinson disease without a gold standard: a latent classes models study.

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INTRODUCTION: It is increasingly known that REM Sleep Behavior Disorder (RBD) in Parkinson's disease (PD) patients may be associated with a more malignant clinical phenotype. Despite its prognostic value, the diagnosis of RBD in PD is often challenging because of mild forms that may go unnoticed. Recent diagnostic criteria, including a quantitative measure of REM sleep without atonia (RSWA), have been defined mainly based on idiopathic RBD population referred to a sleep disorder center for their parasomnia. We aim to ascertain whether current diagnostic criteria for RBD are appropriate in PD population consulting a movement disorder center, and to assess the role of each criterion in a large cohort of patients with PD.

METHODS: One-hundred-eleven PD patients (M=67; mean age: 65.8±8.5 yrs) consecutively evaluated at three movement disorder centers were enrolled. All patients underwent a detailed sleep-focused interview followed by a full-night video-polysomnographic (vPSG) recording. Without a gold standard, latent class models were applied to create an unobserved ("latent") variable. The observed variables used in these models were: 1) history of dream-enactment behaviors 2) Video-PSG-documented REM sleep-related motor behaviors and 3) RSWA according to the proposed cut-off derived from the SINBAR scoring method (i.e. ≥27% of 30-s REM sleep epochs contain any chin EMG activity combined with phasic EMG activity in bilateral Flexor Superficialis Digitorum). Sensitivity analysis were also realized with an alternative RSWA cut-off derived from the Montreal scoring method (i.e. ≥30% of tonic 30-s REM sleep epochs and/or ≥15% of 2-s REM sleep mini-epochs containing phasic activity). Finally, we assessed the respective diagnostic performance of each diagnostic criterion for RBD.

RESULTS: According to the best LCM-derived model, RBD was diagnosed in patients having either "history" or "video" with RSWA; or showing both "history" and "video" without RSWA. In those patients, the criterion "history" showed 85.5% of sensitivity, 95.2% of specificity, 96.7% of PPV and 80% of NPV, with a Cohen's K of 0,78. The criterion "video" showed 88.4% of sensitivity, 95.2% of specificity, 96.8% of PPV and 83.3% of NPV respectively, with a Cohen's K of 0.81. The criterion "RSWA" showed 94.2% of sensitivity, 88.1% of specificity, 92.9% of PPV and 90.2% of NPV, with Cohen's K of 0.83 using the SINBAR cut-off. Using the Montreal cut-off, RSWA showed a sensitivity of 88.4%, a specificity of 88.1%, a PPV of 92.4% and a NPV of 82.2% with a Cohen's K of 0.75. The concomitant presence of both "history" and

"video" showed 73.9% of sensitivity, 100% of specificity, 100% of PPV, 80% of NPV and Cohen's K of 0.68.

CONCLUSIONS: Results of the best latent classes-derived model for diagnosis of RBD in PD were consistent with the current RBD diagnostic criteria. Moreover, the diagnostic criteria "RSWA" showed the highest sensitivity, reducing the risk of false positive, and the concomitance of "history" and "video" reduced the risk of false negative, that it would be crucial in PD population frequently unaware of their RBD status.

Diagnosing REM sleep behaviour disorder in Parkinson disease without a gold standard: a latent-class model study.

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Manuscript to be submitted in Sleep.

Running Title: Diagnosing RBD in PD without a gold standard

Key words: RBD, Parkinson's Disease, diagnostic criteria

ABSTRACT
REM Sleep Behavior Disorder (RBD) in Parkinson's disease (PD) is associated to a more severe clinical phenotype. Despite its prognostic value, the diagnosis of RBD in PD is often challenging because of mild forms that may go unnoticed. Screening questionnaire and current diagnostic criteria, which include quantification of REM sleep without atonia (RSWA), have been mainly defined based on non-PD (idiopathic) RBD population referred to a sleep disorder center for their parasomnia. We aim to ascertain whether current diagnostic criteria for RBD are appropriate in PD population consulting a movement disorder center, to assess the role of each criterion and to determine the value of the screening questionnaire for RBD in in a large cohort of patients with PD,

METHODS: One-twenty-eight PD patients (M=67; mean age: 65.8±8.5 yrs) consecutively evaluated at three movement disorder centers were enrolled. All patients underwent a screening questionnaire, followed by a sleep-focused interview and a full-night video-polysomnographic (vPSG) recording. One-hundred-eleven PD patients were finally included. Without a gold standard, latent class models were applied to create an unobserved ("latent") variable. Sensitivity analysis were also realized with an alternative RSWA cut-off derived from the Montreal scoring method. Finally, we assessed the respective diagnostic performance of each diagnostic criterion for RBD.

RESULTS: According to the best LCM-derived model, RBD was diagnosed in patients having either "history" or "video" with RSWA; or showing both "history" and "video" without RSWA. In those patients, the criterion "history" showed 85.5% of sensitivity, 95.2% of specificity, 96.7% of PPV and 80% of NPV, with a Cohen's K of 0,78. The criterion "video" showed 88.4% of sensitivity, 95.2% of specificity, 96.8% of PPV and 83.3% of NPV respectively, with a Cohen's K of 0.81. The criterion "RSWA" showed 94.2% of sensitivity, 88.1% of specificity, 92.9% of PPV and 90.2% of NPV, with Cohen's K of 0.83 using the SINBAR cut-off. Using the Montreal cut-off, RSWA showed a sensitivity of 88.4%, a specificity of 88.1%, a PPV of 92.4% and a NPV of 82.2% with a Cohen's K of 0.75. The concomitant presence of both "history" and "video" showed 73.9% of sensitivity, 100% of specificity, 100% of PPV, 80% of NPV and Cohen's K of 0.68.

CONCLUSIONS: Results of the best latent classes-derived model for diagnosis of RBD in PD were consistent with the current RBD diagnostic criteria. Moreover, the diagnosis of provisional RBD should be considered as full-blown RBD in PD population.

INTRODUCTION

Up to 60% of patients with Parkinson's disease (PD) have REM sleep behavior disorder (RBD),(1,2) a parasomnia characterized by loss of normal muscle atonia during REM sleep associated with dream-enacting behaviors. (3,4) Nowadays, it is increasingly known that RBD in PD is a marker of a more widespread and aggressive neurodegenerative process (5), associated with a malignant clinical phenotype.(6)(7) Indeed, PDRBD+ patients tend to have more rigid akinetic forms, axial symptoms, and levodopa-induced dyskinesia.(8–10) These patients also have increased autonomic dysfunction,(10,11)more severe neuropsychiatric comorbidities, cognitive deficits, and an increased risk of dementia(12–17). Thus, the correct identification of RBD in PD may bear therapeutic and prognostic implication.

The diagnosis of RBD relies on a history of dream-enactment behaviors, but it has to be confirmed by vPSG. Diagnostic criteria for RBD according to the International classification of sleep disorders-third edition published in 2014 (ICSD-3)(18) include: 1) the presence of repeated episodes of sleep-related vocalization and/or complex motor behaviors; 2) these behaviors are documented by video-polysomnography (vPSG) to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep; 3) polysomnographic recording has to demonstrate REM sleep without atonia (RSWA) that exceed normal values; and 4) the disturbance is not better explained by another sleep disorder, mental disorder, medication or substance use.

Compared to the previous diagnostic criteria, a quantitative assessment of RSWA is currently required for the diagnosis of RBD. Although there is no univocal way to quantify RSWA, the American Academy of Sleep Medicine (AASM) Manual for the Scoring of Sleep and Associated Events (reff) refers to the SINBAR method as the most current evidence-based data for detecting RSWA in the evaluation of RBD, reliably distinguishing RBD patients from controls. Based on these method, RSWA is defined by a value of ≥27% of 30-sec epochs of REM sleep, with any (tonic/phasic) chin electromyographic (EMG) activity combined with bilateral phasic activity of the flexor digitorum superficialis (FDS) muscles.(19–22).

The recent ICSD-3 has also provided the terms “provisional RBD” for all patients who may have a typical clinical history of RBD and/or exhibit typical dream-enacting behaviors during vPSG, but do not fulfill the criterion of RSWA.

RBD manifestations in PD are often milder than those seen idiopathic RBD and may include twitching or jerk-like movements, or simple vocalizations, that can go unnoticed by both patient and bedpartner. This could be due to a peculiar RBD phenotype in PD, as it has been observed that RBD in PD would be characterized by more “tonic” rather than phasic EMG activity.(23) However, a referral bias has also been suggested, since more complex and violent behaviors in idiopathic RBD patients may lead to medical attention, while simple and milder behaviors may not.

Given these premises, the diagnosis of RBD in PD is often challenging, especially in milder forms. On the other hand, ICDS-3 diagnostic criteria for RBD have been mainly established based on the clinical features of I-RBD patients. This is

especially true for the RSWA diagnostic cut-off, that has been established based on norms including only small number (n=15) of PD patients.(19)

Finally, diagnosis of RBD requires vPSG, which is an expensive and time-consuming procedure requiring specific expertise, not always available in clinical or research settings, especially in the field of epidemiological research. For this reason, several screening questionnaires for RBD have been developed, including the most used RBD screening questionnaire (RBDSQ) and RBD single question (RBD1Q). However, these tools have been mainly validated in idiopathic RBD (I-RBD) population or in small cohort of PD patients.(24–28)

In this study, we aimed to ascertain whether current ICSD-3 diagnostic criteria for RBD are appropriate in PD population and to assess the respective role of each single criterion, in a large cohort of PD patients consecutively seen in a Movement Disorder Center and undergoing a vPSG assessment. Furthermore, we wished to assess whether the cut off for RSWA selected by the AASM, reliably distinguish PD patients with RBD patients from those without.

Finally, we aimed to assess the sensitivity and specificity of two RBD screening questionnaires, namely the RBD single question (RBBD1Q)(24) and the RBDSQ,(25) in the same large cohort of consecutive PD patients.

METHODS

Subjects

One hundred twenty-eight (80 male, mean age 65.6 ± 8.3 years) non-demented PD patients consecutively seen for their routine evaluation at three Movement Disorder Center, namely the University Hospital in Clermont-Ferrand, France (n=102), "Le Molinette" University Hospital in Turin, Italy (n=8), and the University Hospital in Cagliari, Italy (n=18), were recruited.

Inclusion criterion was the clinical diagnosis of PD according to United Kingdom Brain Bank Criteria.(29) Exclusion criteria were the presence of other causes of parkinsonism, clinically-defined dementia according to the Diagnostic Statistical Manual V (DSM-V) criteria,(30) psychosis according to DSM-V,(30) the use of device aided therapy, like subcutaneous Apomorphine infusion, intra-duodenal gel infusion or deep brain stimulation, untreated obstructive sleep apnea syndrome with an apnea/hypopnea index ≥15/h.

Demographic and clinical data, such as sex, age, PD duration, PD severity as measured by both the Hoehn & Yahr scale(HY) (31) and the Unified Parkinson's disease rating scale (UPDRS), global cognitive function as assessed by the Montreal Cognitive Assessment (MOCA), and current treatment dose, were collected for all patients by a neurologist expert in movement disorders. The use of selective serotonin/noradrenaline reuptake inhibitors (SSRI/SNRI), tricyclic antidepressant, benzodiazepines, and beta-blockers was assessed. The total levodopa equivalent

daily dose (LEDD) and the dopamine agonist levodopa equivalent daily dose (DA-LEDD) were calculated according to Tomlinson et al.(32)
A detailed sleep-focused interview, including history of parasomnia, was performed by neurologist expert in sleep medicine.

