ORIGINAL ARTICLE

Facilitatory/inhibitory intracortical imbalance in REM sleep behavior disorder: early electrophysiological marker of neurodegeneration?

Giuseppe Lanza[1,2,*], Debora Aricò[2], Bartolo Lanuzza[2], Filomena Irene Ilaria Cosentino[2], Mariangela Tripodi[2], Floriana Giardina[2], Rita Bella[3], Monica Puligheddu[4], Giovanni Pennisi[1], Raffaele Ferri[2] and Manuela Pennisi[5]

[1]Department of Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy, [2]Sleep Research Center, Department of Neurology IC, Oasi Research Institute – IRCCS, Troina, Italy, [3]Department of Medical and Surgical Science and Advanced Technologies, University of Catania, Catania, Italy, [4]Sleep Disorder Research Center, Department of Medical Science and Public Health, University of Cagliari, Cagliari, Italy and [5]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

*Corresponding author: Giuseppe Lanza, Department of Surgery and Medical-Surgical Specialties, University of Catania, Via Santa Sofia, 78 – 95125, Catania, Italy. Email: glanza@oasi.en.it.

Institution where work was performed: Oasi Research Institute – IRCCS, Troina, Italy

Abstract

Study Objectives: Previous studies found an early impairment of the short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) to transcranial magnetic stimulation (TMS) in Parkinson's disease. However, very little is known on the TMS correlates of rapid eye movement (REM) sleep behavior disorder (RBD), which can precede the onset of a α-synucleinopathy.

Methods: The following TMS measures were obtained from 14 de novo patients with isolated RBD and 14 age-matched healthy controls: resting motor threshold, cortical silent period, latency and amplitude of the motor evoked potentials, SICI, and ICF. A cognitive screening and a quantification of subjective sleepiness (Epworth Sleepiness Scale [ESS]) and depressive symptoms were also performed.

Results: Neurological examination, global cognitive functioning, and mood status were normal in all participants. ESS score was higher in patients, although not suggestive of diurnal sleepiness. Compared to controls, patients exhibited a significant decrease of ICF (median 0.8, range 0.5–1.4 vs. 1.9, range 1.4–2.3; $p < 0.01$) and a clear trend, though not significant, towards a reduction of SICI (median 0.55, range 0.1–1.4 vs. 0.25, range 0.1–0.3), with a large effect size (Cohen's d: –0.848). REM Sleep Atonia Index significantly correlated with SICI.

Conclusions: In still asymptomatic patients for a parkinsonian syndrome or neurodegenerative disorder, changes of ICF and, to a lesser extent, SICI (which are largely mediated by glutamatergic and GABAergic transmission, respectively) might precede the onset of a future neurodegeneration. SICI was correlated with the muscle tone alteration, possibly supporting the proposed RBD model of retrograde influence on the cortex from the brainstem.

Statement of Significance

Rapid eye movement (REM) sleep behavior disorder (RBD) is considered to be a strong predictor of future neurodegeneration. In this study, we provide novel insights into the pathomechanism and neurochemical basis underlying isolated RBD and propose the role of specific electrophysiological measures as putative markers of early cortical dysfunction in these patients, who are still asymptomatic for a parkinsonian syndrome. This would allow the possibility of a very early recognition of a neurodegenerative disease and open new evidence-based therapeutic avenues for both RBD and movement disorders. Longitudinal studies are required to verify whether the abnormalities detected at this early stage of the disorder correlate with the clinical progression of RBD.

Introduction

REM sleep behavior disorder

Rapid eye movement (REM) sleep behavior disorder (RBD) is a dream-enacting behavior parasomnia characterized by an intermittent or continue loss of REM sleep muscle atonia and an increase of phasic and/or tonic muscle activity during REM sleep, with complex nocturnal motor behaviors [1]. RBD is usually seen in middle-aged to elderly men [2], and may occur alone (idiopathic form) or in association with a variety of neurological disorders, in particular with a group of neurodegenerative diseases called α-synucleinopathies [3–5] that include Parkinson's disease (PD) [6, 7], dementia with Lewy bodies [8], and multisystem atrophy [9]. Given that RBD can precede the onset of these disorders, often by several years, it is considered to be a strong predictor of neurodegeneration. In particular, up to 65% of patients diagnosed with RBD subsequently develop PD within an average time of 12–13 years. More recently, an appraisal of the body of evidence suggests that RBD does not simply precede but actually "marks" the onset of a α-synucleinopathy [10].

