Università degli Studi di Cagliari

PHD DEGREE

NEUROSCIENCE

Cycle XXXII

TITLE OF THE PHD THESIS

"Autonomic Nervous System and Rem Behavior Sleep Disorder: a new tool to identify Idiopathic or Parkinsonians patients through Heart Rate Variability Polysomnography Analysis"

Scientific Disciplinary Sector(s)

Area 06 Medical Sciences MED/26 Neurology

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Introduction

Materials and Methodology

Aim of the study

Sleep is a complex physiological process regulated by multiple factors which attend in the alternation of Non REM Sleep (NREM), REM Sleep and Wake transition.

One of the main systems involved in this charming process of sleep-wake circadianity is Autonomic Nervous System (ANS), formed by parasympathetic and sympathetic branch that modulate cardiovascular activity (for example cardiac frequency, blood pressure, etc…).

During NREM phase predominates parasympathetic system, which has the function of stabilize cardiovascular parameters when sleep passes through this stage.

Conversely, during REM stage prevails sympathetic activity inducing a sort of "activation" of cardiovascular system.

This ANS circadianity clinically reflects the peak of cardiovascular accidents that arise in the early morning when REM sleep in more expressed end therefore the instability induced by sympathetic system.

Recently, researches focus on investigation of a new tool to indirectly test functions and activities of the ANS during sleep, finding in the Heart Rate Variability (HRV) a good biomarker of the system: the HRV is the time variation of the interval between two consecutive heartbeats, identified on the ECGraphic trace in the RR interval.

The HRV analysis in our study was carried out on patients suffering from Rem Behavior Sleep Disorder (RBD), clinically characterized by violent motor behaviors associated with an offensive-defensive dream content, fairly typified in literature; polysomnographically it is instead diagnosed when, during the REM phase, the absence of the physiological muscular atony of the myloid muscle is detected.

RBD is a sleep disorder known to be associated with Parkinson's disease; patients affected by this neurodegenerative pathology have a very high percentage of this sleep disorder; however, there are patients who present these symptoms and polysomnographic anomalies even in the absence of an extrapyramidal pathology, configuring cases of idiopathic RBD.

Our study analyzed two populations of RBD patients through polysomnography analysis: idiopathic and Parkinson's disease patients.

In these two populations, HRV was analyzed by linear and non-linear methods on each sleep stage even considering every probable physiological transition phase. The goal of this analysis is to identify whether HRV can be a valid biomarker to distinguish the two populations of patients affected by RBD, both idiopathic and affected by Parkinson's disease.

The REM sleep phase allowed to record the greatest number of significant differences in HRV Index (exactly the non-linear parameters) between the two groups of patients, both when the first and the longest REM epochs were studied.

HRV analysis in polysomnography study could represent a new and easy tool to identify, among the REM Behavior Sleep Disorder, patients affected or not by Parkinson’s desease.

It could be even useful when an early diagnosis is needed or it is necessary monitoring a probable conversion from idiopathic form to PD or evaluate the effectiveness of RBD therapies.

Introduction

Sleep-Wake Cycle

Sleep is a complex physiological process involving different biological system characterized by the activation of cortical, subcortical and medullar neural circuits.

Monoaminergic neurons in the pons project to noradrenergic neurons in the locus coeruleus and to dopaminergic and serotoninergic relay station in the raphe.

In wake period monoaminergic neurons activity inhibit ventrolateral preoptic nucleus (VLPO) and neurons which regulate the transition to REM sleep. In turns, in sleep period, the increased activity of VLPO inhibits monoaminergic neurons facilitating sleep onset.

The macrostructure of sleep includes two main physiological stages: NREM and REM sleep.

NREM Sleep is mainly regulated by "sleep-promoting" substances including Adenosine, Prostaglandin D2 and Cytokines (such as interleukin-1 and tumore-necrosis factor-α). In the Basal Forebrain, cortex and hippocampus, extracellular levels of Adenosine increase across prolonged periods of wake and decline during sleep.

The Preoptic Area (POA) contains neurons that promote sleep, in particular the ventrolateral part (VLPO) and the Median Preoptic nucleus (MnPO) contain sleep-active neurons as GABA-ergic and VLPO neurons which produce Galanin inhibiting arousal-promoting brain regions.

REM sleep and NREM sleep can be clearly distinguished based on electroencephalogram and electromyogram recordings, making sleep a directly quantifiable behavior with an alternating cycling of REM sleep occurring every about 90 minutes.

NREM sleep features can be subdivided in three phases called N1, N2 and N3.

N1 sleep is generally referred to as light sleep, marking the transition from wakefulness to sleep. It is determined by the presence of low amplitude, mixed frequency EEG, mainly occurring between 6-8 Hz in what is called the theta waveband. This pattern of brain activity is accompanied by a notable decrease in muscle tone (as compared to waking states) and slow rolling eye movements, which are present as a person begins to fall asleep.

The EEG of **N2 sleep** mainly occurs across a frequency range of 4-7 Hz in the theta waveband, and it is accompanied by a similar decrease in muscle tone as in N1, as well as reduced heart rate, lowered core body temperature, and no eye movements. Most importantly, N2 sleep is typified by distinct bursts of brain activity, known as K-complexes and sleep spindles.

A K-complex is a high amplitude negative wave, followed by a high voltage positive slow wave, first occurring after 6 months of age. Sleep spindles are spurts of high amplitude, high frequency waves, first occurring after 4 weeks of age. Sleep spindles are caused by interactions between neurons in the brain – specifically around the areas of the thalamus and cortex. They operate at varying frequencies between 9-16 Hz, however, mainly occur at 12-14 Hz. The parts of the brain involved in the creation and formation of sleep spindles changes as a person age. Spindles occurring in the frontal region of the brain are more evident in younger children but decrease dramatically at around 13 years of age. Contrastingly, spindles operating in the centroparietal brain region maintain a steady presence throughout life.

N3 sleep is considered to be the deepest stage of sleep. The EEG of N3 consists of high amplitude, low frequency waves of brain activity, mainly in the delta waveband (0 – 4 Hz). Due to these large, slow waves, N3 is commonly referred to as Slow Wave Sleep (SWS). Whilst the EEG of N3 is characteristically different to that of N2, similarities occur in the occasional presence of sleep spindles, and decreased heart rate and core body temperature.

Rapid Eye Movement Sleep (REM) is a recurrent phase of sleep–wake cycle characterized by fast and desynchronized rhythms, hippocampal theta activity in the cortical electroencephalogram (EEG) and autonomic activation.

An important feature of REM sleep is the change in muscle activity, including a complete loss of muscle tone in axial postural muscles (atonia), phasic muscle twitches in distal limb and orofacial muscles, and phasic bursting of oculomotor muscles. Respiratory-related muscles are also tonically suppressed, as genioglossus muscle, except for diaphragm.

The structures in brainstem involved in REM sleep control are reciprocal interactions between mesopontine cholinergic / glutamatergic REM-on and aminergic / GABAercic REM-off neurons. REM-on region consists in the sublaterodorsal nucleus (SLD), Pre-coeruleus (PC), ventrolateral preoptic nucleus (eVLPO), locus coeruleus (LC), laterodorsal tegmental nucleus (LDTN), peduncolopontine nucleus (PPN) and the raphe nucleus (RN).