Twenty-five age and sex matched controls were enrolled (12 male, mean age 61.5 ± 13.7 years). Inclusion criteria for controls were age 40-85 years. Exclusion criteria were presence of neurological disease, sleep disorders, current or past treatment with antipsychotic or antidepressant drugs. The notion of the presence of a bedpartner was also recorded in both groups.

The local ethical committees of each center approved the study ad all participants gave written informed consent, according to the Declaration of Helsinki.

Screening Questionnaire

All patients fulfilled the RBD1Q and the RBDSQ prior to clinical interview. The RBD1Q is a single, "yes or no", question that concerns the dream-enactment behavior of RBD: "*Have you ever been told, or suspected yourself, that you seem to 'act out your dreams' while asleep (for example: punching, flailing your arms in the air, making running movements, etc.)?*".(24)
The RBDSQ is a 10-item patient self-rating questionnaire, with "yes" or "no" questions, with a maximum score of 13 points, covering the clinical features of RBD.(25) In general population, a cut-off value of >=5 has shown a sensitivity of 96% and specificity of 56%, correctly diagnosing 66% of I-RBD subjects.(25) A cut-off value

>=6 was proposed for PD subjects because of item 10 assessing the presence of a comorbid CNS disease is always scored yes in these patients.(33)

Polysomnographic recording

All participants underwent one full-night attended vPSG recording in sleep laboratory with digital polysomnography according to the American Academy of sleep Medicine (AASM) recommendations.(34) All participants were monitored with infrared video recording synchronized with PSG. For both patients and healthy controls, the following montage was used: electroencephalographic leads (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), left and right electrooculography (EOG) channels, bilateral surface EMG channels (submentalis, flexor digitorum superficialis on upper limbs, tibialis anterior on lower limbs), and electrocardiography. The respiratory analysis included nasal thermistor and nasal pressure sensor, thoracic and abdominal respiratory effort, pulse-oxymeter and microphone.

In order to increase the detection of motor activity, all participants slept uncovered, even if a light sheet could be allowed for their comfort.

Sleep stages were scored according to AASM criteria,(34) with allowance to chin EMG tone during REM sleep. The following sleep data were collected: total bed time, total sleep time, sleep efficiency, sleep latency, wake after sleep onset (W), number

of REM sleep episode, percentage of time in each sleep stage (N1, N2, N3, R), arousal index, periodic limb movements index (PLMS-i), apnea-hypopnea index (AHI), oxygen-desaturation index (ODI).

REM sleep without atonia (RSWA) was assessed in patients having spent ≥ 5 minutes in REM sleep, since shorter duration was believed to be insufficient for a reliable assessment of this parameter.(23) RSWA was manually quantified according to two previously published methods, namely the Montréal,(35,36) adapted to 30-sec epochs,(37) and the SINBAR method.(19,21,22) REM sleep epochs were carefully inspected for artifacts, such as increased muscle tone caused by respiratory arousal. The background EMG activity for each participant was considered as the minimum EMG amplitude during NREM sleep.

According to the Montréal method, adapted to 30-s epochs, each epochs was scored as "tonic" if more 50% of the 30-s epoch duration presents increased sustained EMG activity, with an amplitude at least twice the background EMG muscle tone, or more than 10 μV.(36,37) Phasic chin EMG density was the percentage of 2-s mini-epochs containing EMG events lasting 0.1 to 10 sec, with amplitude exceeding four times the amplitude of background activity.(35–37)
As reported by previous findings, RSWA was defined if ≥30% of 30-s REM sleep epochs contains tonic chin EMG activity and/or ≥15% of 2-s REM sleep mini-epochs contains phasic chin activity.(35–37)

On the other hand, the SINBAR method scored each epoch as “tonic” referring to the Montreal method, but phasic activity was scored into 3-s mini-epochs and was defined as any burst of EMG activity lasting 0.1 to 5-s with an amplitude exceeding twice the background activity.(19,21,22) Moreover, phasic chin EMG activity superimposed on a background of tonic activity, during a 3-s mini-epoch, must show at least twice the amplitude of the background activity within the same 3-s mini-epoch. Furthermore, each 3-s mini-epochs was scored as having or not “any” chin EMG activity, when containing either tonic and/or phasic EMG activity within the same mini-epoch, in order to include also EMG activity lasting from 5 to 15 s. Tonic EMG activity was scored only in the chin muscle, while phasic activity was assessed both in the chin muscle and in bilateral FDS muscle. According to this method,(19,21,22) RSWA was defined by presence of ≥ 27% of 30-s REM sleep epochs contains any (either tonic or phasic) chin EMG activity combined with phasic EMG activity at bilateral FDS muscles, (“any chin + FDS 30-s”) as suggested by the ICSD-3 edition.(18) Moreover, the following additional EMG cut-off for RSWA, as defined by the SINBAR group, were also employed, namely ≥16.3% of 3-s REM sleep mini-epochs contains phasic chin activity (“phasic-3”), ≥18% of 3-s REM sleep mini-epochs contains any chin EMG activity (“any chin”), and ≥32% of 3-s REM sleep epochs contains any chin EMG activity combined with phasic EMG activity at bilateral FDS muscles (“any chin + FDS 3-s”).(19,21,22)

The visual scoring of RSWA was performed by a neurologist expert in sleep medicine (MF), who was blinded to RBD history.

Statistical analysis

Statistical analysis was performed using Stata software, version 13 (StataCorp, College Station, TX, US) and R 3.3.3 (http://cran.r-project.org/). All tests were two-sided with a Type I error set at 0.05. Continuous data were expressed as means and standard deviations (SD) or as medians with interquartile range [IQR] according to statistical distribution, and categorical parameters as frequencies and associated percentages.

Without a gold standard, latent class models were applied to create an unobserved ("latent") variable. The latent variable represents an individual's true unobserved disease status, used subsequently as a gold standard to estimate sensitivity and specificity of various diagnostic criteria for RBD. This method was applied using *poLCA*, a package available in R, which uses expectation-maximization and Newton-Raphson algorithms to find maximum likelihood estimates of the model parameters, as, described previously.(38) The observed variables used in these models were: 1) history of dream-enactment behaviors ("history") 2) vPSG-documented REM sleep-related motor behaviors ("video") and 3) RSWA according to the proposed cut-off derived from the SINBAR scoring method. Sensitivity analysis was also realized with an alternative RSWA cut-off derived from the Montreal scoring method. Concordance between each criterion for RBD and the classification obtained with latent class method was then assessed with percent agreement and Cohen's kappa coefficient. Finally, sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated to assess diagnostic

performance of each diagnostic criterion for RBD. These measures were expressed with 95% confidence interval.

The same statistical assessment (percentage of agreement, Cohen's kappa coefficient, Se, Sp, PPV and NPV) was also used for the two screening questionnaires, namely RBD1Q and RBDSQ, administered individually or together.

Finally, we assessed the sensitivity and specificity of RSWA threshold for the diagnosis of RBD in PD. Patients with history of dream enactment behaviors (i.e. repeated episodes of sleep related vocalization and/or complex motor behaviors associated with dream mentation) and/or vPSG documented behaviors were included in the analysis together with control subjects and PD patients without neither history nor video-PSG recorded behaviors.

A receiver operating characteristic (ROC) curve was generated to assess the accuracy of RSWA parameter to discriminate patients having both history of dream-enactment behaviors and vPSG-documented motor behaviors from those who have not. The area under the ROC curve was presented with a 95% confidence interval obtained by the technique of DeLong et al. Finally, in order to study the "optimal" threshold value of RSWA to predict RBD, various statistical indices were used (Youden, Liu, efficiency).

RESULTS

Subjects

Of the original 128 patients, 16 patients were excluded from analysis, 3 of them because of technical reasons, 11 of them had none or insufficient (<5min) REM sleep during vPSG, and 2 of them showed an AHI≥15/h.

Thus, the latent classes analysis was performed in 111 PD patients (67 male, mean age: 65.8 ± 8.5 years). Figure 1 summarizes the flow chart of this study.

A total of 15 patients were treated with drugs known to potentially increase RSWA, namely 10 patients with selective serotonin re-uptake inhibitor (SSRI) and 5 patients with beta-blockers. On the other hand, 9 patients were taking Clonazepam, 2 patients were taking neuroleptics and 1 was taking melatonin.

Twenty-five age and sex matched healthy subjects were enrolled (12 male, mean age 61.5 ± 13.7 years).

The clinical and demographic features of patients and controls are summarized in Table 1.

Polysomnographic results and REM analysis

Polysomnographic results in PD patients and HC are reported in Table 2. The amount of REM sleep in terms of percentage and duration, were significantly lower

in PD patients compared to healthy controls. Both the total number and the index of PLMS were significantly higher in PD patients than in controls.

For the manual quantification of RSWA, a total of 8553 30-sec REM sleep epochs, 85380 3-sec REM sleep mini-epochs, and 128295 2-sec REM sleep mini-epochs of REM sleep have been analyzed.

A total of 77 30-sec REM sleep epochs, 788 3-sec epochs (0,9%) and 1154 2-sec REM sleep mini-epochs (0.9%) were excluded from the analysis because of the presence of respiratory-related arousal EMG activity.

The RSWA parameters obtained in PD patients and HC are shown in Table 3. All RSWA parameters were significantly higher in PD patients than in controls.

Latent class model

The latent class model analysis allows to identify patients considering their characteristics, as having RBD (class 1) or not having RBD (class 2). These models rely on the maximum likelihood estimation.

According to the latent classes model analysis, using the SINBAR cut-off "any chin + FDS 30-s" to define RSWA, patients classified as having RBD showed 82.6% probability to have "history" of RBD, 85.7% to have motor behaviors on vPSG and 92.2% to show RSWA. Using the SINBAR cut-off "any chin" to define RSWA, patients classified as having RBD showed 82.9% probability to have history of RBD, 86.0% of probability to have motor behaviors on vPSG, and 92.3% of having RSWA. According to these two models, patients having both history of RBD and presence of motor

behaviors on vPSG, but without RSWA, also referred as “provisional RBD” according to ICSD-3 criteria, have been classified as having RBD. Conversely, patients with either RBD history or vPSG documented behaviors and without RSWA, have been classified as not having RBD. Likewise, patients showing only RSWA, but without neither history nor vPSG documented motor behaviors, have been identified as not having RBD.

Similarly, using Montreal’s cut-offs to define RSWA, patients classified as RBD showed 83% likelihood to have a history of RBD, 85% to have motor behaviors on vPSG and 86% to have RSWA. Using the SINBAR cut-off “any chin + FDS 3-s” for RSWA, patients classified as RBD had 83.2% of chance to have history of RBD, 84.7% of probability to have video-documented motor behaviors, and 86.3% of RSWA.

According to these two models, patients showing only history of RBD, or both history and video-documented behaviors, but without RSWA, has been classified as having RBD. On the other hand, patients showing vPSG documented motor behaviors without RSWA have been classified as not having RBD.

According to the best latent class analysis-derived model, RBD was diagnosed in patients having either “history” or “video” with RSWA; or showing both “history” and “video” without RSWA. Table 4 summarizes the latent classes model results.