The pathogenesis of idiopathic RBD still remains a matter of debate. Evidence of an involvement of several brainstem structures mainly located in the pons, which include the ventral meso-pontine junction, the pedunculo-pontine nucleus (PPN), the sublatero-dorsal tegmental nucleus (SLD, equivalent to the subcoeruleus nucleus in humans), the locus coeruleus (LC), and the peri-LC area, comes from both animal models [11] and neuropathological observations in humans [12–14]. The most numerous cholinergic and aminergic fibers within the brainstem reticular formation originate from the PPN and LC, respectively, and both play a crucial role in arousal and cortical activation [15–17]. Nevertheless, several in vivo and in vitro experimental studies have also examined how motor neurons are controlled during REM sleep, leading to the identification of an upstream brain circuitry that ultimately seems to trigger motor atonia [18–20].

In this context, a diagnostic tool directed to specifically explore and possibly measure other alterations beyond the brainstem in RBD would be relevant to determine whether a prodromic neurodegenerative disorder underlies this condition occurring at the stage of isolated RBD, i.e. before the onset of additional signs and symptoms. Recently, a number of studies have been recently carried out to evaluate the neurophysiological pattern of cortical excitability and cortico-spinal conductivity to transcranial magnetic stimulation (TMS) in different sleep disorders [21–24].

Single and paired-pulse TMS

TMS is a safe, noninvasive, and painless technique by which hypotheses regarding cortical excitation and inhibition can be explored in vivo and in real time in humans [25]. Different paradigms of stimulation are applied to obtain a direct measure of cortical excitability and, indirectly, to gain information regarding the functioning of underlying neurotransmitter systems [26, 27] in neurological and psychiatric conditions [28–33], as well as in systemic diseases with central nervous system (CNS) involvement [34–38]. Several variables, such as the resting motor threshold (rMT), the cortical silent period (CSP), the latency and the amplitude of motor evoked potentials (MEPs), and the central motor conduction time (CMCT), are assessed by means of the single-pulse technique [26].

Paired-pulse technique allows the measurement of more sensible indexes of intracortical functioning, namely the short-interval intracortical inhibition (SICI) and the intracortical facilitation (ICF) [26], thus providing physiological insights on the cortical circuitry activated by TMS [39]. Kujirai and coworkers [40] first described SICI by reporting that a subthreshold conditioning stimulus (CS) could suppress a MEP to a later suprathreshold test stimulus (TS) if the interstimulus interval (ISI) was ≤5 ms. Because the CS is below the MT, the interaction likely occurs at the cortical level, where the CS suppresses the recruitment of descending volleys evoked by the TS, as confirmed by a direct recording of these volleys [41]. A CS, that itself does not evoke any cortico-spinal activity, can actually produce a strong suppression of late I-waves if the interval to the TS is between 1 and 5 ms. Notably, the I1 wave is virtually unaffected, whereas inhibition affects the I3 and later waves [41], suggesting that SICI does not modify directly the excitability of pyramidal neurons and, therefore, the inhibition is due to other intracortical elements [42].

Conversely, ICF occurs when a CS increases the response to a later TS with an ISI from 8 to 20 ms [40, 43]. This mechanism has been further investigated in patients with high cervical epidural electrodes by recording the descending volleys produced by single and paired-pulse TMS [44]. Although the MEP was facilitated, there was no significant change in the amplitude or number of the descending volleys. To explain the dissociation between MEP and epidural findings, it has been suggested that ICF may result from a recruitment of circuits in addition to those involved in I-wave generation, evoking a more desynchronized activity not evident in the epidural recordings [44].

Literature background

Most of previous studies found changes of both SICI and ICF in patients with PD, including those in the early stage, in terms of decreased SICI and reduced ICF, suggesting a disinhibition and hypofacilitation of the motor cortex [45–51]. To date, conversely, only one TMS study has been carried out in idiopathic RBD patients [52], reporting an impairment of the short-latency afferent inhibition (SAI) and supporting the hypothesis of cholinergic dysfunction in those who develop cognitive impairment. This finding was also found in a second study by the same research group on patients with RBD associated to PD, again interpreted as the result of a cholinergic involvement correlated with their cognitive decline [53]. The authors conclude that cholinergic degeneration is an important contributor to nonmotor parkinsonian features, raising the possibility that RBD exposes PD to an increased risk of cognitive impairment [53]. Overall, these data help the early recognition of PD with cholinergic system degeneration and stimulate future targeted cholinergic treatments [54].

In line with these findings, electroencephalographic (EEG) slowing (i.e. increased slow-wave sleep and theta and delta power in the frontal and posterior cortical regions), both in wakefulness and during REM sleep (but without changes on sleep architecture), was associated with cognitive decline in several studies on idiopathic RBD patients, thus providing a potential marker of future α-synucleinopathy [55–61]. Similar EEG changes have

been observed in parkinsonian patients, including those with mild cognitive impairment [62, 63]. Nevertheless, given that also RBD patients without cognitive impairment showed EEG alterations during wakefulness [64], longitudinal studies are warranted to confirm a reliable prediction of neurodegeneration.