REM-off region consists in the ventrolateral part of periacqueductal gray matter (vl-PAG) and lateral pontine tegmentum (LPT). Lesions at this level increase the amount of REM sleep.
REM-off region is physiologically inhibited by GABA-ergic and galaninergic projections from eVLPO and cholinergic projections from PPN and LDTN. It is instead activated from the noradrenergic LC, serotoninergic raphe nucleus and hypocretinergic pathways from lateral hypothalamus.
The pontine tegmentum and the ventral medulla are critical areas for muscles atonia generation in REM sleep.
There are two motor system, one for generating muscle atonia and one for suppressing motor activity. The absence of motor activity in normal REM sleep occurs via active inhibition of spinal motorneurons plus reduced drive within locomotor generators. Normal phenomena such as phasic oculomotor and locomotor activity, rapid eyes movements and brief/low-amplitude muscles twitches, is directly or indirectly suppressed. The SLD glutamatergic neurons is the major structure in the rostral tegmentum pontis responsible for REM sleep.
Several studies indicate that SLD glutamatergic neurons are divided in at least two subpopulations; one that directs descending projection responsible for muscle atonia, and one ascending to the cortex responsible for the REM-state and EEG activation. This finding suggests that only the descending subpopulation would be destroyed in RBD patient that show loss of muscle atonia but preserve REM sleep.

It is hypothesized that SLD projections to GABAergic/glycinergic premotorneurons, located in the medullary level spinal motoneurons ("direct route"), are the final common pathway that cause active inhibition of skeletal muscles activity in REM sleep. The "indirect route" starts from SLD but projects to medullary magnocellular reticular formation (MCFR) located in the pontomedullary junction. Those neurons suppress anterior horn cell activity via ventrolateral reticulospinal tract (VLST) and also contribute to the reduced inhibition of muscles activity but it seems not to be enough to be the only lesion causing RBD in humans.

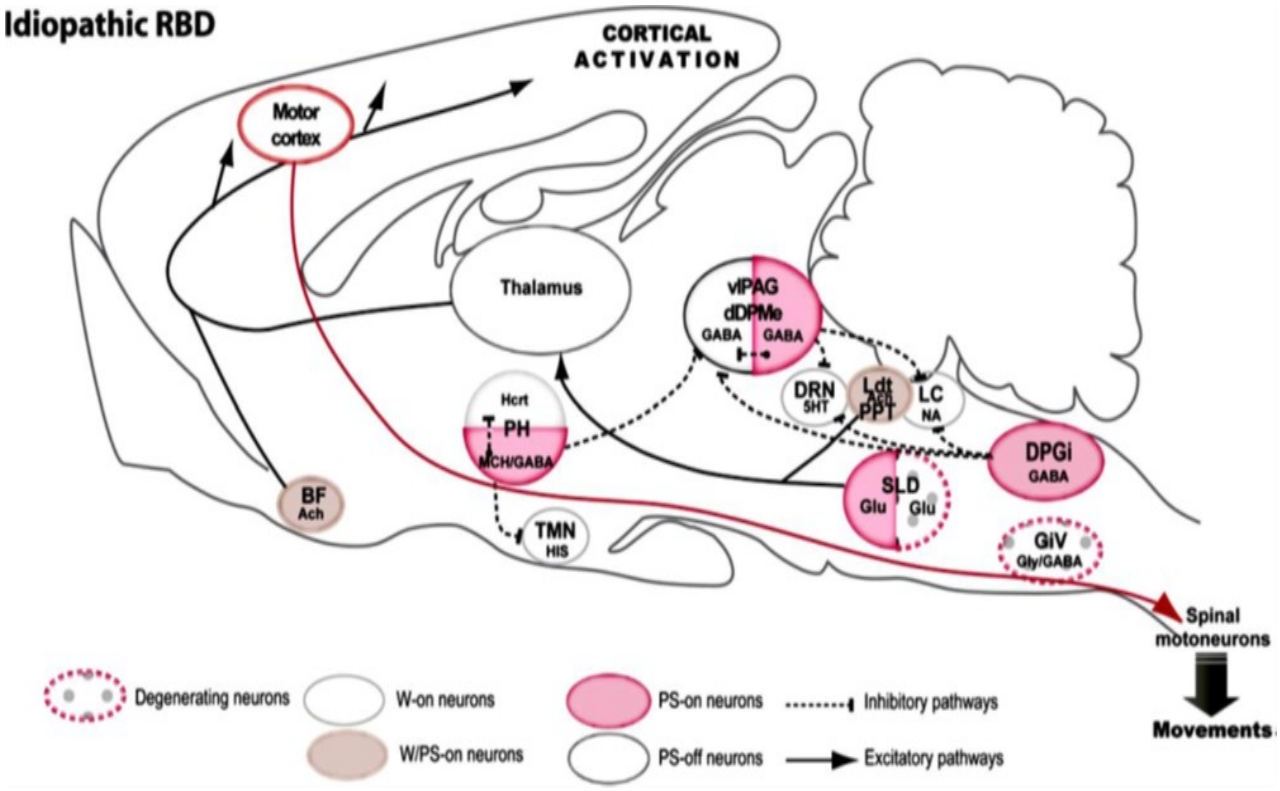

Fig 1. Pierre-Hervé Luppi, Olivier Clément, Emilie Sapin, Damien Gervasoni, Christelle Peyron, Lucienne Léger,Denise Salvert, Patrice Fort*

"The neuronal network responsible for paradoxical sleep and its dysfunctions causing narcolepsy and rapid eye movement (REM) behavior disorder

" Sleep Medicine Reviews 15 (2011) 153e163

Rem Sleep Behavior Disorder

Rem Sleep Behavior Disorder (RBD) is a parasomnia clinically characterized by simple or complex motor activity related to vivid dreams during Rem sleep stage. The three main aspect of RBD are abnormal vocalization, abnormal motor behavior and abnormal dream mentation. The adjectives "abnormal" intend that every aspect tends to be different from other physiological manifestation or other parasomnia that can occur during non-REM sleep.

Vocalization in RBD tend to be loud: shouting, screaming, and swearing are common and are described to be unlike the typical soft-spoken of normal people during wakefulness.

The motor activity in RBD often begins with nonspecific movements followed by purposeful activity such as punching, flailing as like protect oneself, running, jumping out of bed. In some cases, bruxism, somniloquy or periodic limb movements in sleep may be the primary manifestation of a RBD disorder. During these behaviors patients can injury the bed partner which refers that he/she is dreaming something distressing.

Dream mentation is described by patients as a nightmare that often involved insect, animals, or people chasing or attaching them or their familiar and friends. Many patients are able to recount the content of their dreams when awakened by the bed partner because they usually return to rapid alertness and orientation. Others can't remember what they are dreaming but the spouse tells that the patient is distressing in sleep and that the behavior mirrors the dream content, as they are "acting out their dreams".

The timing of RBD episodes reflects when patient is in REM sleep; it can starts after 90 minutes from sleep onset but generally in the latter half of the sleep period, that, for most individuals, is after 3 am and in the few hour prior to wake onset.

The frequency varies widely; some exhibit RBD several episodes each night and others only one or less episode per month.

Most patient with RBD are male typically over 50 years of age, usually between 40 and 70, and, interestingly, this aggressive dream contents is non-associated with an increase daytime patient aggressive. There is evidence that RBD is different in its presentation between male and female where there is less violent dream-enacting behavior.

We can identify and idiopathic or secondary RBD, and an acute or chronic disorder.

Acute onset of RBD is almost always caused by medications, usually tricyclic antidepressant, monoamine oxidase inhibitors, serotonin-specific and serotonin-norepinephrine reuptake inhibitors. An acute form may also occur during alcohol or hypnotic sedative withdrawal.

Idiopathic RBD refers to the disorder that occurs in the absence of any other neurologic disorder. Secondary or symptomatic RBD refers to the combination between RBD plus a neurologic disorder. A high rate of RBD are strongly reported in association with degenerative disorders as Parkinson's disease (PD) and strongly with dementia with Lewy bodies (LBD) and multiple system atrophy (MSA), collectively termed "synucleopathies" because of their prominent α-synuclein-positive pathology. RBD, preceding the motor and cognitive features of a neurodegenerative disorder, supports the concept of selective vulnerability occurring in brainstem network. The evolution of clinical features maybe reflects the topography of degeneration over time and their timing may reflect when the critical threshold of neuronal network degeneration are reached.