According to the best latent class analysis-derived model, n=69 PD patients were classified as having RBD while n=44 PD patients not.

Furthermore, sensitivity analysis has been performed considering different thresholds of RSWA (any chin EMG + FDS 30-s) ranging from 25% to 28%, leading to same results.

A ROC curve was generated to assess the assess the accuracy of the RSWA to discriminate patients having both history of dream-enactment behaviors and vPSG-documented motor behaviors from those who have not, finding AUC at 0.95. The optimal threshold value of 27% of any chin EMG + FDS 30-s showed 90.4% of sensitivity and 92.1% of specificity.

Figure 2 resumes the ROC analysis results.

RBD screening questionnaires.

Ninety-seven patients fulfilled the RBD1Q and the RBDSQ, before a sleep-focused interview. Table 5 reports the performance of screening questionnaires.

The RBD1Q showed 67.7% (95% CI: 54.7; 79.1) of sensitivity, 82.9% (95% CI: 66.4; 93.4) of specificity, 87.5% (95% CI: 74.8; 95.3) of PPV and 59.2% (95% CI: 44.2; 73.0) of NPV, with Cohen's K of 0.47. The RBDSQ showed 55.7% (95% CI: 42.4; 68.5) of sensitivity, 71.4% (95% CI: 53.7; 85.4) of specificity, 77.3% (95% CI: 62.2; 88.5) of PPV and 48.1% (95% CI: 34.0; 62.4) of NPV, with Cohen's K of 0.25.

If RBD1Q and RBDSQ were considered together, with at least one of them positively scoring for RBD, the combined questionnaires showed 72.6% (95% CI:

59.8; 83.1) of sensitivity, 65.7% (95% CI: 47.8; 80.9) of specificity, 78.9% (95% CI: 66.1; 88.6) of PPV and 57.5% (95% CI: 40.9; 73.0) of NPV, with Cohen's K of 0.37.

DISCUSSION

To the best of our knowledge, this is the first study that assessed, in a large cohort of PD patients, whether the ICSD-3 diagnostic criteria for RBD published by the AASM are appropriate in patients with Parkinson's disease.(18,34) Indeed, current diagnostic criteria for RBD as defined by the AASM are mainly based on findings in I-RBD population.

According to the best latent classes-derived model, patients has been classified as having RBD if showing either "history" or "video" with RSWA; or showing both "history" and "video" without RSWA. Using both SINBAR and Montreal scoring methods, RSWA criterion showed the highest sensitivity in identify RBD, so reducing the risk of false negative. Similarly, concomitance of history of RBD and vPSG documented behaviors, regardless to presence of RSWA, presented the highest specificity, hence reduced to zero the risk of false positive. On one hand, these results highlight the importance of quantification of RSWA in detecting the true no-RBD patients. Quantification of RSWA might be time-consuming and not suitable routinely in clinical practice. Recently, substantial agreement was found between an automatic scoring method, namely the REM sleep Atonia Index (RAI) ,(39) and both the Montreal(36) and SINBAR(22) methods, suggesting that RAI might be used as the

first method to quantify RSWA in PD patients, while the visual scoring may be employed in uncertain cases.(23)

However, results of the present study suggest that, in those PD patients who have a clear history of RBD and presence of REM sleep video-recorded behaviors, RSWA assessment is not mandatory for the diagnosis of RBD.

As a matter of fact, a recent study on de-novo PD patients, longitudinally assessed by vPSG, have found that subjects with REM sleep associated motor behaviors not fulfilling RSWA diagnostic cut-offs, named “provisional RBD” according to ICDS-3, at baseline developed a full-blown RBD after two-years follow-up.(40) Thus, REM sleep behavioral events not associated with RSWA might be precursors to RBD, and it should be considered as “prodromal” RBD.

In light of these results, the visual inspection of vPSG may be crucial to detect all range of REM behavioral events. Nevertheless, minor movements, like twitching or jerking, may be not easily discernable, especially if patients sleep with sheets or blanket. Given the role of video-documented behaviors in the diagnosis of RBD, it is advisable that vPSG should be performed without blanket or at least with light sheets.

RSWA is a core feature of RBD diagnosis. In the present study, we found that current diagnostic cut-off for RSWA, as defined by the presence of ≥ 27% of 30-s REM sleep epochs contains any (either tonic or phasic) chin EMG activity combined with phasic EMG activity at bilateral FDS muscles, (“any chin + FDS 30-s”) suggested by the ICSD-3 edition, are suitable for PD patients.(18,19) Additionally, sensitivity

analysis has been performed considering different thresholds of RSWA (any chin EMG + FDS 30-s) ranging from 25% to 28%, leading to same results.

Moreover, the cut-off of RSWA included in the ICSD-3(18) (i.e. ≥27% of "any chin EMG + FDS 30-2) was found to be optimal in our large cohort of PD patients, as shown by the ROC curve analysis.

In the absence of vPSG recording, screening tools are available to detect the presence of clinical probable RBD. As well as clinical and PSG diagnostic criteria, these screening questionnaires have been mainly validated in idiopathic RBD patients consulting a sleep clinic, showing a very good sensitivity and specificity.(24,25) This high sensitivity was probably due to the characteristics of this population, who were seeking medical attention for their sleep problem, and were aware of their RBD condition.(25) Nomura and colleagues (33) evaluated the validity of RBDSQ in forty-five PD patients finding a sensitivity of 84% and specificity of 96% in detecting RBD. However, the clinical setting of that study potentially leading to referral bias (movement disorder vs sleep clinic) was not specified.
Recently, Stiasny-Kolster and colleagues have assessed the diagnostic value of the RBD screening questionnaire in two different samples of patients with PD consulting a sleep clinic, one of which included patients who underwent a RBD-focused interview prior to administration of RBDSQ, whereas the other underwent the screening questionnaire during routine work-up.(41) The authors found that diagnostic value of the RBDSQ strongly depends on the clinical setting and may be prejudiced by the individual's awareness on RBD.

In the present study, the RBDSQ administered prior the clinical interview showed 55.7% of sensitivity and 71.4% of specificity, with a positive predictive value of 77.3% and negative predictive value of 48.1%. On the other hand, the RBD1Q performed slightly better, showing 67.7% of sensitivity, 82.9% of specificity, with 87.5% of positive predictive value and 59.2% of negative predictive value. Interestingly, the two questionnaires showed a poor agreement with Cohen's K of 0.37, probably because they explore different symptoms dimensions. On the other hand, combined RBD1Q and RBDSQ performed better in detecting RBD in PD patients with 72.6% of sensitivity, 65.7% of specificity, 78.9% of PPV and 57.5% of NPV.

Our findings confirm the notion that RBD screening questionnaires alone are of limited usefulness in PD population. This is especially true in case of large sample epidemiological studies in PD whose results should be interpreted with caution. Also, our findings point out the importance of a comprehensive interview focused in sleep and conducted by a neurologist expert in sleep medicine.

In conclusion, using the best latent classes-derived model for diagnosis of RBD in PD, our study indicates that current RBD diagnostic criteria, including RSWA measures, are appropriate in PD population.(18) Moreover, results of the present study suggested that the diagnosis of provisional RBD according to ICSD-3 might be considered as a full-blown RBD in patients with Parkinson's disease.

As RBD in PD may be a marker of a malignant PD phenotype, with a heavier burden of both motor and non-motor symptoms as well as an increased frequency of a more rapid cognitive decline evolving toward dementia, the correct identification of RBD in PD appears to be critical. This may bear prognostic and perhaps therapeutic implication, when disease-modifying and neuroprotective therapies would be hopefully accessible.

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Table 1. Clinical and demographic features of PD patients and Healthy Control (HC).

	PD (n=111)	HC (n=25)
Males (n,%) [a]	67 (60.4%)	12 (48.0%)
Age (years) [a]	65.8±8.5	61.5±13.7
Bed partner (n,%)	43 (62.3%)	17 (68%)
PD duration (years)	7.9±5.0	NA
H&Y stage	2.0±0.7	NA
UPDRS III	17.8±10.0	NA
UPDRS-tot	33.4±17.5	NA
MoCA	25.4±3.6	NA
LEDD (mg)	748.9±454.0	NA
DA-LEDD (mg)	119.3±117.1	NA
SSRI (n)	10 (9.0)	0 (0)
Beta-Blockers (n)	5 (4.5)	0 (0)
Clonazepam (n)	9 (8.1)	0 (0)
Neuroleptics (n)	2 (1.8)	0 (0)
Melatonin (n)	1 (0.9)	0 (0)

PD: Parkinson's Disease;HC: healthy controls, H&Y: Hoehn and Yahr; UPDRS III: Unified Parkinson's disease rating scale III; MoCA: Montreal Cognitive Assessment; LEDD: Levodopa equivalent daily dose; DA-EDD: Dopamine-agonist equivalent daily dose; SSRI: selective serotonin re-uptake inhibitor. [a] age and sex were part of the frequency matching.

Table 2. Polysomnographic features of PD patients and healthy controls.

	PD (n=111)	HC (n=25)	p
TST (min)	329.7±60.3	490.6±71.6	NS
Sleep efficiency (%)	77.6±14.4	75.1±15.8	NS
WASO (min)	96.7±74.4	71.6±61.8	NS
N1 (%)	9.6±7.4	9.5±6.6	NS
N2 (%)	57.1±13.6	55.3±11.5	NS
N3 (%)	21.4±12.9	17.9±9.5	NS
REM (%)	11.7±6.8	17.4±4.3	0.0003*
REM (min)	38.8±24.7	66.9±23.0	0.0005*
PLMS (n)	121.5±142.8	65.6±71.6	0.03*
PLMS index	25.2±33.6	12.5±15.8	0.03*
AHI	4.7±7.6	4.9±9.4	NS

TST= Total Sleep Time; WASO= wake after sleep onset; N1= NREM sleep stage N1; N2= NREM sleep stage N2; N3= NREM sleep stage N3; PLMS = Periodic leg movements during sleep, AHI= Apnea/hypopnea index

Table 3. REM sleep EMG tone parameters in PD patients and healthy controls

	PD (n=111)	HC (n=25)	P<
Tonic EMG chin 30 s (%)	38.9±31.2	3.2±6.7	<0.001
Phasic EMG 2 s (%)	9.5±9.4	5.7±9.2	<0.001
Phasic EMG chin 3 s (%)	10.3±8.9	4.5±3.9	<0.001
Any EMG Chin 3 s (%)	38.0±27.5	7.0±5.5	<0.001
Any EMG chin + FSD 3 s (%)	42.2±27.5	9.7±7.1	<0.001
Any EMG chin + FSD 30 s (%)	43.6±31.2	3.8±6.8	<0.001

PD: Parkinson's Disease, RBD: REM Sleep Behavior Disorder, EMG: electromyography, 30-s: 30 seconds epoch; 2-s: 2 seconds mini-epochs; 3-s: 3 seconds mini-epochs; FDS: Flexor Digitorum Superficialis, REM: Rapid Eye Movements.