Aim and hypothesis

The aim of this study was to apply single- and paired-pulse TMS in de novo patients with isolated RBD, in order to detect any change in their electrocortical profile and to correlate TMS measures with the REM Sleep Atonia Index (RAI). We hypothesize that, compared to healthy controls, isolated RBD patients might exhibit a pattern similar to that reported in early PD, thus confirming the role of RBD as a predictor of an α-synucleinopathy also at the TMS level.

Materials and Methods

Participants and assessment

Fourteen patients with clinical and videopolysomnography-confirmed isolated RBD (11 males; median age 65.5 years, range 57.0–69.0; median disease duration 2.5 years, range 1.0–4.0) were consecutively recruited at the Sleep Research Centre of the "Oasi Research Institute – IRCCS" in Troina (Italy), and age-matched with 14 healthy controls (9 males; median age 65.0 years, range 60.0–70.0). All patients fulfilled the current standard clinical criteria for RBD [65].

Exclusion criteria were: age <18 years; history or presence of psychiatric illness (e.g. major depression disorder, bipolar disorder, schizophrenia, etc.) or other neurological diseases (e.g. PD, atypical parkinsonisms, epilepsy, stroke, dementia, head trauma, multiple sclerosis, peripheral nervous system disorders, etc.); other sleep disorders, such as obstructive sleep apnea syndrome, restless legs syndrome, periodic limb movements (index >15/hour), abnormal sleep–wake rhythm, insomnia, narcolepsy; acute or chronic noncompensated medical illness; any medical or drug-related condition affecting cognitive performance or mood status; history or current alcohol or illicit drug abuse; current intake of psychoactive medications or other drugs able to modulate cortical excitability [66–68]; Mini Mental State Examination (MMSE) [69] <24, Geriatric Depression Scale (GDS, short form) [70] >5, and Unified Parkinson's Disease Rating Scale – part III (UPDRS-III) [71] >0; any condition precluding TMS or magnetic resonance imaging (MRI).

The clinical-demographic assessment included: age, gender, education, handedness, social and living conditions, family and clinical history, general and neurological examinations. The right handedness of all individuals was checked with the Edinburgh Handedness Inventory [72]. No significant comorbidity was reported; two patients had a family history of RBD. At the time of the examination, none of the patients had been treated with drugs for RBD or any other psychoactive drugs; in particular, none assumed benzodiazepines and/or melatonin before undergoing TMS.

All patients preliminarily completed the UPDRS-III to clinically exclude any early motor manifestations of PD. A global cognitive test (MMSE) and a quantification of depressive symptoms (GDS) and subjective sleepiness (Epworth Sleepiness Scale [ESS]) [73] were also carried out. Standard EEG was performed to rule out predisposition to seizure. In addition, in order to exclude a spinal or peripheral contribution to cortical excitability, a routine conduction study of the right ulnar nerve was performed prior to the entry into the study, which was normal in all patients. A conventional 1.5 T brain MRI was also unremarkable in all patients.

The study was approved by the local Ethics Committee (prot. 2018/07/18/CE-IRCCS-OASI/14; approval date 18/07/2018) and conducted in accordance with the Declaration of Helsinki of 1964 and its later amendments. All participants gave written informed consent prior to the study and after a full explanation and acceptance of the whole procedure.

Computation of the REM Atonia Index

Standard nocturnal laboratory polysomnografic recordings were obtained in all subjects enrolled in this study, including, in particular, the electromyogram (EMG) of the submentalis muscle (bipolar derivations with two electrodes placed 3 cm apart and affixed using a collodion-soaked gauze pad). Impedance was kept less than 10 KΩ (typically <5 KΩ).

For the quantitative computation of RAI, we used an established automatic algorithm [74–76]. The submentalis muscle EMG signal was digitally band-pass filtered at 10–100 Hz, with a notch filter at 50 Hz and rectified. Subsequently, each REM sleep epoch included in the analysis was divided into 30 mini-epochs of 1 s each. The average amplitude of the rectified submentalis muscle EMG signal was then obtained for each mini-epoch. After a noise reduction procedure [76], the average submentalis muscle EMG signal amplitude in each 1 s mini-epoch was used to compute the percentage of mini-epochs falling into the following 20 amplitude (amp) classes (in μV): amp ≤1, 1 <amp ≤2, …, 18 <amp ≤19, and amp >19. Muscle atonia is expected to be reflected by high values of the first class (amp≤1), while phasic and tonic activations are expected to increase the value of the remaining classes [77–79]. RAI was then computed, summarizing in a single value the degree of preponderance of the first class in REM sleep: RAI = amp ≤1 / (100 – 1 < amp ≤2). Mathematically, this index can vary from 0 (absence of mini-epochs with amp ≤1), i.e. complete absence of EMG atonia, to 1 (all mini-epochs with amp ≤1) or stable EMG atonia in the epoch. The algorithm was run blind to the condition of the subject, even though no manual modification of the parameters is possible.