Braak and colleagues have proposed a staging system for the neuropathological characterization of the phenotype of RBD and this may be applicable to the timing of the evaluation of RBD. This staging system (VI stages) posits a temporal sequence of α-synuclein pathology in the brain, beginning mainly in the medulla then gradually ascending to cortex. In stage I the dorsal IX-X motor nucleus and the olfactory bulb are affected with presumably coexisting degenerative changes in these structures. Disfunction in caudal raphe nuclei, SLD and/or MCRF (Stage II) could lead to RSWA and RBD. In stage III there is a rostral progression to PPN, SN and nucleus basalis of Meynert (NBM) and when sufficient degeneration respectively occurs, parkinsonism and cognitive changes become manifest. These temporal sequences of pathology could explain why RBD precedes parkinsonism, cognitive decline and dementia (Stages IV).

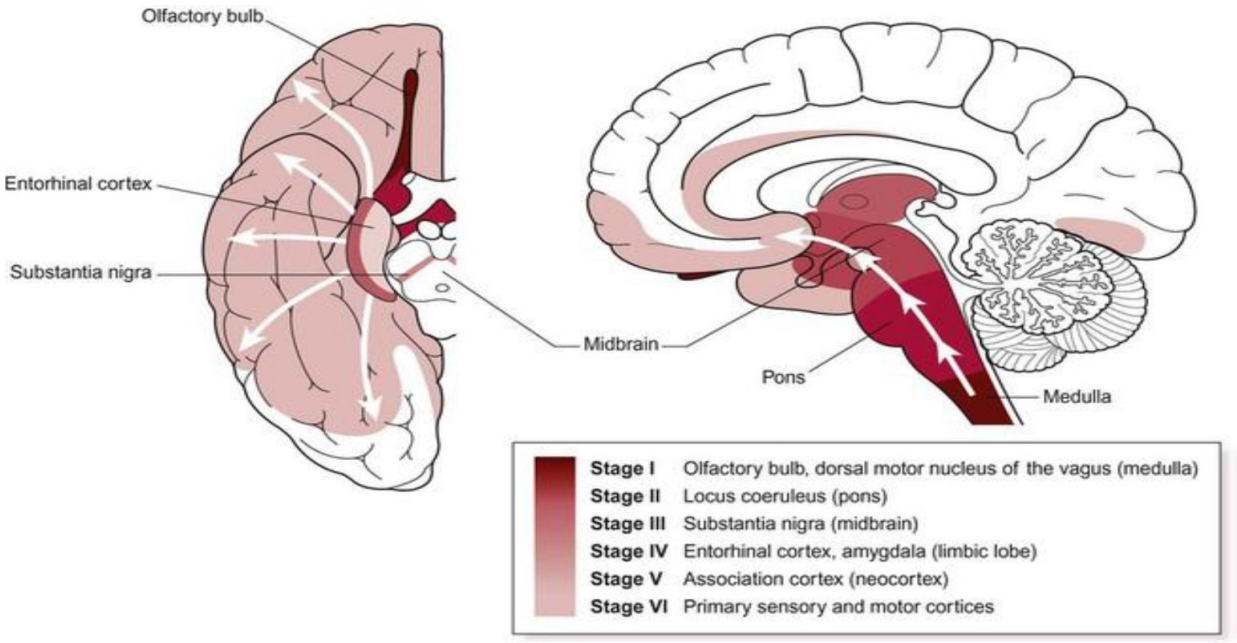

Fig 2. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K .

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Cell Tissue Res. 2004 Oct;318(1):121-34. Epub 2004 Aug 24.

Polysomnography and Hypnogram

Polysomnography refers to a systematic process used to collect physiologic parameters during sleep. A polysomnogram (PSG) is a procedure that utilizes electroencephalogram (EEG), electro-oculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and pulse-oximetry, as well as airflow and respiratory effort, to evaluate for underlying causes of sleep disturbances. PSG is the gold standard for diagnosing of several sleep disorders, including rapid eye movement sleep behavior disorder (RBD).

The EEG is necessary to perform sleep scoring recording spontaneous electrical activity that is generated by the brain using surface electrodes positioned on the scalp.

In a relaxed waking condition and with the subject with closed eyes, it is possible to detect an activity that has one frequency between 8 and 13 Hz which takes the name of alpha activity. This is more intense in the occipital regions but it is also detectable in the parietal and frontal regions.

As the subject falls asleep there is the disappearance of the alpha and the appearance of activity in the band of theta (4-8 Hz) or delta (0.5-4 Hz) which is the typical frequency of deep sleep.

The EOG records ocular activity of orbicular eye muscles, and it is very useful to investigate both the presence of Slow Eyes Movements or SEMs in N1 stage of NREM sleep, and Rapid Eyes Movements during REM Sleep phase.

The EMG records the electrical activity of muscle origin from anterior tibialis and mylohyoid muscles, this one progressively reduces in voltage during NREM sleep until it physiologically becomes completely atonic during REM sleep.

The ECG, or the recording of the electrical activity generated by the heart muscle, is of fundamental importance to monitor heart rate variation during the night.

Lead II is generally chosen, placing the electrodes below the right clavicle and at the height of the seventh rib, as it is the derivation in which the QRS complex has the greatest amplitude.

According to the *American Academy of Sleep Medicine* (AASM 2013), it is recommended that all those digital recordings sampling rates are set between a desirable and minimum value, respectively of

500 and 200 Hz. Routinely recorder filter settings are: 0.3 Hz for low-frequency referring to EEG, EOG and ECG, and 10 Hz to EMG; 53 Hz for high-frequency applied for EEG and EOG, 100 Hz for EMG and 70 Hz for ECG.

Definitely, Wakefulness, NREM and REM sleep can be distinguished on polysomnography by a combination of their EEG, EOG and EMG features. Those different stages (N1, N2, N3 and REM) are visible, in function of the night-time, on a graph named ***Hypnogram*** that allows us to see the *sleep macrostructure*.

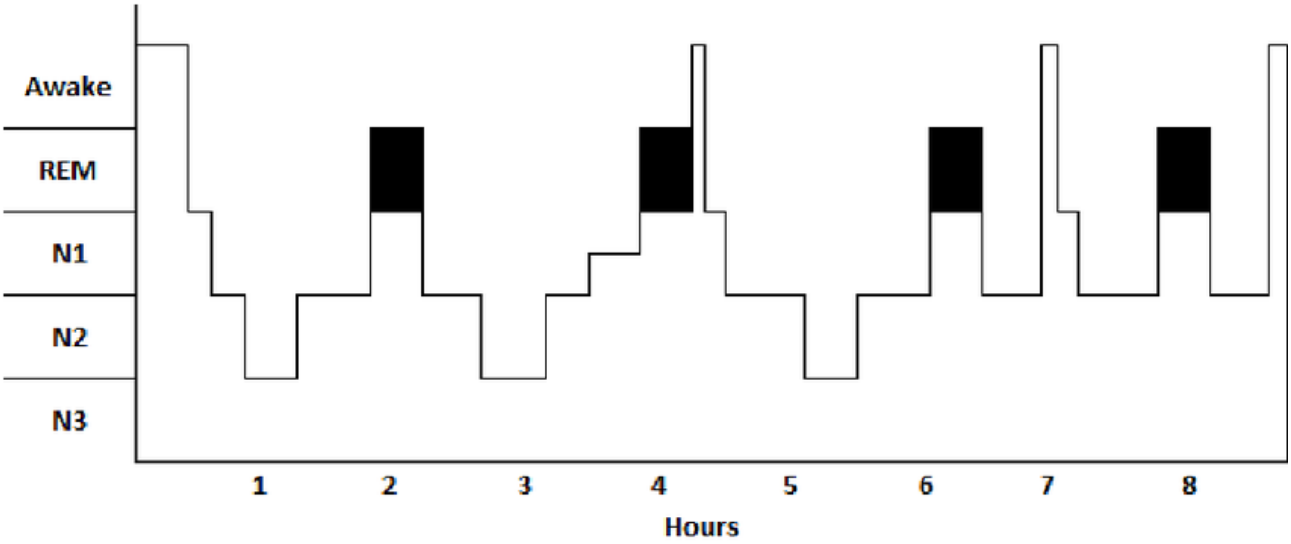

Fig. 3 *Hypnogram graph showing physiological succession of sleep stages during eight hours of sleep.*

Autonomic Nervous System

A key role in the physiology of sleep is played by Autonomic Nervous System (ANS) whose regulation modulates a wide range of physiological conditions during sleep onset and transition to different sleep stages. Cardiovascular, respiratory, gastrointestinal system are based on the reciprocal activation of the two branches of ANS, sympathetic and parasympathetic nervous system, also called sympato-vagal balance.