Table 4. Latent class model analysis

	PDRBD- (n=42)	PDRBD+ (n=69)	Accord %	Kappa	Se [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
History	2 (4.8%)	59 (85.5%)	89.2	0.78	85.5 [75.0;92.8]	95.2 [83.8;99.4]	96.7 [88.7;99.6]	80.0 [66.3;90.0]
Video	2 (4.8%)	61 (88.4%)	91.0	0.81	88.4 [78.4;94.9]	95.2 [83.8;99.4]	96.8 [89.0;99.6]	83.3 [69.8;92.5]
History + video	0 (0.0%)	51 (73.59%)	83.8	0.83	94.2 [85.5;98.4]	100 [91.6;100]	100 [93.9;100]	70.0 [56.8;81.2]
Any chin+ FDS (30-s)	5 (11.9%)	65 (94.2%)	91.9	0.83	94.2 [85.8;98.4]	88.1 [74.4;96.0]	92.9 [84.1;97.6]	90.2 [76.9;97.3]
Montréal	5 (11.9%)	61 (88.4%)	88.3	0.75	88.4 [78.4; 4.9]	88.1 [74.4;96.0]	92.4 [83.2;97.5]	82.2 [67.9;92.0]
Any chin	9 (21.4%)	65 (94.2%)	88.3	0.75	94.2 [85.8;98.4]	78.6 [63.2;89.7]	87.8 [78.2;94.3]	89.2 [74.6;97.0]
Any chin +FDS (3-s)	4 (9.5%)	61 (88.4%)	89.2	0.77	88.4 [78.4;94.9]	90.5 [77.4;97.3]	93.8 [85.0;98.3]	82.6 [68.6;92.2]

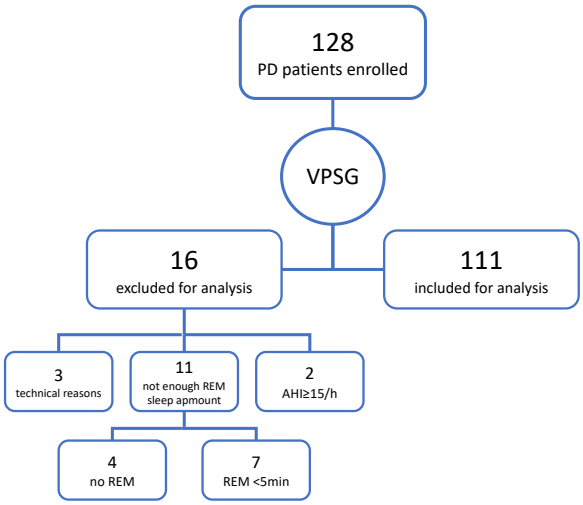
PDRBD-: Parkinson's disease patients without REM Sleep Behavior Disorder, PDRBD+: Parkinson's disease patients with REM Sleep Behavior Disorder, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, 30-s: 30 seconds epoch, 3-s: 3 seconds mini-epochs; FDS: Flexor Digitorum Superficialis; CI: confidence interval

Table 5. Performances of two screening questionnaires: RBD single question (RBD1Q) and RBD screening questionnaire (RBDSQ)

	PDRBD- (n=42)	**PDRBD+ (n=69)**	**Accord**	**K**	**Se % [95% CI]**	**Sp % [95% CI]**	**PPV % [95% CI]**	**NPV % [95% CI]**
RBD1Q (n=97)	6/35 (17.1%)	42/62 (67.7%)	73.2%	0.47	67.7 [54.7; 79.1]	82.9 [66.4; 93.4]	87.5 [74.8; 95.3]	59.2 [44.2; 73.0]
RBDSQ (n=97)	10/35 (28.6%)	34/62 (55.7%)	61.5%	0.25	55.7 [42.4; 68.5]	71.4 [53.7; 85.4]	77.3 [62.2; 88.5]	48.1 [34.0; 62.4]
QUEST (n=97)	12/35 (34.3%)	45/62 (72.6%)	70.1%	0.37	72.6 [59.8; 83.1]	65.7 [47.8; 80.9]	78.9 [66.1; 88.6]	57.5 [40.9; 73.0]

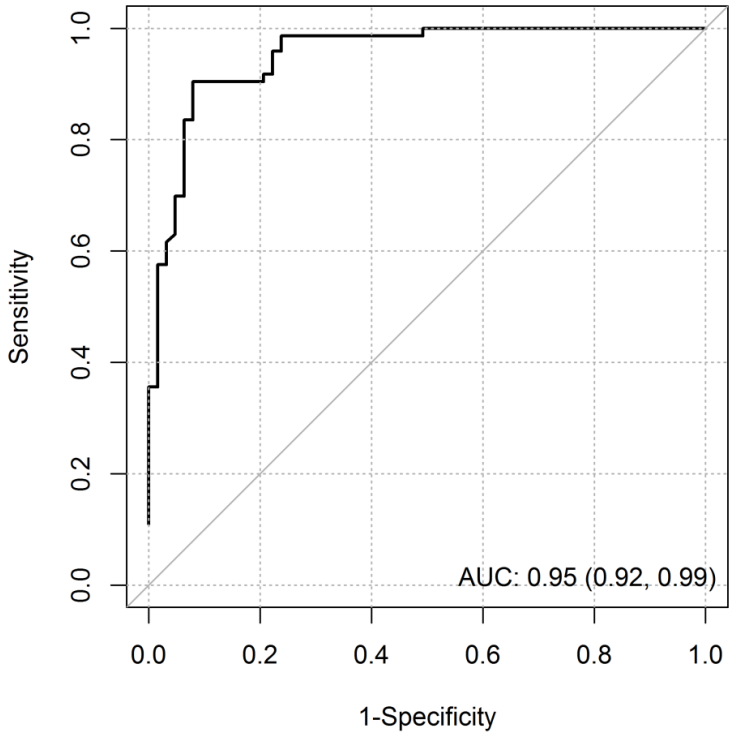
PDRBD-: Parkinson's disease patients without REM Sleep Behavior Disorder, PDRBD+: Parkinson's disease patients with REM Sleep Behavior Disorder, K: Cohen's K, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, QUEST: at least one of these questionnaires, RBD1Q and RBDSQ, scored positively for RBD.

Figure 1. Flow chart of the present study.



PD: Parkinson's Disease, VPSG: video-polysomnography, AHI: apnea/hypopnea index

Figure 2. ROC analysis results.



STUDY 3: LONGITUDINAL ASSESSMENT OF CLINICAL AND POLYSOMNOGRAPHIC FEATURES OF PARKINSON DISEASE WITH REM SLEEP BEHAVIOR DISORDER

Article to be submitted to Movement Disorders.

THE EVOLUTION OF REM SLEEP BEHAVIOR DISORDER IN PARKINSON'S DISEASE

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To be submitted to Movement Disorders

Running Title: Evolution RBD in PD

Key words: REM sleep behavior disorder, Parkinson's Disease,

ABSTRACT

INTRODUCTION: REM Sleep Behavior Disorder (RBD) in Parkinson's disease (PD) may be associated with a more malignant clinical phenotype. Despite its prognostic value, little is known about the evolution of RBD in PD. Motivated by the recurrent observation of an improvement of RBD symptoms in PD patients over time, we aimed to ascertain whether or not RBD is a stable feature in PD. We then prospectively evaluated clinical and neurophysiological features of RBD, including REM Sleep Without Atonia (RSWA), in PDRBD patients at baseline and at 3 years follow-up and assessed whether or not the changes in measures of RSWA over time parallel the progression of motor and non-motor symptoms of PD.

METHODS: Twenty-four (17 male, mean age 64.0±6.9 years) non-demented moderate to advanced PD patients (mean PD duration at baseline: 7.6±4.8 years) with RBD, underwent one-night full vPSG, and an extensive clinical and neuropsychological assessment at baseline and after a 3-years follow-up.

RESULTS: At follow-up, self-assessed frequency of RBD symptoms increased in 6 patients, decreased in 6 and remained stable in 10, while RSWA measures significantly increased in all subjects. At follow-up, patients had worse Hoehn and Yahr stage (p=0.02), increased dopaminergic dose (p=0.05) and they performed significantly worse in phonetic and semantic fluency tests (p=0.02; p=0.04). Changes in RSWA significantly correlated with the increase in dyskinesia (r=0.61, p=0.05) and motor fluctuation (r:0.54, p=0.03) scores, and with the worsening in executive functions (r0.78, p=0.001) and in visuo-spatial perception (r=-0.57,p=0.04).

CONCLUSION: Despite subjective improvement of RBD symptoms in one-fourth of PD patients, all RSWA measures increased significantly at follow up, and their change correlated with clinical evolution of certain motor and non-motor symptoms. RBD is a long-lasting feature in PD and RSWA is a marker of progression of the disease.

INTRODUCTION

REM sleep behavior disorder (RBD) is a parasomnia characterized by intermittent or complete loss of the normal muscle atonia during REM sleep associated to dream-enacting behaviors.[1,2] RBD is found in up to 60% of patients with Parkinson's disease (PD) and is usually associated with a heavier burden of disease in terms of both motor and non-motor symptoms.[3–8]

Indeed, PD patients with RBD (PDRBD) were found to have more rigid akinetic forms, axial symptoms, and levodopa-induced dyskinesia[9–11] These patients also have increased autonomic dysfunction, especially orthostatic hypotension (OH), [12,13] cognitive deficits, and an increased risk of dementia[3–5,14,15] Actually, prospective studies have shown a higher incidence of mild cognitive impairment (MCI) and dementia in PDRBD, concluding that RBD is the strongest determinant for dementia development in PD, followed by MCI and orthostatic hypotension.[16,17] Recently, a prospective cohort study using cluster analysis have confirmed that PD associated with RBD, associated to orthostatic hypotension and multidomain MCI, represents a diffuse/malignant phenotype characterized by with a more rapid decline and worst prognosis.[18] Finally, PDRBD patients may show an increased risk to develop neuropsychiatric complication related to dopaminergic replacement therapy (DRT), like impulse control disorder, with possible management implications.[19,20] Therefore, the diagnosis of RBD in PD may bear therapeutic and prognostic implications, requiring careful monitoring of motor and non-motor complications, in order to adjust early symptomatic treatments. The prevalence of RBD in de novo PD is up to 60%. A longitudinal vPSG study performed in de novo PD has recently showed a significant increase of RSWA after two years of follow-up.[21]

However, it is not known whether RBD represent a stable marker in PD over time. Actually, an improvement of RBD symptoms, such a decrease in the frequency or in the intensity of sleep motor behaviors, is occasionally reported by some PD patients as their disease progresses. Longitudinal studies on RBD assessed by questionnaires in PD patients led to controversial results, reporting both improvement and worsening or stability of RBD symptoms over time. [6,8,22]

S longitudinal vPSG study reports a stability of RVBD over time but patients were only tested at a very early stage of PD for a period of two years of follow up. [21]

In the present study, we aimed to prospectively evaluate clinical and neurophysiological features of RBD in moderate to advanced PD patients, including measures of RSWA, by means of vPSG recording, at baseline and after a mean interval of 3 years, in order to ascertain whether the diagnosis of RBD remain stable over time. Second, we aimed to assess the relationships between the evolution of both clinical and vPSG measures of RBD and the progression of motor and non-motor symptoms of PD, including neuropsychiatric and behavioral aspects, in order to ascertain whether or not the changes in measures of RSWA over time parallel the progression of the clinical symptoms in PD.

METHODS

Subjects

Twenty-four (17 male, mean age 64.0±6.9 years) PD patients with vPSG confirmed RBD were enrolled. All patients recruited had a clinical diagnosis of PD according to the United Kingdom Parkinson Disease brain bank criteria and had a diagnosis of RBD, according to current diagnostic criteria.[1,23]

All patients underwent one-night full attended vPSG recording, together with an extensive clinical and neuropsychological assessment at baseline and at 3-years follow-up.