TMS

TMS was performed using a high-power Magstim 200² magnetic stimulator (Magstim Co., Whitland, Dyfed, UK). A 90 mm (external loop) figure-of-eight coil was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral First Dorsal Interosseous (FDI) muscle of the dominant hand. Since all subjects were right-handed, the left primary motor cortex was selected, with the induced current flowing in a postero-anterior direction, as recommended [80]. EMG activity was recorded with silver/silver-chloride disposable self-adhesive and self-conductive surface electrodes. The active electrode was placed over the muscular belly of the target muscle (FDI), the reference distally at the metacarpal-phalangeal joint of the index finger, and the ground on the dorsal face of the wrist. MEPs were amplified and filtered (bandwidth 3-3,000 Hz).

The resting motor threshold (rMT) was defined as the lowest stimulus intensity able to elicit MEP at rest of an amplitude >50 μV in at least 5 of 10 trials, according to the international guidelines [80]. CMCT was calculated by subtracting the conduction time in peripheral nerves obtained by magnetic stimulation of the cervical root, from the MEP cortical latency obtained during moderate active muscle contraction, with a stimulus intensity set at 130% of the rMT. Peak-to-peak MEP amplitude during active contraction level was calculated. The CSP was determined with an approximately 50% of maximum tonic voluntary contraction of the FDI muscle, induced by single TMS pulses delivered at 130% of rMT. The mean CSP duration of 10 rectified trials was calculated [80].

Paired-pulse TMS was performed using a 90-mm figure-of-eight coil deriving pulses from a couple of Magstim 200^2 Stimulators, connected each other through a BiStim module. SICI and ICF were studied using the conditioning-test paradigm by applying two magnetic stimuli in rapid succession [40, 43]. The conditioning stimulus was set at 80% of the individual rMT, whereas the test stimulus was set at 130%, thus allowing to evoke a MEP in the relaxed FDI with a peak-to-peak amplitude of approximately 1 mV. The ISIs tested were 3 and 10 ms, as they are known to be representative intervals able to produce a clear inhibition and facilitation of the test response, respectively [25, 40, 43]. Indeed, it has been shown that SICI at 3 ms best represents the inhibitory postsynaptic potentials mediated by the gamma-aminobutyric acid (GABA)-A receptor [81, 82], whereas the facilitation at 10 ms is consistent with the optimal activation of the excitatory postsynaptic potentials reflecting the time course of ICF [66]. Ten trials for both ISIs were recorded in a random way, with an 8-s interval among each trial. Responses were expressed as the ratio between the MEP amplitude produced by the paired-stimulation and that produced by the test stimulus alone [40, 43]. A continuous EMG audio-visual feedback at high gain assisted the participants in maintaining a complete muscle relaxation.

All measurements were conducted while subjects were seated in a comfortable chair, in the same laboratory, equipment, and experimental conditions, by the same trained operators for each subject, and at the same time of the day (approximately 9:30–11:30 am). Data were collected on a dedicated computer and stored with an ad hoc software for off-line analysis [83].

Statistical analysis

In order to have a preliminary indication on the sample size needed for this study and in consideration of the strict inclusion and exclusion criteria that precluded to program the enrollment of a large number of patients, we performed a sample size analysis by assuming an effect size of 1, with power 80%, and alpha 0.05, in a one-tailed comparison between two independent groups. With these parameters, we obtained a total number of subjects required of 28 (14 in each group).

We first evaluated the normality of the distribution of continuous variables by means of the Shapiro-Wilk W test which was significant in all instances, then the hypothesis that the distribution were normal was rejected. For this reason, all comparisons were performed by means of the nonparametric Mann–Whitney test for unpaired datasets. However, because of the relatively low power of this test, we also used the Student's *t* test after log-transformation of data and, to rule out possible type II errors, we also calculated effect sizes using the Cohen's *d* [84]. Cohen's *d* is defined as the difference between two means divided by their pooled standard deviation. According to Cohen, 0.2 is indicative of a small effect, 0.5 of a medium, and 0.8 of a large effect size. Differences were considered as statistically significant when they were below the $p < 0.05$ level.