The anatomic distribution of the autonomic nervous system is complex and involves multiple areas of both the central and peripheral nervous system.

The central autonomic nervous system comprises several interconnected regions of the forebrain, brainstem, and spinal cord. The spinal cord coordinates segmental sympathetic or sacral parasympathetic reflexes that are modulated by higher levels. The lower brainstem (bulbopontine) is involved in control of circulation, respiration, gastrointestinal function, and micturition. The upper brainstem (pontomesencephalic) system integrates control of autonomic function with pain and the behavioral responses to physiologic stress. The forebrain system includes the hypothalamus (control of homeostasis and adaptation) and the anteriorlimbic circuits, which comprise the insula, anterior cingulate cortex, and the amygdala. Through these diverse relays the anterior cingulate cortex exerts control over both sympathetic and parasympathetic functions; the amygdala is a key element of the "emotional" autonomic response and exerts downstream control of both the neuroendocrine and autonomic outputs to stress and fear.

The hypothalamus is responsible for homeostasis and adaptation to changes in body temperature, osmolarity, hypoglycemia, circadian rhythms, sleep, arousal, or other external environ-mental changes.

The rostral ventrolateral medulla is centrally involved in control of blood pressure through connections to *sympathetic* preganglionic neurons that control cardiac output and total peripheral resistance and mediates baroreflex, chemoreflex, and cardiopulmonary reflexes. The caudal ventrolateral medulla provides inhibitory control over the rostral ventrolateral medulla (via GABAergic neurons) and serves as a relay for the inhibitory inputs from the NTS to the arterial baroreflex.

The parasympathetic preganglionic neurons are located in the dorsal motornucleus of the medulla and the ventrolateral portion of the nucleus ambiguus. These preganglionic neurons control *vagal output* and connect to local ganglia in the heart, respiratory tract, enteric nervous system, liver, and pancreas.

The *parasympathetic* fibers of the sacral spinal cord originate in the S2–S4 region. These preganglionic fibers extend to the pelvic viscera where local ganglia act as therelay station for the particular organ of interest.

The thoracolumbar segments, from T1 to L2, house **preganglionic sympathetic neurons** that maintain blood pressure, control thermoregulation, and control vascular flow redistribution during activity or stress.

The preganglionic neurotransmitter for the sympathetic nervous system is acetylcholine, and the receptors on the postganglionic neurons are of the nicotinic type. The postganglionic neurotransmitter is norepinephrine for all end-organ connections, with adrenergic receptors on peripheral target tissues.

The autonomic responses to the sympathetic and parasympathetic systems are often antagonistic and reflect the highly coordinated interactions within the central nervous system.

During NREM sleep the cardiovascular system is stable and parasympathetic cardiac modulation is stronger; *parasympathetic effects* are localized and generally related to the maintenance of restorative and energy-saving function for a longer period of time, for example bradycardia, hypotension, myosis, bronchospasm. *During REM sleep* cardiovascular system is unstable and greatly influenced by sympathetic activity which effects are diffuse, generalized, fast, and serve physiological defense mechanism in response to extremi stimuli or conditions (fear, danger, hypoxia, etc.…) inducing tachycardia, hypertension, vasoconstriction, mydriasis, bronchodilation.

Adverse cardiovascular events reflect this circadian variation of sympato-vagal balance during sleep, showing a higher incidence in the morning hours during REM phase.

In fact from N1 to N3, the stages of highest synchronization, a gradual decrease is observed in heart rate, blood pressure and MSNA, with minimum values reached during N3. REM sleep has a sort of activation of cardiovascular system to levels sometimes higher than wakefulness.

Heart Rate Variability (HRV)

Heart Rate Variability is a simple and non – invasive approach to study Autonomic Nervous System circadianity during the night though ECG trace on polysomnography.

Heart rate is modulated on a beat-to-beat basis by the combined effects of the sympathetic and parasympathetic nervous system on the sino-atrial node. Variability of heart rate is the measure of the variation of the interbeat interval (IBI), or the interval between two consecutive heartbeats (R-R on ECG trace).

Adverse cardiovascular events exhibit a clear circadian pattern, with maximal occurrence during morning hours. Indeed, there is a ~40% higher incidence in acute myocardial infarction, sudden cardiac death, atrioventricular block, ventricular fibrillation, ventricular tachycardia, and ischemic events between 06:00-12:00 relative to the rest of the day. We and others have recently provided evidence that an interaction between circadian and sleep-wake dependent processes regulates heart rate variability (HRV), an accepted method to quantify autonomic nervous system (ANS) cardiac modulation. These observations could contribute to peak cardiovascular vulnerability observed in the morning. Although nighttime is assumed to be cardioprotective, a meta-analysis revealed a bimodal distribution of myocardial infarctions, sudden cardiac death, and implanted cardioverter-defibrillator at night with peak cardiovascular risk at the beginning and end of the night.

Sleep stages alternate throughout a normal sleep period and correlate with changes in HRV. During NREM sleep, the cardiovascular system is stable and parasympathetic cardiac modulation is stronger. During REM sleep, the cardiovascular system is unstable and greatly influenced by surges in sympathetic activity. The increased circadian propensity to REM sleep in the early morning could, in part, explain the coincidental increased cardiac risk. Indeed, REM sleep could precipitate numerous adverse cardiac events such as arrhythmia, acute myocardial infarction, and sudden cardiac death, whereas NREM sleep could increase the risk of ischemic events in susceptible patients. Sleep disruption associated with a variety of sleep disorders such as sleep disordered breathing, periodic limb movements (PLMs), insomnia, and other medical conditions including nocturia and depression has also been associated with cardiovascular diseases.

Prior studies have shown that misalignment between the sleep schedule and the endogenous circadian clock as observed in night shift workers may lead to elevated risk of adverse cardiovascular events.

MATERIALS AND METHODOLOGY

Patients Recruitment

The study examines the clinical data and polysomnographic records of 37 patients aged 53 years and older (median 72.7; mean 72.3 ± 7.4; range 53-84), from the Center for Sleep Medicine and from the Neurology Unit of the University Hospital of Monserrato. The population under examination is made up of 7 women (18.9%) and 30 men (81.1%).

Among the 37 patients of the sample, 22 received a diagnosis of idiopathic REM sleep behavior disorder (59.5%; age: median 72.5; mean 74.5 ± 5.2; range 68-83), of which 3 women (13.6%) and 19 men (86.4%); 15 persons had a diagnosis of REM sleep behavior disorder secondary to Parkinson's disease (40.5%; age: median 73; mean 69.5 ± 9 ; range 53-84), including 4 women (26.7%) and 11 men (73.3%).