Demographic and clinical data, such as sex, age, duration of PD, PD severity as measured by the Unified Parkinson Disease Rating Scale (UPDRS) and the Hoehn and Yahr staging system, dose and duration of DRT and other treatment were assessed at baseline at after 3-years follow-up. Non-motor symptoms were investigated using the Non-motor symptoms questionnaire (NMSSQ), UPDRS part 1, and the Epworth sleepiness scale (ESS).

An in-depth sleep-focused interview including RBD duration, current self-reported frequency of RBD episodes during the last month, and presence of bedpartner, was performed in all patients by a neurologist expert in both movement disorders and sleep medicine (MLF, MF, AM).

The use of drugs potentially affecting RSWA such as selective serotonin/noradrenaline reuptake inhibitors (SSRI, SNRI), tricyclic antidepressant, benzodiazepines, melatonin and beta-blockers, was assessed. The total Levodopa equivalent daily dose (LEDD), and the dopamine agonist Levodopa equivalent daily dose (DA-LEDD) were calculated according to Tomlinson.[24]

All participants gave informed and written consent for participate, according to the Declaration of Helsinki, and the local ethical committee approved the study.

Polysomnographic recordings and REM analysis

All patients underwent a full night attended vPSG recording in sleep laboratory with digital polysomnography according to the American Academy of sleep Medicine (AASM) recommendations, at baseline at after 3-years follow-up.[25] vPSG were performed with digitally synchronized videography and the following montage: electroencephalographic (EEG) leads (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), left and right electrooculography (EOG) channels, bilateral surface electromyographic (EMG) channels (submentalis, flexor digitorum superficialis (FDS) on

upper limbs, tibialis anterior on lower limbs), and electrocardiography (ECG). The respiratory analysis included nasal airflow, which was recorded by both thermistor and nasal pressure sensor, thoracic and abdominal respiratory effort, oxygen saturation recording by cutaneous finger pulse-oxymeter and microphone. In order to detect all motor activity, patients slept uncovered with toleration of a light sheet for their comfort.

Sleep stage will be scored according to AASM criteria, with allowance to chin EMG muscle tone during REM sleep.[25] The following sleep data were collected for descriptive purpose: total sleep time, sleep efficiency (SE), wake after sleep onset (WASO), number of REM sleep episode, percentage of time in each sleep stage (N1, N2, N3, R), arousal index (arousal-i), periodic limb movements index (PLMSi), Apnea-hypopnea index (AHI), oxygen-desaturation index (ADI).[25]

REM sleep without atonia (RSWA) was assessed if REM sleep lasted ≥5 minutes, since shorter duration was believed to be insufficient for a reliable assessment of this parameters.[26] RSWA was manually quantified according to two previously published methods, namely the Montréal,[27,28] adapted to 30-sec epochs,[29] and the SINBAR method.[30–32] REM sleep epochs were carefully inspected for artifacts, such as increased muscle tone caused by respiratory arousal. The background EMG activity for each participant was considered as the minimum EMG amplitude during NREM sleep.

The visual scoring of RSWA was performed by a neurologist expert in sleep medicine (MF), who was blinded to RBD clinical status.

RSWA was assessed according to its different components. First, tonic EMG activity was assessed according to the Montréal method, adapted to 30-s epochs.[28,29] Indeed, each epochs was

scored as “tonic” if more 50% of the 30-s epoch duration includes increased sustained EMG activity, with an amplitude at least twice the background EMG activity, or more than 10 μV.[28,29] RSWA was defined if ≥30% of 30-s REM sleep epochs contains tonic chin EMG activity.[28,29] Phasic chin EMG density was assessed according to the SINBAR method as the percentage of 3-s mini-epochs containing phasic EMG events (“phasic 3-s”) lasting 0.1 to 5-s with an amplitude exceeding twice the background activity.[30,32] Furthermore, each 3-s REM sleep mini-epochs was scored as having or not “any” chin EMG activity, namely either tonic and/or phasic EMG activity within the same mini-epoch (“any chin 3-s), in order to taking into account also EMG activity lasting from 5 to 15 s.[30,32] Finally, percentage of any (phasic and/or tonic) chin EMG activity combined with phasic EMG activity in bilateral FDS muscle was assessed in both 3-sec (“any chin + FDS 3-s”) and in 30-s epochs (“any chin + FDS 30-s”).[30,32] According to the SINBAR method, RSWA is currently defined by either ≥16.3% of 3-s REM sleep mini-epochs contains phasic chin activity (“phasic 3-s”), ≥18% of 3-s REM sleep mini-epochs contains any chin EMG activity (“any chin”), ≥32% of 3-s REM sleep epochs contains any chin EMG activity combined with phasic EMG activity at bilateral FDS muscles (“any chin + FDS 3-s”), and by ≥ 27% of 30-s REM sleep epochs containing any (either tonic or phasic) chin EMG activity combined with phasic EMG activity at bilateral FDS muscles, (“any chin + FDS 30-s”).[30–32]

Neuropsychological assessment

A broad spectrum of cognitive functions was assessed at baseline at after 3-years follow-up. Neuropsychological features were evaluated by means of Montreal Cognitive Assessment (MoCA) for global cognitive functions; Digit span for verbal short-term memory; California Verbal Learning test (CVLT) for episodic verbal memory; semantic and phonemic verbal fluency test for fluency and sematic memory; modified Wisconsin Card Sorting Test (MWCST) for abstract reasoning, attention,

conceptualization, and ability to change problem-solving strategy; Stroop test for measure selective attention, cognitive flexibility and processive speed; Digit span backward to evaluate working memory; Visual Object and Space Perception Battery (VOSP) and Rey-Osterrieth complex figure for visuospatial function; Ekman Test to assess emotion recognition.

Psycho-behavioral aspects were also assessed, namely impulse control disorders according to standard diagnostic criteria,[33] hypo- and hyperdopaminergic behaviors with the Ardouin scale of behavior in Parkinson's Disease,[34] apathy using the Lille Apathy Rating Scale (LARS)[35], depression with the Hospital Anxiety Depression Scale-depression sub-score (HADS), [36] aggressiveness by means of the Aggression Questionnaire (AQ), and impulsivity with the Urgency, Premeditation, Perseverance and Sensation Seeking Scale (UPPSS).[37]

Statistical analysis

The statistical analyses were carried out using the statistical software Stata (version 13, StataCorp, College Station, US). All statistical tests were conducted for a two-sided type I error at 5%. Continuous variables were described as mean and standard-deviation or median and interquartile range, according to statistical distribution (assumption of normality studied using Shapiro-Wilk test). Then, paired comparisons were conducted using paired Student t-test or Wilcoxon test if the assumptions of t-test were not met. The results were expressed with Hedge's effects-size and 95% confidence interval. Finally, the relationships between quantitative variables were analyzed with correlation coefficients (Pearson or Spearman, according to statistical distribution), applying a Sidak's type I error correction in order to consider multiple comparisons.

RESULTS

Subjects

Of the original 24 patients, two did not performed vPSG recording at follow-up for personal reasons, while all of them had clinical assessment.

At baseline, only 1 patient was treated with drugs known to potentially increase RSWA (beta-blockers), while, at 3-years follow-up, a total of 3 patients were taking selective serotonin re-uptake inhibitor (SSRI) and 2 patients were on beta-blockers. On the other hand, 1 only patient was taking clonazepam at baseline, while 2 patients were on this treatment at follow-up.

The clinical and demographic features of patients at baseline and at 3-years follow-up are summarize in Table 1.

At 3-years follow-up, patients had significantly higher H&Y (p=0.02), and LEDD (p=0.05), while the others severity measure of PD symptoms did not show a significant worsening compared to baseline assessment. However, neuropsychological assessment showed that patients performed significantly worse in the phonetic and semantic fluency (p=0.02; p=004) at follow-up.

Six out of 22 patients reported reduced frequency of RBD behaviors at follow-up, 6/22 an increased frequency of RBD episodes, while 10/22 reported no changes in terms of frequency at follow-up compared to baseline. Also, 17 (77%) out of 22 patients had a bed-partner. No difference was observed in the percentage of bedpartners between those who reported improvement and those who did not.

Polysomnographic results and REM analysis.

The polysomnographic results at baseline and at 3-years follow-up are reported in Table 2.

PD patients had a significant higher TST (p=0.02) and a significant higher amount of NREM sleep stage N1 (p=0.05) at follow-up compared to baseline.

For the manual quantification of RSWA, a total of 1784 30-s REM sleep epochs, 17840 3-s REM sleep mini-epochs have been analyzed at baseline. On the other hand, a total of 1949 30-s REM sleep epochs, and 19490 3-s REM sleep mini-epochs, were analyzed at follow-up.

A total of 80 (0.04%) REM sleep epochs, 802 (0.05%) 3-s REM sleep mini-epochs were rejected for the analysis, because of the presence of respiratory-related arousal EMG activity, at baseline. Similarly, a total of 83 (0.04%) 30-s REM sleep epochs, and 833 (0.04%) 3-s REM sleep mini-epochs were excluded from the analysis, at follow-up. The RSWA parameters are shown in Table 2.

At follow-up, all PDRBD patients were still found to fulfill the diagnostic criteria for RBD.[1] Overall, RSWA parameters were found significantly increased at follow-up compared to baseline. In particular, all but 4 patients showed an increase of tonic EMG activity, while the totality of patients showed an increase in chin phasic EMG. Furthermore, increase in “any chin” EMG activity was found in all but 3 patients, increase in “any chin +FDS 3-s” in all but 5 patients, while “any-chin +FDS 30-s” increased in all but 4 patients. Therefore, it has to be pointed out that, despite a few isolated decreases in some of the RSWA parameters, all RSWA measures exceeded their normal threshold at follow-up.

No correlations were found between RSWA measures and subjective symptoms of RBD assessed at both baseline and follow-up. Furthermore, no difference in the increase of RSWA measures over time were observed between patients reporting a clinical improvement of their RBD symptoms vs. those who worsened or remained stable over time.

Figure 1 resumes the changes of sleep and RSWA features together with clinical and neuropsychological features of PD at 3-years follow-up compared to baseline. At follow-up, patients were found to have a worse H&Y score, a higher LEDD and a worse performance in some executive functions, namely in phonemic and semantic fluencies.

Relationship between RSWA changes and progression of Parkinson's Disease

A significant correlation was found between changes in RSWA and variations in the severity of Levodopa induced dyskinesia (LiDs) ($r=0.61$, $p=0.05$) and the severity of motor complications assessed with the UPDRS-IV ($r=0.54$, $p=0.03$), the three increasing over time. Increase in RSWA was also found to correlated with worsening in executive functions, namely in episodic verbal memory ($r=0.78$, $p=0.001$), and in visuo-spatial perception ($r=-0.57$, $p=0.04$).

No other significant relationships were found between RSWA changes and variations in motor and non-motor symptoms of PD overtime.

DISCUSSION

In the present study, self-assessed frequency of RBD symptoms increased in 6 out of 22 (27%) patients, decreased in 6/22 (27%) and remained stable in 10/22 (42%), while all RSWA measures significantly increased over time. Moreover, no correlations were found between RSWA measures and subjective symptoms of RBD assessed at both baseline and follow-up. Furthermore, no difference in the increase of RSWA measures over time were observed between patients reporting a clinical improvement of their RBD vs. those who worsened or remained stable over time.