Finally, the correlation between RAI and TMS measures of intracortical inhibition (conditioned MEP amplitude at ISI 3 ms and SICI) and facilitation (MEP conditioned amplitude at ISI 10 ms and ICF) was analyzed by means of the Spearman R rank correlation coefficient.

Results

All subjects completed all TMS procedures without any discomfort or undesired effect. Although some patients complained of excessive sleepiness, none of them became drowsy during the stimulation sessions and no particular solicitation was needed to maintain vigilance. Clinical-demographic characteristics of the participants are summarized in Table 1. The two groups were similar in terms of age, sex, education, social and living conditions, global cognitive functioning, mood status, and neurological examination (completely normal in all patients). Median ESS was higher in patients than in controls, although none of the reported scores was suggestive of a significant diurnal sleepiness.

As shown in Table 2, single-pulse TMS parameters did not differ between the two groups. Conversely, when paired-pulse indexes of intracortical excitability were considered, a statistically significant decrease of median ICF and a clear, though not significant, trend towards a reduction of SICI in patients

Table 1. Clinical and demographic features of patients and controls

	Controls (*n* = 14)	RBD patients (*n* = 14)	Mann–Whitney U-test		Student's t-test*		Cohen's *d*
	Median (interquartile range)	Median interquartile range)	U	*p* ≤	t	*p* ≤	Effect size
Age, years	65.00 (60.00–70.00)	65.50 (57.00–69.00)	87.5	NS	0.727	NS	0.243
Education, years	8.00 (5.00–13.00)	8.00 (5.00–13.00)	97.5	NS	0.114	NS	−0.077
Disease duration, years		2.50 (1.00–4.00)					
Mini-Mental State Examination	28.00 (26.70–29.50)	27.00 (26.00–29.00)	86.5	NS	0.689	NS	0.257
Epworth Sleepiness Scale	2.00 (0.00–5.00)	4.00 (3.00–9.00)	54.5	**0.048**	−2.387	**0.025**	**−0.880**
Geriatric Depression Scale	3.00 (0.00–4.00)	1.00 (0.00–4.00)	91.5	NS	0.625	NS	0.032
REM Sleep Atonia Index		0.77 (0.73–0.89)					

*Log-transformed data. NS, not significant; numbers in bold = statistically significant *p* values or large effect size.

Table 2. Comparison between the TMS data obtained in controls and RBD patients

	Controls (n = 14)	RBD patients (n = 14)	Mann–Whitney U-test		Student's t-test*		Cohen's d
	Median (interquartile range)	Median interquartile (range)	U	p ≤	t	p ≤	Effect size
rMT, %	43.00 (40.00–47.00)	43.00 (38.00–46.00)	96.5	NS	0.204	NS	0.075
CSP, ms	66.90 (51.00–80.50)	69.00 (64.80–78.90)	82.0	NS	−0.831	NS	−0.162
MEP latency, ms	21.20 (20.20–21.90)	21.25 (20.30–21.90)	97.5	NS	−0.243	NS	−0.112
MEP amplitude, mV	1.70 (1.40–3.00)	2.20 (1.30–3.00)	93.0	NS	−0.141	NS	0.049
PMCT, ms	14.75 (14.30–15.10)	15.15 (13.70–15.30)	86.0	NS	−0.470	NS	−0.202
CMCT, ms	6.45 (6.00–7.60)	6.35 (6.00–7.30)	97.0	NS	0.103	NS	0.044
MEP amplitude ISI 3 ms, mV	0.20 (0.10–0.30)	0.50 (0.10–0.90)	70.5	NS	−1.857	NS	**−0.877**
MEP amplitude ISI 10 ms, mV	2.25 (1.40–2.60)	0.80 (0.80–0.90)	13.5	**0.00011**	4.781	**0.00006**	**1.212**
SICI, ratio	0.25 (0.10–0.30)	0.55 (0.10–1.40)	66.0	NS	−1.908	NS	**−0.848**
ICF, ratio	1.90 (1.40–2.30)	0.80 (0.50–1.40)	29.0	**0.00165**	3.759	**0.0009**	**1.084**

*Log-transformed data.

CSP, cortical silent period; MEP, motor evoked potential; CMCT, central motor conduction time; ISI, interstimulus interval; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; PMCT, peripheral motor conduction time; rMT, resting motor threshold.

NS, not significant; numbers in bold = statistically significant p values or large effect size.

compared to controls were observed, accompanied by a large effect size.