Patients were recruited according to the following inclusion criteria: diagnosis of PD according to the clinical criteria of the Movement Disorder Society and / or a diagnosis of RBD according to the criteria of the International Classification of Sleep Disorders (ICSD-3).

Patients were excluded from the study when did not give their informed consent, have being treated for REM sleep behavior disorder, had Parkinson's disease, heart disease, taking electrical devices or when polygraphic traces had artifacts.

Every patients ruled out a complete full-night polysomnography that analyzed sleep score in EEG traces, cardio-respiratory assessment, ECG and EMG.

For each polysomnography we created its hypnogram on which, individuating five minutes epochs, we applied HRV index analysis (HR, SDNN, SNSN, RMSSD, NN50, NN20, pNN50, pNN20, VLF, LF, HF, LF/HF, ApEn, SampEN, LZC, KC, LTI, STV, II). Results were then analyzed with Wilcoxon test e considering significant when p-value were < 0,05.

RBD and Polysomnography

Full night video-polysomnoghraphy recording is mandatory to establish the diagnosis of RBD.

We used the Micromed Polysomnograph and the Sleep-RT program to score sleep and create the hypnogram of the night study according to American Academy Sleep Medicine (AASM 2013).

- 12 EEG channels with common reference;
- 12 switchable in bipolar / common reference;

- 2 DC channels;

- 2 integrated channels for breath with inductive bands;

- 2 integrated channels for pressure flow (Diff. And Gauge);

- 1 Body Position channel integrated in the recorder;

- 3 digital channels for SpO2, heart rate and plethysmogram.

According to the last International Classification Of Sleep Disorders (ICSD-3), diagnostics of RBD contemplates the whole following criteria:

- ✓ Repeated episodes of sleep related vocalization and/or complex motor behaviors witch can be observed during a single night of video-PSG.
- ✓ These behaviors are documented by PSG to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep.
- ✓ PSG recording demonstrates REM sleep without atonia (RSWA)
- ✓ The disturbance is not better explained by another sleep or mental disorder, medication or substance use.

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 almost exclusively arm and hand behaviors during REM sleep.

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 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) reliably distinguishes RBD patients from controls.

Sleep architecture and the customary cycling among REM and NREM sleep stages are usually preserved in RBD, although some patients show a shift toward N1 sleep.

Upon awakening, the individual is tipically awake, alert, coherent and oriented; 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 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.

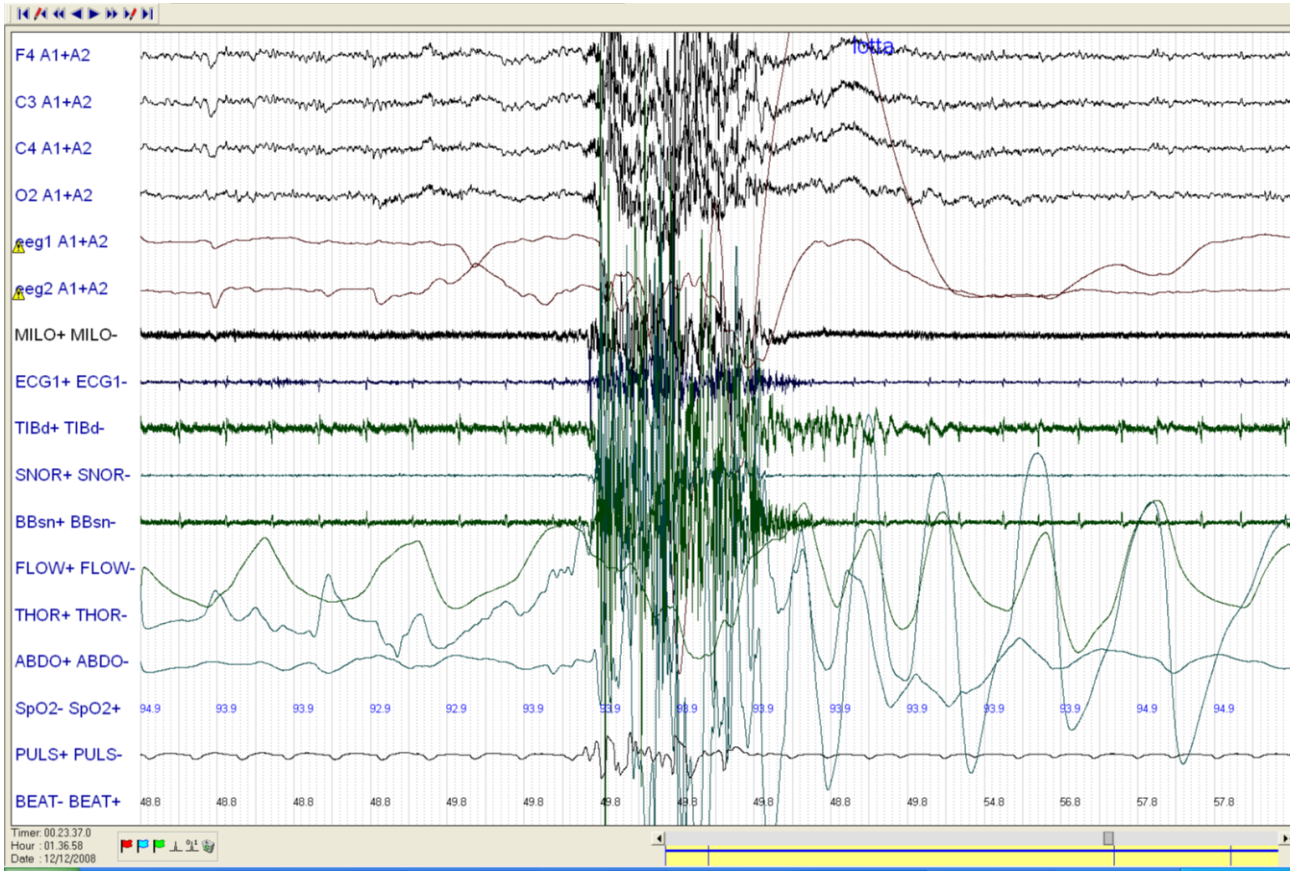

Fig 4. Polysomnography Trace showing a REM sleep stage with loss of myloid and limbs muscles atonia.

Hypnogram

The Hypnogram shows a graphic representation of the different sleep stages succession during the night.

It could be associated with the analysis of different parameters: entropy, tools about sleep continuity or fragmentation, and REM sleep quantification advices.

The Sleep Diversity Index (SDI) is a parameter we estimated on hypnogram that calculates Shannon-Wiener Entropy analysis on 15 epochs of 30 seconds. Its value is minimum (zero) when there is no change in sleep stage in those 15 epochs while it's a maximum value when all the five sleep stages can be appear equally on hypnogram. We estimated other several parameters on hypnogram:

Sleep Continuity:

- Awakening Index (number of awakenings during the night)
- Deep Sleep Efficiency (ratio between the number of minutes spent in N3 stage and the number of minutes spent in bed)
- Number of sleep stages transitions during the night
- Number of sleep transition from N3 stage to N1 or N2 phase
- Sleep Efficiency (ratio between minutes of sleep and minutes in bed)
- Wake After Sleep Onset (or WASO, total amount of minutes awake after the first sleep epoch is achieved)

REM Sleep Stage

- Average duration of all Rem Stages
- Ratio between all Rem sleep stages minutes and total sleep minutes
- Ratio between the number of REM sleep stages and all sleep stages

Aforementioned Hypnogram analysis parameters were made on two different graphs.

The first one is the classic hypnogram where visual representation of sleep macrostructure is created by the succession of small horizontal segments representing 30 seconds (one epoch) of one sleep phase. Every stage corresponds to a number: wake – 0, N1 – 1, N2 – 2, N3 – 3, REM – 5.

If there was an epoch without sleep staging, a gap is visible instead of the linear segment so they could be eliminated for our analysis.