This is the first study assessing the evolution of RBD in moderate to advanced PD patients after 3-years of follow-up. On the clinical ground, RBD symptoms are occasionally reported to improve or disappear in PD patients as the disease progresses. So far, only another longitudinal vPSG study has assessed RSWA evolution in de-novo PD patients after 2 years of follow-up, finding that RSWA increased significantly after 2 years and RBD does not resolve over time or with dopaminergic treatment.[22] However, in this study patients were evaluated at the very early stage of PD and for a shorter 2-years period. In the present study, we prospectively evaluated clinical and v-PSG features of RBD in more advanced PD patients, with a mean disease duration of 7.5±4.7 years, and a mean duration of treatment of 6.7±4.5 years, at baseline. The main finding of the present study was that all PDRBD patients at baseline were found to still fulfill the diagnostic criteria for RBD at follow-up and that all RSWA measures significantly increased after 3-years from baseline, regardless subjective evolution of clinical symptoms.[28,31] Furthermore, despite the fact that we observed isolated decreases in RSWA parameters in some patients, the totality of RSWA measures in each patients exceeded their normal threshold at follow-up.

Thus, these results complete the results obtained in PD at very early stage and support the notion that v-PSG features of RBD in moderate to advanced PD patients does not resolve over time or with DRT, but rather show a worsening. This suggests an aggravation in motor control during REM sleep over time from the onset of PD up to advanced stage of the disease, possibly paralleled by a progressive neurodegenerative process within the neuronal network that modulate muscle tone during REM sleep. Based on our results, it may be suggested that RBD status is a stable marker in PD patients, while RSWA appears to progress over time. On the other hand, similarly to PD, also in idiopathic RBD (i.e. RBD without evidence of other neurological disease, that may often precede by several years the clinical onset of PD), longitudinal studies have shown that RSWA increases over time, as a dynamic marker.[38,39]

In our study, a significant worsening in Hoehn & Yahr stage, but not in UPDRS motor score, as well as in executive functions, particularly in semantic and phonemic verbal fluency, was observed at three-years follow-up in PDRBD. It is known that RBD strongly predict cognitive decline in PD.[18,40] The prominent worsening in executive functions, rather than in motor functions, observed in our study may herald the rapid cognitive decline observed in PDRBD by other studies, although the lack of a control group represented by PD patients without RBD prevents to draw conclusions. No significant worsening was observed in other motor or non-motor symptoms and in psycho-behavioral features. Limitations of the study are the small sample size and the relatively short follow-up. Indeed, longer follow-up might allow to better appreciate the correlation between RSWA changes and motor and non-motor symptoms progression.

Despite no significant worsening of motor function was observed in our patients at 3-years follow-up, except for H&Y stage, in the present study, a significant correlation was observed

between the extent of the increase in RSWA and worsening of LiDs and motor complications, the three increasing over time. Increase in RSWA was also found to correlated with worsening in executive functions, namely in episodic verbal memory, and in visuo-spatial perception. The fact that increasing RSWA parallels the development of motor complication, rather than the simple worsening in motor performance, and that RSWA changes also correlate with impending cognitive dysfunction, strongly support the notion of PDRBD as a diffuse/malignant subtype with a more rapid progression than the usual forms, although the lack of a control group represented by PD patients without RBD again prevents to draw formal conclusions.

Another limitation of the study was that the subjective severity of RBD was assessed mainly in terms of frequency of RBD episodes, as we did not precisely assess the intensity (i.e. the degree of violence displayed during the behavior or its complexity), which is a different component of the RBD severity. Intensity of episodes may broadly fluctuate in the same patient within the same night and between nights, as an expression of a night-to-night variability, and its evaluation mostly rely on witness.[41] Actually, a severity scale of RBD episodes has been developed and used by the investigators to rate the behaviors obtained during in-lab vPSG, while subjective at-home assessment of RBD severity is not easy. Currently, it is not known whether a decrease in RBD frequency would be paralleled by an increase or a decrease in the intensity of episodes and subjective assessment is of limited value. Further studies on the evolution of RBD should try to assess different dimensions of at-home sleep motor behaviors, perhaps developing new tools to assess RBD severity.

To sum up, the present study found for the first time that RBD status represent a stable feature in moderate to advanced PD, and that RSWA consistently worsens over time, regardless

clinical subjective symptoms. In light of the role of RBD in predicting more rapid decline in PD, especially in cognitive functions, the presence of RBD as a stable feature may prompt the inclusion of early PDRBD patients in neuroprotective and disease-modifying trials, when they will be hopefully available. Further studies in larger cohort of PD patients are needed to confirm the role of RSWA as a marker of disease progression in PD.

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Table 1. Demographical and clinical data of PD patients at baseline and at 3-years follow-up

	Baseline (n=24)	Follow-up (n=24)	P	Effect Size
Age (Yrs)	64.0±6.9	66.6±7.0	nc	nc
Sex (n, %)	17 (68)	17 (68)	nc	nc
Bedpartner (n, %)	17 (68)	17 (68)	nc	nc
PD Duration (Yrs)	7.6±4.8	10.8±5.2	nc	nc
RBD Duration (Yrs)	6.4±5.6	9.4±5.7	nc	nc
H&Y	2.0±0.6	2.2±0.6	0.0242*	-.4078
UPDRS-I	2.4±1.8	2.6±1.9	0.6612	-.1027
UPDRS-II	9.3±5.6	10.8±6.0	0.1015	-.2416
UPDRS-III	20.2±10.6	22.3±13.8	0.2714	-.1649
UPDRS-IV	3.0±3.3	4.2±3.9	0.1596	-.3191
UPDRS-TOT	35.0±17.3	39.8±20.6	0.0977	-.2497
Dysk-Score	0.9±1.4	1.0±1.7	0.2735	-.0701
Fluct-Score	0.9±1.6	1.8±1.9	0.2053	-.5066
NMS	9.1±3.3	11.1±4.9	0.2505	-.4488
ESS	11.1±5.4	10.0±5.3	0.3388	-.2161
DRT Duration (Yrs)	6.7±4.5	9.2±5.0	nc	nc
LEDD (mg/day)	735.4±356.7	921.9±433.9	0.0515*	-.4716
DA-LEDD (mg/day)	102.4±139.8	136.9±128.5	0.1260	-.2526
Clonazepam	1	2	nc	nc
Melatonin	0	0	nc	nc
Ssri/Snri	0	3	nc	nc
Betablocker	1	2	nc	nc

PD= Parkinson's disease; RBD= REM sleep behavior disorders; H&Y= Hoehn and Yahr; UPDRS= Unified Parkinson's disease rating scale; Dysk= dyskinesia; Fluct= fluctuation; NMS= non-motor symptoms questionnaire; ESS= Epworth sleepiness scale; DRT= dopaminergic replacement theraphy; LEDD= Levodopa equivalent daily dose; DA-LEDD= dopamine agonist Levodopa equivalent daily dose; SSRI = selective serotonin re-uptake inhibitor. NC= not calculated. Data are expressed as mean and standard deviation or number (percentage of total).

Table 2. Neuropsychiatric features of PD patients at baseline and at 3-years follow-up

	Baseline (n=24)	follow-up (n=24)	p	effect size
Global cognitive fx (MoCA)	24.6±3.7	24.3±4.0	0.5159	.0890
Impulsivity (UPPSS)	97.17±38.8	86.3±15.2	0.3498	.0994
Aggressiveness (AQ)	60.4±3.4	63.9±3.9	0.4117	-.4664
Psycho-behaviors (ECMP_TOT)	6.8±4.9	7.6±5.0	0.5934	-.1177
Impulsive behaviors (ECMP_ICD)	1.5±1.9	1.1±1.3	0.4688	.2673
Visuo-constructional fx (Rey figure)	31.9±2.6	31.8±3.1	0.9165	-.2292
Apathy (LARS)	–24.3±8.2	–22.9±11.5	0.5813	-.1938
Long term verbal memory (CVLT)	1.0±2.0	0.6±0.9	0.9289	.1748
Inhibition and selective attention (Stroop)	2.4±9.9	–0.7±10.4	0.2583	.1708
Short-term memory (Digit span forward)	6.1±1.0	6.1±0.8	0.8364	.1120
Working memory (Digit span backward)	4.6±1.2	4.4±1.0	0.5328	.0489
Visuo spatial perception (Vosp Lett)	18.5±1.7	18.6±1.6	0.6714	-.0871
Visuo spatial perception (Vosp Locch)	8.7±1.4	8.4±3.5	0.7688	.0670
Semantic fluency	30.3±9.4	25.4±10.4	0.0039*	.6181
Phonemich fluency	22.7±7.7	19.3±8.2	0.0167*	.6418
Emotion recognition (Ekman)	46.9±8.2	39.2.2±19.9	0.0821	.4283
Executive functions (MWSCT)	4.0±4.8	5.9±7.6	0.1244	-.1233
Depression (HADS)	6.2±4.3	3.6±2.8	0.0525	.7451

Fx= functions; Moca= Montreal Cognitive Assessment; UPPSS= Urgency, Premeditation, Perseverance and Sensation Seeking Scale; AQ= aggression questionnaire; ECMP= Ardouin scale of behavior in Parkinson's Disease; ECMP-ICD= ECMP sub-score Impulsion control disorder; LARS= Lille Apathy Rating Scale; CVLT= California Verbal Learning test; VOSP= Visual Object and Space Perception Battery; MWCST= modified Wisconsin Card Sorting Test; HADS= Hospital Anxiety Depression Scale-depression sub-score

Table 3. Polysomnography features of PD patients at baseline and at 3-years follow-up

	Baseline (n=24)	follow-up (n=22)	p	effect size
TST (min)	350.7±67.1	389.8±85.8	0.0186*	-.6834
SE (%)	73.8±10.1	75.0±13.6	0.7154	-.3733
Awakening (n)	26.0±16.5	28.2±15.8	0.5393	-.1302
Arousal-i (events/h)	8.2±4.7	9.9±6.5	0.2832	-.0935
WASO (min)	92.1±48.9	99.1±61.7	0.6365	.0177
N1 (%)	11.9±6.7	16.0±8.1	0.0521*	-.3834
N2 (%)	55.1±12.2	54.4±7.6	0.7738	.1258
N3 (%)	22.0±14.3	18.3±8.6	0.2008	.2321
R (%)	11.0±5.8	11.4±6.8	0.7644	-.1425
REM duration (min)	39.0±22.6	46.2±33.1	0.1267	-.3653
PLMSi (events/h)	21.1±19.2	21.8±22.4	0.8839	.0292
AHI (events/h)	5.2±9.7	6.5±9.8	0.6539	-.1948