Finally, the correlation between RAI and TMS measures of intracortical inhibition (conditioned MEP amplitude at ISI 3 ms and SICI, left panels) and facilitation (conditioned MEP amplitude at ISI 10 ms and ICF, right panels) are shown in Figure 1. Statistically significant Spearman R values were found for both correlations between RAI and TMS measures of intracortical inhibition. On the contrary, the correlation between RAI and TMS measures of intracortical facilitation did not reach statistical significance. However, following the Cohen's indications [84], we considered correlations 0.10, 0.30, and 0.50 as corresponding to small, medium, and large sizes, respectively; thus, while both R values found for the correlation between RAI and TMS measures of intracortical inhibition are of large size, only the correlation between RAI and ICF had a medium size.

Discussion

Main findings

A neurophysiological pattern characterized by a significant hypofacilitation and a tendency towards a disinhibition of the motor cortex in RBD is the main finding of the present study. This suggests that, in awake subjects with idiopathic RBD but still asymptomatic for parkinsonism or dementia, changes of ICF and, to a lesser extent, SICI might reflect an intracortical imbalance between facilitatory and inhibitory microcircuits, mainly mediated by the glutamate and GABA-A transmission, respectively [85]. Therefore, in addition to the well-known brainstem-related mechanisms, an unbalanced motor cortex excitability may be part of the RBD pathophysiology. Although it is still controversial where dream behaviors in RBD patients come from [86], a decreased SICI might contribute to generate motor behaviors in response to sensory feedback from elementary movement during dreaming in these patients.

Notably, no difference between patients and controls was observed for single-pulse TMS indexes (i.e. rMT, CSP, MEP latency, and amplitude, CMCT). As known, the rMT is a basic parameter of brain excitability, as it is a compound measure of the excitation of cortical motor neuron membranes, neural inputs into pyramidal cells within the cortex, spinal motor neurons, neuromuscular junctions, and muscles. The CSP (a suppression of EMG activity that occurs when a suprathreshold TMS is applied to the primary motor cortex during a tonic voluntary contraction of the contralateral muscle) is a functional measure of intracortical inhibitory circuits mainly mediated by GABA-B transmission. The MEP latency and CMCT are indexes of integrity of the cortico-spinal pathways, whereas the MEP amplitude reflects an aggregate measure of the excitation state of output cells in the motor cortex, motor axons, and peripheral motor nerves till the muscles [25, 80]. Therefore, the observation of normality of these measures indicates that the global cortical excitability and cortico-spinal conductivity seem to be unaffected in RBD at this stage.

Overall, these data show that TMS can identify even subtle changes in the pathophysiology of the motor cortex and, as such, it may be more useful in the detection of very early stages of neurodegeneration than clinical observation alone. Indeed, the TMS profile we observed is similar to that reported by most of the studies in patients with early PD [45–51], especially in the off-state [87], thus suggesting that an early impairment of both glutamate and GABA might be detectable in RBD even in the absence of an overt extrapyramidal syndrome [54]. In agreement with this hypothesis, the occurrence of RBD in PD patients has been found to be associated more with neocortical, limbic, and thalamic denervation rather than nigro-striatal dopaminergic denervation, thus reasonably indicating a different etiology and pathophysiology compared to the typical PD manifestations [88].