The second graph reports arousal appearing in one second of sleep as two vertical lines representing respectively the onset and the end of one arousal.

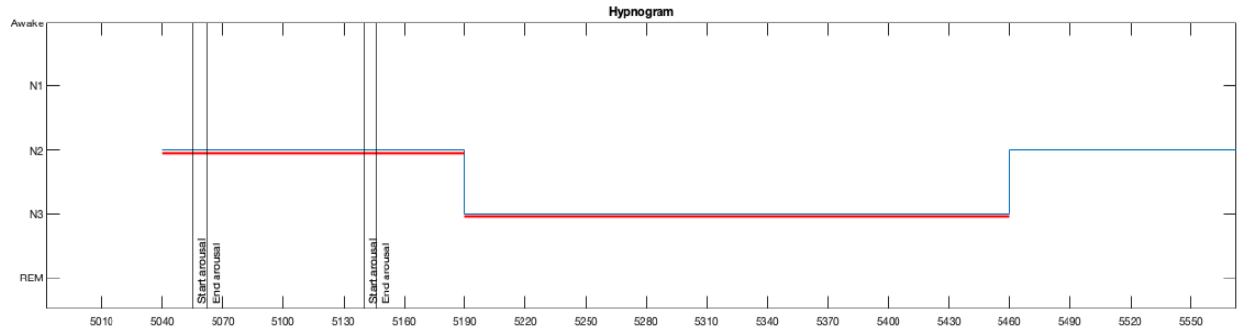

Fig 5. Hypnogram graph showing an horizontal 30 seconds line for each sleep stage (N1, N2, N3 and REM) and a vertical line for both onset and ending arousal.

Heart Rate Variability

Heart Rate Variability study is based on the Normal-to-Normal (NN) series, obtained from the R-R peak of QRS complex, after excluding ectopic heart beats because they're not originated in the SA node, so they do not depend on the functionality of the SNA. It is educed by ECG polysomnography trace witch sample rate was set to 512 Hz to guarantee a best precision in R-peak identification. On the ECG trace of this 5 minutes segment, we applied the ***Pan-Tompkins algorithm*** to identify QRS complex according to its gradient, amplitude and width.

Tacogram is the graphic representation of NN series interval and on Tacogram we can analyze several HRV parameters subdivided in linear or non-linear indices.

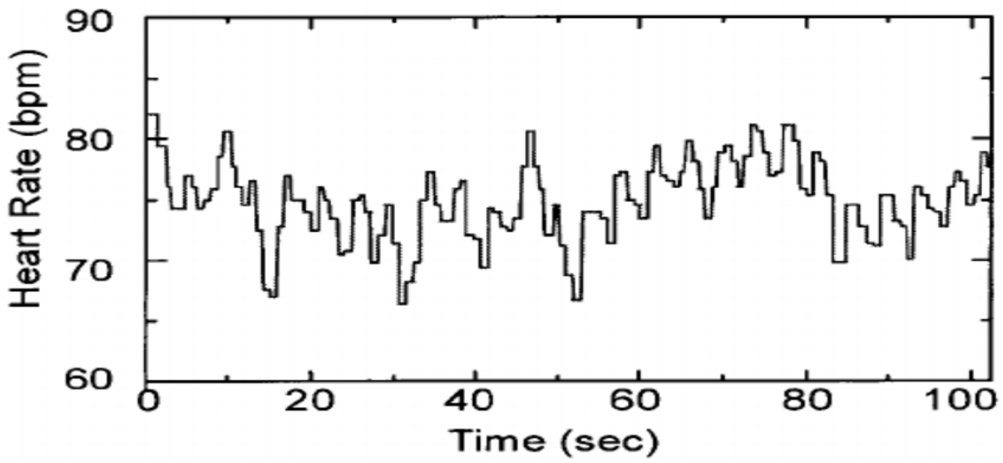

Fig 6. Graphic representation of Tacogram

Linear Indices:

- **Time-domain** indices of HRV quantify the amount of variability in measurements of the interbeat interval (IBI), which is the time period between successive heartbeats. These values may be expressed in original units or as the natural logarithm (Ln) to achieve a more normal distribution. These metrics include the SDNN, SDRR, SDANN, SDNN Index, RMSSD, NN50, pNN50, HR Max − HR Min, the HRV triangular index (HTI), and the Triangular Interpolation of the NN Interval Histogram.

  The standard deviation of the IBI of normal sinus beats (SDNN) is measured in ms. "Normal" means that abnormal beats, like ectopic beats (heartbeats that originate outside the right atrium's sinoatrial node), have been removed.

  The standard deviation of the IBIs for all sinus beats (SDRR), including abnormal or false beats, is measured in ms.

  The standard deviation of the average normal-to-normal (NN) intervals for each of the 5 min segments during a 24 h recording (SDANN) is measured and reported in ms like the SDNN.

  The SDNNI is the mean of the standard deviations of all the NN intervals for each 5 min segment of a 24-h HRV recording. Therefore, this measurement only estimates variability due to the factors affecting HRV within a 5-min period.

  The number of adjacent NN intervals that differ from each other by more than 50 ms (NN50) requires a 2 min epoch. The percentage of adjacent NN intervals that differ from each other by more than 50 ms (pNN50) also requires a 2-min epoch.

  The root mean square of successive differences between normal heartbeats (RMSSD) is obtained by first calculating each successive time difference between heartbeats in ms. Then, each of the values is squared and the result is averaged before the square root of the total is obtained.

  The RMSSD reflects the beat-to-beat variance in HR and is the primary time-domain measure used to estimate the vagally mediated changes reflected in HRV.

Parameter	Unit	Description
SDNN	ms	Standard deviation of NN intervals
SDRR	ms	Standard deviation of RR intervals
SDANN	ms	Standard deviation of the average NN intervals for each 5 min segment of a 24 h HRV recording
SDNN index (SDNNI)	ms	Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording
pNN50	%	Percentage of successive RR intervals that differ by more than 50 ms
RMSSD	ms	Root mean square of successive RR interval differences

Fig 7. HRV time-domain Indices

- **Frequency-domain** measurements estimate the distribution of absolute or relative power into four frequency bands. *Power* is the signal energy found within a frequency band. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) divided heart rate (HR) oscillations into ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands. We used Fast Fourier Transformation (FFT) modeling to separate HRV into its components that operate within different frequency ranges.

Frequency-domain measurements can be expressed in absolute or relative power. *Absolute power* is calculated as ms squared divided by cycles per second (ms^2/Hz). *Relative power* is estimated as the percentage of total HRV power or in normal units (nu), which divides the absolute power for a specific frequency band by the summed absolute power of the LF and HF bands.

The ultra-low-frequency (**ULF**) band (≤0.003 Hz) is highly correlated with the SDANN time-domain index. There is disagreement about the contribution by the PNS and SNS to this band.

The very low frequency (**VLF)** band (0.0033–0.04 Hz) requires a recording period of at least 5 min and s strongly correlated with the SDNNI time-domain measure. There is uncertainty regarding the physiological mechanisms responsible for activity within this band; the heart's

intrinsic nervous system appears to contribute to the VLF rhythm and the SNS influences the amplitude and frequency of its oscillations.

The Low Frequency (**LF)** band (0.04–0.15 Hz) mainly reflects baroreceptor activity during resting conditions. LF power may be produced by both the PNS and SNS, and BP regulation *via* baroreceptors, primarily by the PNS or by baroreflex activity alone. During periods of slow respiration rates, vagal activity can easily generate oscillations in the heart rhythms that cross over into the LF band.