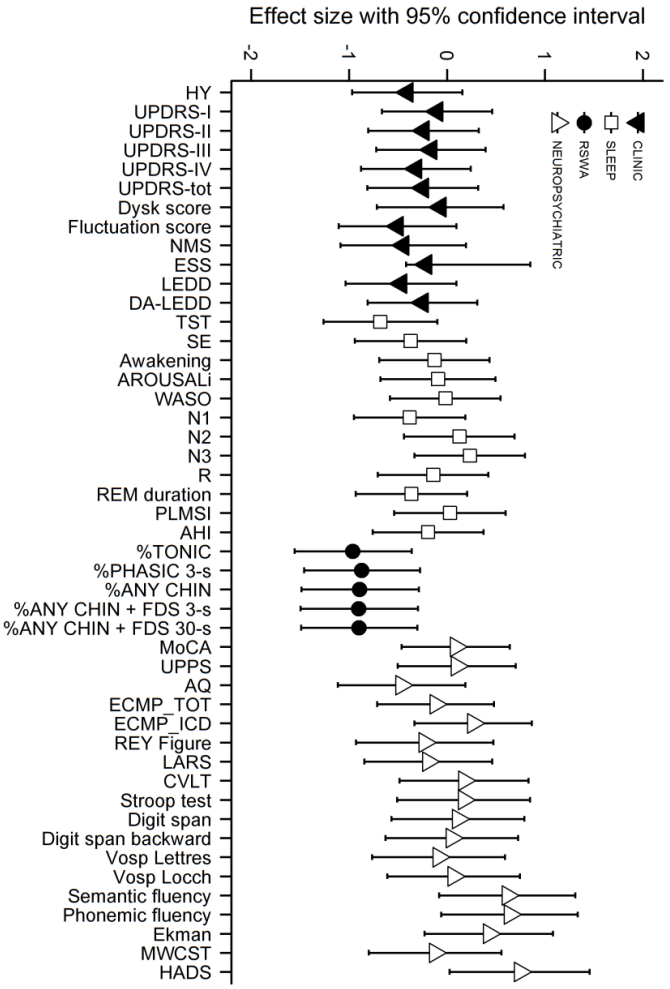
PD= Parkinson's Disease; TST= total sleep time; SE= sleep efficiency; Arousal-i= arousal index; WASO= wake after sleep inset; N1= NREM sleep stage 1; N2= NREM sleep stage 2; N3= NREM sleep stage 3; R = REM sleep; PLMSi= periodic limb movements in sleep index; AHI apnea hypopnea index. Data are expressed as mean and standard deviation or number (percentage of total).

Table 4. REM sleep without atonia measures in PD patients at baseline and at 3-years follow-up

	Baseline (n=24)	follow-up (n=22)	p	effect size
Tonic (%)	51.2±21.5	73.1±23.4	0.0002*	.9621
Phasic 3-s (%)	10.0±7.3	18.0±11.0	0.0068*	-.8706
Any chin (%)	43.7±20.5	68.6±25.7	0.0007*	-.8916
Any chin + FDS 3-s (%)	48.0±18.5	71.5±24.2	0.0006*	-.9004
Any chin + FDS 30-s (%)	55.3±21.4	74.3±25.3	0.0007*	-.8992

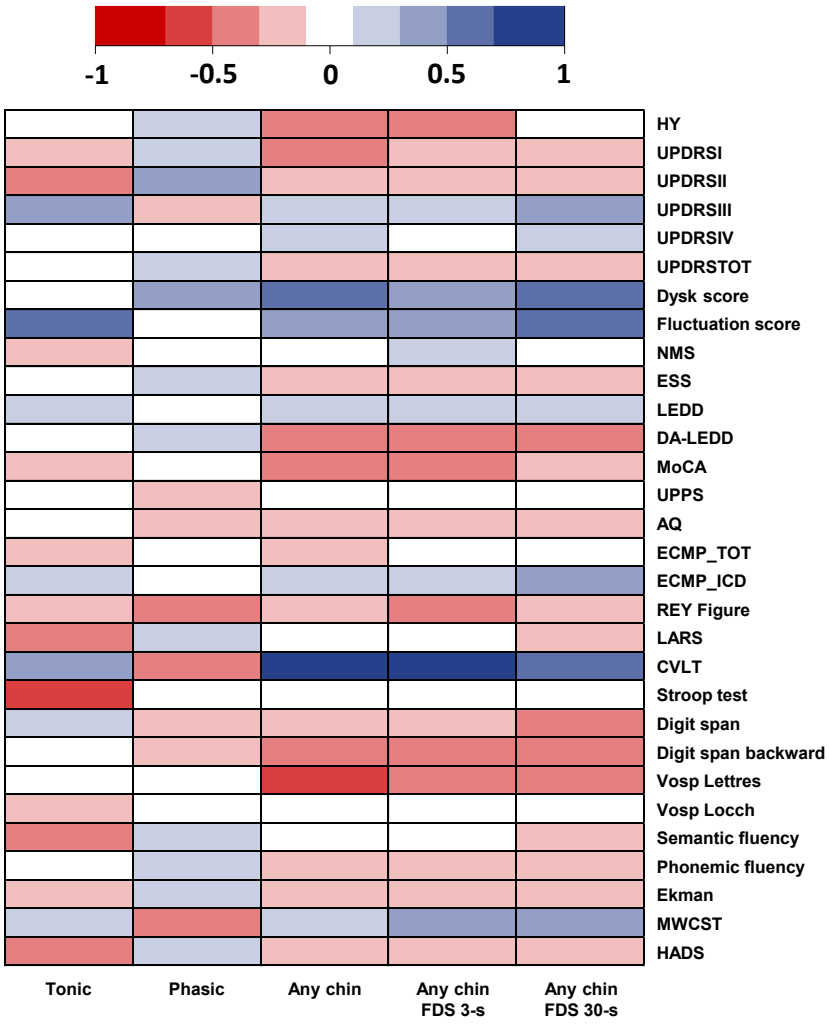
PD= Parkinson's Disease; FDS= Flexor digitorum superficialis. Data are expressed as mean and standard deviation.

Figure 1. Clinical, neuropsychological and polysomnographic changes at 3-years follow-up in PDRBD patients.



H&Y= Hoehn and Yahr; UPDRS= Unified Parkinson's disease rating scale; Dysk= dyskinesia; Fluct= fluctuation; NMS= non-motor symptoms questionnaire; ESS= Epworth sleepiness scale; DRT= dopaminergic replacement theraphy; LEDD= Levodopa equivalent daily dose; DA-LEDD= dopamine agonist Levodopa equivalent daily dose; SSRI = selective serotonin re-uptake inhibitor. Data are expressed as mean and standard deviation or number (percentage of total); TST= total sleep time; SE= sleep efficiency; AROUSALi= arousal index; WASO= wake after sleep inset; N1= NREM sleep stage 1; N2= NREM sleep stage 2; N3= NREM sleep stage 3; R = REM sleep; PLMSi= periodic limb movements in sleep index; AHI apnea hypopnea index. Data are expressed as mean and standard deviation or number (percentage of total); Moca= Montreal Cognitive Assessment; UPPSS= Urgency, Premeditation, Perseverance and Sensation Seeking Scale; AQ= aggression questionnaire; ECMP= Ardouin scale of behavior in Parkinson's Disease; ECMP-ICD= ECMP sub-score Impulsion control disorder; LARS= Lille Apathy Rating Scale; CVLT= California Verbal Learning test; VOSP= Visual Object and Space Perception Battery; MWCST= modified Wisconsin Card Sorting Test; HADS= Hospital Anxiety Depression Scale-depression sub-score.

Figure 2. Correlation between RSWA changes and progression of PD symptoms.



H&Y= Hoehn and Yahr; UPDRS= Unified Parkinson's disease rating scale; Dysk= dyskinesia; Fluct= fluctuation; NMS= non-motor symptoms questionnaire; ESS= Epworth sleepiness scale; LEDD= Levodopa equivalent daily dose; DA-LEDD= dopamine agonist Levodopa equivalent daily dose; Moca= Montreal Cognitive Assessment; UPPSS= Urgency, Premeditation, Perseverance and Sensation Seeking Scale; AQ= aggression questionnaire; ECMP= Ardouin scale of behavior in Parkinson's Disease; ECMP-ICD= ECMP sub-score Impulsion control disorder; LARS= Lille Apathy Rating Scale; CVLT= California Verbal Learning test; VOSP= Visual Object and Space Perception Battery; MWCST= modified Wisconsin Card Sorting Test; HADS= Hospital Anxiety Depression Scale-depression sub-score; FDS= flexor digitorum superficialis muscles.

Conclusions and perspective

Converging evidences indicate that REM sleep behavior disorder in Parkinson's disease represent a biomarker of a more widespread neurodegenerative process associated with a malignant phenotype with worse prognosis. However, diagnosing RBD in PD patients is often challenging for several reasons, mostly because PD patients may have mild RBD episodes, characterized by presence of RSWA with isolated muscle jerking or simple vocalization that may go unnoticed by the patient or the bed-partner. On the other hand, screening tools and current diagnostic criteria for RBD have been mainly established based on clinical features of I-RBD. This is especially true for the RSWA diagnostic cut-off. Additionally, the evolution of PSG markers of RBD in moderate to advanced PD is currently unknown. Indeed, elucidating the time course of RBD would be crucial to determine whether RBD is a stable and reliable marker in PD over time.

In the present thesis, we first focused on methodological aspects related to quantification of RSWA in PD. We aimed to assess the concordance of two visual scoring methods, namely the Montreal and the SINBAR approaches, and to compare them to the REM sleep Atonia Index (RAI) automated method, in a large cohort of PD patients consecutively seen in Movement Disorder Centers, in order to evaluate their correct classification accuracy and reciprocal agreement, as well as their role in the clinical diagnosis of RBD in PD. Results of the first study showed high sensitivity, specificity and accuracy of both visual scoring methods for the assessment of RSWA, with perfect agreement between them, especially when considering tonic EMG activities alone or in combination with phasic activity (e.g. "any chin EMG activity"), in PD patients. On the other hand, visual parameters related to phasic EMG activity alone, assessed by any manual scoring method, showed lowest sensitivity and accuracy. Moreover, we found substantial agreement between the two visual scoring methods, Montreal and SINBAR, and the automatic RAI, when they consider tonic EMG activity alone or in combination with phasic activities, but not for phasic EMG activity alone. Finally,

these findings confirm previous published data which have shown good correlation between Montreal method and RAI in patients with I-RBD, or RBD associated to MSA or narcolepsy.[113,117,127]

Based on results of our study, we proposed that the automatic scoring of RSWA together with visual inspection of video recorded behaviors would be the first-line method to assess RSWA in PD patients, while visual scoring methods for RSWA should be used in doubtful cases.

Furthermore, our findings have shown that the addition of FDS leads to the usual full PSG montage did not seem to provide an enhanced diagnostic power compared to the assessment of the chin EMG activity alone, in quantifying RSWA in PD patients. Indeed, including FDS channels within the routine vPSG work-up might be time-consuming and less suitable in clinical setting. However, the SINBAR group have shown that isolated recording of the chin EMG activity in I-RBD patients did not detect the totality of motor events seen in the video, mainly because they often involved only the limbs, and that the simultaneous evaluation of the chin and of bilateral FDS EMG activity detected the majority of the behavioral manifestations.[115]. Although FDS leads did not increase the diagnostic power in our cohort of PD patients, we agreed that recording bilateral FDS activity might be of great help in detecting behavioral episodes when increased EMG activity is observed in these leads on PSG recordings.