Proposed pathomechanisms

Overall, it can be argued that RBD modifies the global neurochemical balance broader than the specific atonia-generating brainstem circuitry (i.e. subcoeruleus nucleus and ventro-lateral medulla), and including, through ascending pathways (reticular formation and thalamo-cortical projections), also the cortical level [52]. Whether the signatures of glutamatergic and GABA/Glycinergic dysfunction found in our study may be caused by a direct cortical pathology or, indirectly, through a damage arising from the brainstem structures that regulate REM sleep and then activate the neocortex, is still unknown. What we know is that both neuropathologic and brain imaging studies in RBD have shown alterations in several brainstem nuclei and
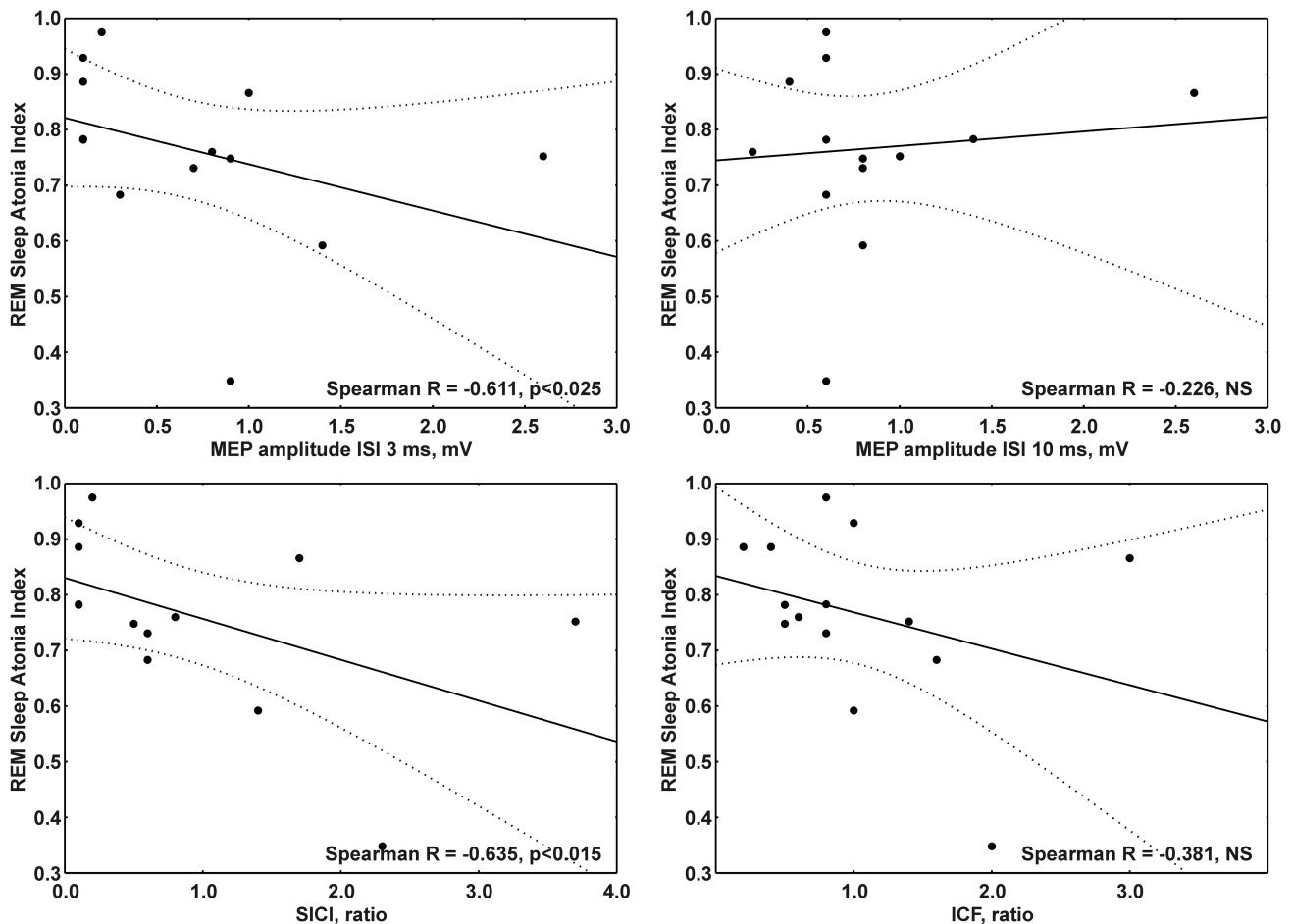
Figure 1. Correlation between REM Sleep Atonia Index and TMS measures of intracortical inhibition (conditioned MEP amplitude at ISI 3 ms and SICI, left panels) and facilitation (conditioned MEP amplitude at ISI 10 ms and ICF, right panels). The linear regression line is also shown (continuous line), along with its 95% confidence intervals (dashed lines); also the values of the corresponding Spearman R values are reported with their statistical significance (MEP, motor evoked potential; ISI, interstimulus interval; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; NS, not significant).

corresponding neurotransmitters. All of these structures have diffuse projections to the cerebral cortex and, therefore, perturbations of these neural networks may explain the presence of cortical dysfunction in patients with RBD [52], as also demonstrated by a recent evidence linking cortical thinning in RBD with clinical progression [89].

Moreover, a recent study using volumetric and diffusion tensor imaging did not show any significant differences in PPN neuronal loss according to RBD symptoms, suggesting that a cholinergic dysfunction of the pontine tegmentum alone is probably not sufficient to explain the whole symptomatology of RBD [90], as also supported by using the Vestibular Evoked Myogenic Potentials (VEMPs) [91]. Indeed, the correlation we found between TMS measures of intracortical inhibition and RAI (i.e. the higher is SICI, the lower is RAI or, in other words, the lower is the MEP inhibition, the higher is the muscle tone) seems to be in agreement with that recently demonstrated with VEMPs, suggesting that RAI is a sensitive neurophysiological measure of impairment of the cerebral regions and networks involved in RBD [91]. As VEMP reflects the brainstem circuitry of the vestibular system, which is located close to the subcoeruleus nucleus and other REM sleep regulating structures, it is reasonable to expect a correlation between VEMPs and RAI. Conversely, since SICI primarily evaluates the motor cortex, the correlation with RAI seems to be apparently unlikely. However, recent basic and clinical evidences, indicating that RBD results from the breakdown of a broad network underlying REM sleep atonia, provide a dynamic model of interaction between the brainstem and both rostral and caudal CNS structures [92]. Moreover, the finding of REM sleep EEG instability in RBD patients [61] and the observation that the dream content cannot be generated by the brainstem [93] open new avenues to future studies aiming at further clarifying how the brainstem and the cortex interact in the pathophysiology of RBD. Taken together, these findings are in line with the proposed model of the retrograde influence of the motor cortex on brainstem nuclei [85, 92] and support the view of RBD as a widespread network dysfunction that goes far beyond the brainstem and acetylcholine only.