The Hight Frequency (**HF**) band (0.15–0.40 Hz) reflects parasympathetic activity and is called the respiratory band because it corresponds to the HR variations related to the respiratory cycle. High-frequency power is highly correlated with the pNN50 and RMSSD time-domain measures. During inhalation, the cardiovascular center inhibits vagal outflow resulting in speeding the HR. Conversely, during exhalation, it restores vagal outflow resulting in slowing the HR *via* the release of acetylcholine. HF band power may increase at night and decrease during the day. Lower HF power is correlated with stress, panic, anxiety, or worry. The modulation of vagal tone helps maintain the dynamic autonomic regulation important for cardiovascular health. Deficient vagal inhibition is implicated in increased morbidity.

The ratio of LF to HF power (**LF/HF ratio**) shows both PNS and SNS activity contribute to LF power, and PNS activity primarily contributes to HF power. The intent was to estimate the ratio between SNS and PNS activity. The assumptions underlying the LF/HF ratio is that LF power may be generated by the SNS while HF power is produced by the PNS. In this model, a low LF/HF ratio reflects parasympathetic dominance. In contrast, a high LF/HF ratio indicates sympathetic dominance, which occurs when we engage in fight-or-flight behaviors or parasympathetic withdrawal.

Parameter	Unit	Description
ULF power	ms^2	Absolute power of the ultra-low-frequency band (≤0.003 Hz)
VLF power	ms^2	Absolute power of the very-low-frequency band (0.0033–0.04 Hz)
LF peak	Hz	Peak frequency of the low-frequency band (0.04–0.15 Hz)
LF power	ms^2	Absolute power of the low-frequency band (0.04–0.15 Hz)
LF power	nu	Relative power of the low-frequency band (0.04–0.15 Hz) in normal units
LF power	%	Relative power of the low-frequency band (0.04–0.15 Hz)
HF peak	Hz	Peak frequency of the high-frequency band (0.15–0.4 Hz)
HF power	ms^2	Absolute power of the high-frequency band (0.15–0.4 Hz)
HF power	nu	Relative power of the high-frequency band (0.15–0.4 Hz) in normal units
HF power	%	Relative power of the high-frequency band (0.15–0.4 Hz)
LF/HF	%	Ratio of LF-to-HF power

Fig 8. HRV frequency-domain Indices

Non Linear Indices

Non-linearity means that a relationship between variables cannot be plotted as a straight line. Non-linear index measurements the unpredictability of a time series, which results from the complexity of the mechanisms that regulate HRV. Non-linear indices correlate with specific frequency- and time-domain measurements when they are generated by the same processes. While stressors and disorders like diabetes can depress some non-linear measurements, elevated values do not always signal health. For example, in post myocardial infarction (post-MI) patients, increased non-linear HRV is an independent risk factor for mortality. This section reviews S, SD1, SD2, SD1/ SD2, approximate entropy (ApEn), sample entropy (SampEn), detrended fluctuation analysis (DFA) α1 and DFA α2, and D2 non-linear measures.

Parameter	Unit	Description
S	ms	Area of the ellipse which represents total HRV
SD1	ms	Poincaré plot standard deviation perpendicular the line of identity
SD2	ms	Poincaré plot standard deviation along the line of identity
SD1/SD2	%	Ratio of SD1-to-SD2
ApEn		Approximate entropy, which measures the regularity and complexity of a time series
SampEn		Sample entropy, which measures the regularity and complexity of a time series
DFA α1		Detrended fluctuation analysis, which describes short-term fluctuations
DFA α2		Detrended fluctuation analysis, which describes long-term fluctuations
D_2		Correlation dimension, which estimates the minimum number of variables required to construct a model of system dynamics

Fig 9. HRV non-linear Indices

Non-linear measurements are able to describe the unpredictability of a time series (an example of which is given by the NN series) but, although useful in the description of a complex systems; their use in the clinical setting is still limited by the lack of a standard.

Some examples of non-linear indices, however used in the context of this thesis, are: approximate entropy, simple entropy, Lempel-Ziv complexity and Kolmogorov complexity.

Approximate entropy (ApEn) is a measure of the unpredictability of fluctuations on a time series of data, used in all those cases in which it is not possible to trace the nature of the observed system. Specifically, the ApEn studies the repetitiveness of the model by observing the NN intervals: similar observations that make up a pattern will have a low value of ApEn, different observations belonging to a complex model will have a high value of ApEn.

Simple entropy (SampEn) describes the conditional probability in which similar observations maintain similarity even in the next sample. Unlike the ApEn, it is independent of the length of the series.

The Lempel-Ziv (LZC) and Kolmogorov (KC) entropies introduce higher levels of complexity. In the analysis of complex series of biological data, each of these is translated into a symbolic sequence based on a finite alphabet: binary, in the case of both complexities, or ternary, in the case of Kolmogorov's complexity alone. The encoding of the data in a binary string provides for the attribution of 1 to each signal increase and of 0 to each decrease; in addition, coding in a ternary system attributes 2 to each stationary state. Since the string encoded in this way is dependent on the quantization level with which the signal was obtained, the introduction of the factor p (p = 0%; p = 0.5%; p = 1%; p = 2%) establishes the minimum quantization level needed to observe a symbol change in the string.

Analysis

Each patient of our study was subjected to a complete full-night polysomnography.

ECG trace has been extrapolated and cleaned by possible saturations; then QRS complex were identified to get the Tacogram. Analyzes are all based on the tacogram, the series of NN intervals**.** It is a vector in which each element is the difference in samples between two successive QRS complexes (RR interval), splits then for the sample rate and multiply by 1000 to get the intervals in ms. Since that we are working on 5 minutes segments, we expect about 300 beats.

From Tacogram linear and non-liner indices of HRV was calculated.

A parallel analysis was conducted on the Hypnogram.

For each stage of sleep we tried to take both the longest epoch and the first epoch that occurred during the night. In both cases, we calculated the average value obtained in the 5-minute floating windows with overlap of 4 and a half minutes used to cover it.

In addition, we made another analysis considering which phase preceded the one analyzed. Phase transitions were thus identified, but only for N1-N2, N2-N3, N3-N2, N2-REM it was possible to apply the statistical analysis, also in this case to due to the lack of an adequate number of patients on which to perform the tests. The choice of which epoch to analyze in reference to phase transitions fell on the first epoch.

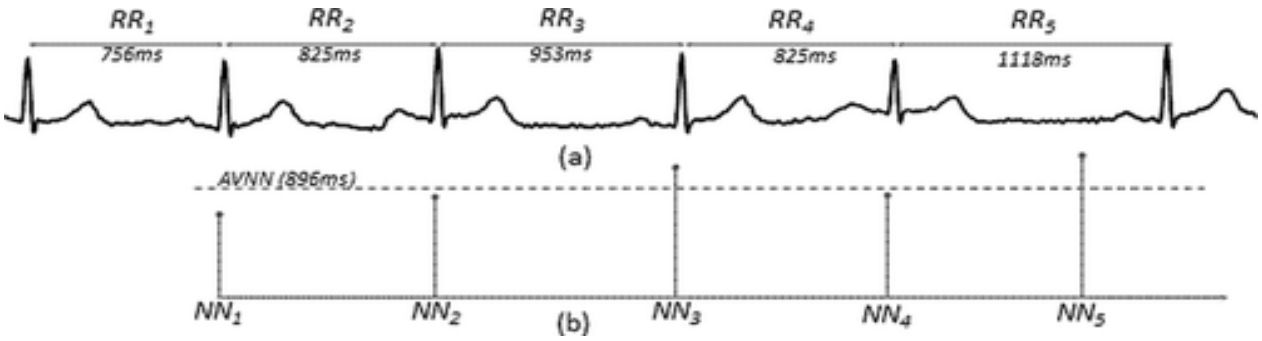

Fig 10. Tacogram

On tacogram we then calculated HRV time-domain and frequency domain index. This last one, a non-parametric method, was used for the calculation of the PSD, based on simple algorithm and processing speed. The method chosen takes advantage of the Fast Fourier Transform and takes the Welch method name. Then, we calculated power value in all the three bands (VLF. LF, HF) and LF/HF ratio.