Interestingly, in the present study we found that PD patients with RBD showed more tonic rather than phasic EMG activity increase during REM sleep, suggesting a peculiar neurophysiological RBD phenotype in PD, different from those idiopathic or associated with narcolepsy and more similar to that found in patients with MSA.[117,127–129] Although Iranzo et al. did not find any difference in RSWA pattern between 45 PDRBD and 39 idiopathic RBD, other studies have shown that PDRBD may differ from iRBD.[117,127,129] The peculiar neurophysiological RBD phenotype in PD patients,

characterized by more tonic rather than phasic EMG activity, might be related to different paces of degeneration within neuronal networks implicated in modulation of muscle tone in REM sleep.

Then, in a second study, we sought to determine the validity and accuracy of both screening tools and diagnostic criteria for RBD, including RSWA parameters, in PD patients consecutively consulting a Movement Disorder Clinic. ICDS-3 diagnostic criteria for RBD, published by the American Academy of Sleep Medicine, have been mainly established based on clinical features of I-RBD.[2,85] This is especially true for the RSWA diagnostic cut-off, that has been established based on norms including only small number (n=15) of PD patients.[86]

To this aim, latent class models were applied to create an unobserved (latent) variable. The latent class model analysis allows to identify patients considering their characteristics, as having RBD (class 1) or not having RBD (class 2). These models rely on the maximum likelihood estimation.

According to the best latent classes-derived model, patients has been classified as having RBD if showing either history of dream-enactment behaviors ("history") and vPSG-documented REM sleep-related motor behaviors ("video") with RSWA; or showing both "history" and "video" without RSWA. Using both SINBAR and Montreal scoring methods, RSWA criterion showed the highest sensitivity in identify RBD. Analogously, concomitance of history of RBD and vPSG documented behaviors, regardless to presence of RSWA, presented the highest specificity.

Therefore, results of the present study suggest that, in those PD patients who have a clear history of RBD and presence of REM sleep video-recorded behaviors, RSWA assessment would not be mandatory for the diagnosis of RBD. Indeed, according to the ICDS-3, some patients might have a typical history and/or vPSG-documented motor behavior during REM sleep without fulfilling the RSWA criteria, and therefore being diagnosed with "provisional" RBD.[2] Currently, it is not

completely understood whether these provisional status represent a prodromal of full-blown RBD. As a matter of fact, a recent study on de-novo PD patients, longitudinally assessed by vPSG, have found that subjects with REM sleep associated motor events (RBE) not reaching RSWA diagnostic thresholds at baseline, developed a full-blown RBD after a two-years follow-up.[108] Thus, RBE not associated with RSWA might be a precursor of RBD, and it should be named "prodromal" RBD. Results of our study showing the diagnostic value of concomitant history and video-recorder RBE without RSWA are in line with this view.

In light of these results, the visual inspection of vPSG may be crucial to detect all range of REM behavioral events. Nevertheless, minor movements, like twitching or jerking, may be not easily discernable, especially if patients sleep with sheets or blanket. Given the role of video-documented behaviors in the diagnosis of RBD, it is advisable that vPSG should be performed without blanket or at least with light sheets.

Finally, these results of the best latent classes-derived model for diagnosis of RBD in PD were consistent with the current RBD diagnostic criteria. Moreover, the diagnosis of provisional RBD should be considered as a full RBD diagnosis in patients with Parkinson's disease.

Moreover, our study showed that the current diagnostic cut-off for RSWA, as defined by the of presence of ≥ 27% of 30-s REM sleep epochs contains any (either tonic or phasic) chin EMG activity combined with phasic EMG activity at bilateral FDS muscles, ("any chin + FDS 30-s") suggested by the ICSD-3 edition, are suitable for PD patients.[2,86] Sensitivity analysis has been performed considering different thresholds of RSWA (any chin EMG + FDS 30-s) ranging from 25% to 28%, leading to same results. Thus, the cut-off of RSWA suggested by the ICSD-3[2] (i.e. ≥27% of "any chin EMG + FDS 30-2) was found to be optimal in our large cohort of PD patients, as shown by the ROC curve analysis.

As vPSG workup is not always accessible in clinical practice or in epidemiological research field, screening tools have been developed to detect the presence of probable clinical RBD. As well as diagnostic criteria, these screening questionnaires for RBD have mainly validated in I-RBD patients consulting Sleep Center, or in small cohorts of PD patients. Among those, the RBD screening questionnaire (RBDSQ) and the RBD single question (RBD1Q) have shown very good sensitivity and specificity when assessed in I-RBD population.[118,122]

In the present thesis, we have assessed sensitivity and specificity of the abovementioned RBD screening tools in a large group of PD patients routinely evaluated in a movement disorder outpatient clinic who later underwent in-lab full v-PSG. In our study, the RBSDQ and the RBD1Q were administered prior clinical sleep-focused interview, showing respectively 55.7% and 67.7% of sensitivity and 71.4% and 82.9% of specificity, with a PPV respectively of 77.3% and 87.5%, and NPV of 48.1% and 59.2%. Interestingly, the two questionnaires showed poor agreement with Cohen's K of 0.37, probably because they explore different symptoms dimension of RBD. On the other hand, when the two questionnaires were administered together, they performed better in detecting probable clinical RBD in PD patients, with 72.6% of sensitivity, 65.7% of specificity, 78.9% of PPV and 57.5% of NPV.

Recently, the diagnostic value of the RBDSQ has been evaluated in two different samples of PD patients consulting a sleep clinic, one of which included patients who underwent RBD-focused interview prior to administration of RBDSQ, whereas the other underwent the screening questionnaire during routine work-up.[130] In that study, RBDSQ showed 64% of sensitivity and 68% of specificity when patients fulfilled RBDSQ prior to interview, while 78% of sensitivity and 100% of specificity when they fulfill the questionnaire during sleep-focused interview. Thus, it was shown that the diagnostic value of the RBDSQ strongly depends on the clinical setting and may be prejudiced by the individual's awareness on RBD.

Our results confirm the notion that RBD screening questionnaires alone, performed out of clinical interview, are of limited value in PD population. Probably, this is related to the fact that PD patients are sometimes unaware of their RBD condition, possibly due to the presence of mild forms of RBD. This is especially critical in epidemiological research studies involving large cohort of PD patients, whose results should be interpreted with carefulness.

After focusing on methodological aspects related to the diagnosis of RBD, the present thesis aimed to elucidate the time course of RBD in PD in order to ascertain whether RBD represent a stable marker in PD.

We prospectively evaluated clinical and vPSG features of RBD in PD patients at a moderate to advanced stage, after at least 3-years of follow-up, with a mean disease duration of 7.5±4.7 years.

In the present thesis, we found that 12 out of 24 (50%) of PDRBD+ patients did not show changes in the self-reported frequency of RBD symptoms, while 6/24 (25%) reported an increased frequency and 6/24 (25%) a reduced frequency in RBD at follow-up compared to baseline. Moreover, we found no correlations between RSWA measures and subjective symptoms of RBD assessed at both baseline and follow-up. Furthermore, we didn't observe difference in the increase of RSWA measures over time between patients reporting a clinical improvement of their RBD vs. those who worsened or remained stable over time.

As far as we known, this is the first study assessing the evolution of RBD in moderate to advanced PD patients after 3-years follow-up. Anecdotally, RBD symptoms might improve or even disappear in PD patients over time. So far, only another longitudinal vPSG study has been performed in patients with PDRBD+. The latter has explored the evolution of RBD in de-novo diagnosed

Parkinson's disease, showing that RBD increased significantly in PD patients in two years of follow-up and suggesting that RBD itself represents a robust and stable marker of early PD.[108] However, in this study patients were tested at the very early stage of PD and for a short 2-years period.

In the present thesis, we found that all PDRBD+ patients were found to still fulfill the diagnostic criteria at 3-years follow-up, and that all RSWA measures had significantly worsened after 3-years of follow-up compared to baseline, regardless subjective impressions of clinical symptoms.[89,114] Furthermore, although we observed isolated improvement in RSWA parameters in some patients, the totality of RSWA measures in each patients exceed normal threshold at follow-up.

Thus, these results confirm previous findings that neurophysiological features of RBD in PD patients does not resolve over time or with DRT, but rather show a worsening.

In particular, a deterioration in motor control during REM sleep, as indicated by an increase of both tonic and phasic components of RSWA, was observed in our cohort of PD patients, suggesting a progressive neurodegenerative process within the neuronal circuits that modulate muscle tone during REM sleep.

Based on our results, it may be suggested that RBD status is a stable marker in PD patients, while RSWA appears to progress over time. On the other hand, similarly to PD, also in idiopathic RBD (i.e. RBD without evidence of other neurological disease, that may often precede by several years the clinical onset of PD), longitudinal studies have shown an increase of RSWA over time, as a dynamic marker.[125,126]

Moreover, in the present thesis, we observed a worsening in Hoehn & Yahr stage and a significant increase in LEDD, as well as a significant worsening in executive functions, particularly in semantic and phonemic fluency at 3-years follow-up compared to baseline in PDRBD+.

Previous studies provide evidences that RBD in PD represent a marker of a more severe disease, in terms of motor and non-motor symptoms, with early cognitive decline and more rapid progression towards dementia.[17,131] The worsening in executive functions, rather than in motor functions, that we have observed in this thesis, might be related to a forthcoming cognitive decline in PDRBD+ reported by other studies, though the lack of a control group of PD patients without RBD prevents to draw conclusions. Indeed, we did not show significant worsening in other motor or non-motor symptoms and in psycho-behavioral features. The present thesis has limitations, namely the small sample size and the relatively short follow-up timing. Thus, longer follow-up time might allow to better appreciate the correlation between RSWA changes and motor and non-motor symptoms progression.

However, in the present thesis we showed a significant correlation between the extent of the increase in RSWA measures and the extent of worsening of dyskinesia and fluctuation score, the three increase over time, indeed. Moreover, we also observed that increase in RSWA was found to significantly correlate with worsening in executive functions, namely episodic verbal memory, and in visuo-spatial perception. The correlation between the increase of RSWA and the development of motor complications at three years, or the impairment in cognitive functions, strongly support the notion that PDRBD+ represent a diffuse/malignant phenotype with a more rapid progression than other more benign subtypes. Although again, the lack of control group of PD patients without RBD precludes to draw conclusions.

Another limitation of the present thesis was that we did not assess the intensity of RBD severity, namely the degree of violence of motor behaviors. As a matter of fact, it is not known whether a decrease in RBD frequency would be paralleled by modifications in the intensity of motor behaviors, thus subjective assessment is of limited utility. Further studies are needed to develop new tools to assess RBD severity.

In summary, the ensemble of our results underlines on one hand, the need of appropriate screening questionnaire for RBD in PD population. On the other hand, we demonstrated the reliability of an automated scoring method of RSWA compared to two visual methods in a large cohort of PD patients. Moreover, we showed that current diagnostic criteria for RBD are suitable in PD population, and that the diagnosis of provisional RBD, as defined by the ICDS-3 might be considered as a clear-cut RBD diagnosis in this population.

Finally, we were able to demonstrate in this cohort of PD patients that RBD status is a stable marker, while RSWA is an evolving feature of RBD. In light of the role of RBD as a marker of a malignant subtype of PD with a more rapid decline in cognitive functions, the notion that RBD represents a stable feature in PD may be of special interest for eventual clinical trials with potential treatments or disease-modifying therapies or for longitudinal studies assessing the evolution of other aspects associated to RBD.

Further large cohort studies are needed to confirm the role of RSWA as a marker of disease progression in PD.

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