On the other hand, this study is in line with the most recent data on the neuronal network underlying RBD. According to an integrated animal model of REM sleep, RBD would be caused by the degeneration of a subpopulation of glutamatergic neurons, specifically involved in the generation of muscle atonia, and localized in the SLD [85]. Alternatively, a specific lesion of the inhibitory (largely GABAergic) premotoneurons, mainly localized

in the medullary ventral gigantocellular reticular nucleus, might occur [94, 95].

Based on this consideration, the activity of the brainstem centers might be implicated in the physiological suppression of muscle activity also during wakefulness [96]. Conversely, in RBD an impaired control, arising from the brainstem and ascending to the supratentorial structures, might probably cause both the reduction of REM sleep atonia and an imbalance between SICI and ICF, in favor of the former. A recent EEG study may support this hypothesis: the REM sleep microstructure EEG changes indicate subtle but significant alterations in the cortical electrophysiology in isolated RBD, possibly representing the early stage of a future degenerative process [61].

Translational implications

The fact that clonazepam (a benzodiazepine-facilitating GABAergic inhibitory transmission, mediated by GABA-A receptor) decreases phasic movement but not muscle tone in RBD suggests that it acts on the neurons generating the phasic movements, i.e. the glutamatergic neurons located in the motor cortex or their relays in the pontine and medullary reticular formation and the spinal cord [94, 95, 97, 98]. Therefore, the empirical but efficacious therapeutic effect of clonazepam is probably exerted by acting on supratentorial rather than subtentorial networks, reducing the negative effects of the brainstem dysfunction on the supratentorial regions but without affecting the pathogenetic core of the disease. Unlike clonazepam, melatonin probably decreases muscle tone without diminishing phasic movements and, as such, it acts by enhancing the effect of GABA-A receptors at the motoneurons level [99].

Therapeutically, glutamate and GABA activities may emerge as new targets for RBD or even early symptoms of PD. Their enhancement by pharmacological or neuromodulatory interventions might represent an innovative "TMS-guided" therapeutic strategy [100, 101]. In this framework, the use of TMS as a proxy measure of neurochemical system integrity would be of great clinical and scientific interest given the need to validate objective measures to identify patients at risk or those candidate for treatment [102]. Moreover, since SICI and ICF are very sensitive to pharmacological agents [66–68], they could also be useful to follow up their efficacy over time.

Limitations

Some limitations of this study need to be acknowledged. The first limitation, as usually occurs in TMS research, is the relatively small number of patients. Although they were all drug-free and very homogeneous in terms of demographics, clinical, and sleep-related features, the small sample size did not allow us to obtain a statistical significance in some of the comparisons which, indeed, had a relatively large effect size. This was especially true for SICI, for which our analysis was probably underpowered and it is likely that a larger sample size would have yielded a statistical significance.

Second, the study was performed in the awake state, and thus in a condition different from sleep; however, as recently reviewed [103], nearly all studies, even the more recent ones, were performed during wakefulness, probably due to technical/procedural reasons.

Third, although glutamate and GABA are thought to largely underlie ICF and SICI, respectively [25], these remain rather complex phenomena and the contribution of other neurotransmitters, especially for ICF (such as dopamine, serotonin, noradrenalin, and acetylcholine) [66–68], cannot be excluded, although their activity was not probed in the present investigation. Furthermore, while it has been convincingly shown that ICF is a cortical phenomenon [44], the exact nature of microcircuits and neurotransmitters specifically involved are still unknown [104]. However, ICF does not seem to be related to N-methyl-D-aspartate (NMDA) receptor transmission, since ketamine (a NMDA antagonist) does not suppress its activity [105].

Conclusions

This study provides novel insights into the mechanisms underlying cortical dysfunction in RBD and might help to open future therapeutic avenues. Integrated with clinical, neuroimaging, and sleep-related data, these TMS findings are suggestive of an electrocortical imbalance in patients with RBD. Longitudinal studies are required to verify whether the abnormalities detected at this early stage of the disorder correlate with the clinical progression of RBD. This would allow the possibility of a very early recognition of this significant predictor of neurodegeneration.

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