Statistical analysis of HRV Index was calculated on two matrices: one for idiopathic RBD and one for RBD in Parkinson's patients. Each column refers to a sleep stage (N1, N2, N3, REM) and each strip for the number of the patients of both groups.

For each sleep stage (N2-N3-REM) we had examined the first and the longest epoch of the polysomnography recording. Each epoch was superimposed in a range of 4,30 minutes.

After calculated HRV index analysis, we compared the two matrices with Wilcoxon Rank Sum Test of MatLab

Results

Our HRV data-analysis is based on 34 patients because of the lack of three valid recordings. Analysis of HRV indices was performed on each sleep stage (N2, N3, REM) and phase transition (N1-N2, N2-N3, N3-N2, N2-REM). Results were then subjected to the Wilcoxon test and considered significant to distinguish our two RBD populations, Idiopathic RBD from Parkinson's Disease RBD, for a p-value <0.05.

Once the null hypothesis has been declared, i.e. that the data obtained for each matrix belong to a family of normal distributions, this can be rejected (MatLab program attributes the value of 1 to the rejection of the hypothesis) or not-rejected (assuming the value 0).

The rejection of the null hypothesis in almost all of the analysis carried out the choice of using the non-parametric Wilcoxon test as the study statistic.

The same test also represents the statistic we used for the clinical-demographic analysis and the indices referring to the hypnogram.

The results reported below, therefore, were acquired from the application of the aforementioned test in the Wilcoxon Rank Sum Test form of MatLab.

Fig. 11 Clinical-demographic and polysomnographic data results reported a significance coefficient p-value <0.05 in: duration in years of RBD (p = 0.0284); the number of periodic leg movements during sleep (p = 0.0297); the PLMS index (p = 0.0229) and the apnea-hypopnea index (p = 0.0487).

	Sample of the study (n = 34)				
	IRBD (n = 20)		PD-RBD (n = 14)		p-value
Age	74,5 ± 5,2	72,5	69,5 ± 9,0	73	0,1336
Gender	1,14 ± 0,35	1	1,27 ± 0,46	1	0,5060
RBD	5,65 ± 5,04	4,5	11,23 ± 8,77	9	0,0284
PD*			9,4 ± 7	8,5	
TBT*	518,4 ± 26,5	514	525,7 ± 36,5	528,3	0,2858
TST*	337,6 ± 96,3	357,5	357,4 ± 128,3	400,8	0,2554
Arousals	27,1 ± 16	22	24,7 ± 16	17,5	0,4109
N1 (%)	16,62 ± 7,98	14,25	14,46 ± 6,88	14,3	0,5061
N2 (%)	45 ± 11,9	46	45,7 ± 9	44,3	0,9442
N3 (%)	25,77 ± 13,11	24,65	25,67 ± 10,03	25,85	0,7264
PLMS*	198,2 ± 168,3	186,5	76,5 ± 101,5	23	0,0297
PLMi*	39,2 ± 38	27,8	13,3 ± 16,1	6,1	0,0229
AHI*	9,49 ± 8,81	9,05	3,05 ± 4,64	0,8	0,0487
RAI*	0,64 ± 0,21	0,70	0,71 ± 0,12	0,75	> 0,05

**PD Parkinson's Disease, TBT total bed time, TST total sleep time, PLMS Perdiodic Limb Movements Syndrome, PLMi Perdiodic Limb Movements Index, AHI Apnea/Hypopnea Index, RAI Respiratory Arousal Index*

Fig 12. **HRV Time-domain Linear Indices** shows useful p-value in the standard deviation of NN interval, in the number of pairs of successive NNs that differ by more than 20 ms, and in the proportion of NN20 divided by total number of NNs, in particular in the longest REM stage and in N2-REM transition.

Index	Longest REM stage	First N2	First N3	First REM	N1 - N2 transition	N2-N3 transition	N3-N2 transition	N1 – REM transition	N2- REM transition
SDNN	0,0453	0,5004	0,9488	0,0695	0,3254	1	0,5726	0,8520	0,0042
HR	0,6669	0,6413	0,7361	0,8369	0,5705	0,7361	0,7618	0,9702	0,8429
SDSD	0,0695	0,6766	0,5421	0,0640	0,4334	0,5854	0,4598	0,1675	0,0338
RMSSD	0,0695	0,6766	0,5421	0,0640	0,4334	0,5854	0,4598	0,1675	0,0338
NN50	0,0746	0,5109	0,4668	0,0897	0,4261	0,4668	0,5686	0,1888	0,0332
NN20	0,0286	0,6303	0,4224	0,0414	0,4046	0,4412	0,3944	0,0676	0,0293
pNN50	0,0632	0,5541	0,5178	0,0972	0,4666	0,5178	0,5686	0,1648	0,0440
pNN20	0,0214	0,7002	0,4605	0,0588	0,5157	0,5004	0,3599	0,1087	0,0338

Fig 13. **HRV Frequency-domain Linear Indices** show significant values too, specially for N2-REM transition.

Index	Longest REM stage	First N2	First N3	First REM	N1 - N2 transition	N2-N3 transition	N3-N2 transition	N1 – REM transition	N2- REM transition
VLF	0,6669	0,0890	0,9488	**0,0260**	**0,0473**	0,9488	0,7618	0,4334	**0,0020**
LF	0,4887	0,9233	0,6303	0,0539	0,6054	0,6303	0,4082	0,2475	**0,0338**
HF	0,7222	0,6303	0,4041	0,2099	0,6289	0,4041	0,3154	0,2794	**0,0388**
LF/HF	0,6944	0,8725	0,6077	0,1204	0,7515	0,6077	0,5726	1	**0,0138**

Fig 14. HRV Non-linear Indices demonstrates not enough significant results for entropy analysis.

Index	Longest REM stage	First N2	First N3	First REM	N1 - N2 transition	N2-N3 transition	N3-N2 transition	N1 – REM transition	N2- REM transition
Approximate Entropy	0,3998	0,7483	0,4412	0,8958	0,9336	0,3357	0,5148	0,2794	0,8429
Sample Entropy	0,3399	0,5004	0,5004	0,2537	0,5597	0,6533	0,6965	0,1457	0,6711
Long Term Irregularity	0,0755	0,7727	1	0,2239	0,6289	0,9233	0,4598	0,5758	0,0100
Short Term Variability	0,0958	0,5004	0,9233	0,3034	0,4334	0,9233	0,7618	0,3909	0,0388
Interval Index	1	0,9233	0,5854	0,8078	0,7515	0,7002	0,6965	0,2794	0,7989

Fig 15. HRV Complexity non Linear Indices according to Lempel-Ziv and Kolmogorov Complexity show much more indices statistically significant for our study instead of Linear Indices.

Index	Longest REM stage	First N2	First N3	First REM	N1 - N2 transition	N2-N3 transition	N3-N2 transition	N1 – REM transition	N2- REM transition
LZC (binary, p=0%)	0,7504	0,2753	0,6077	0,8078	0,0244	0,6303	0,5148	0,0277	0,0161
LZC (binary, p=0,5%)	0,1393	0,1677	0,0021	0,0014	0,6054	0,0021	0,3154	0,5260	0,0219
LZC (binary, p=1%)	0,0640	0,0400	7,5393e-04	5,7440e-05	0,0343	7,5393e-04	0,8968	0,9109	0,0060
LZC (binary, p=2%)	0,0453	0,5854	1,5290e-04	4,0535e-04	0,2237	1,5290e-04	0,3599	0,5758	0,0338
KC (binario, p=0%)	0,6944	0,2614	0,6303	0,8369	0,0244	0,6533	0,5726	0,0229	0,0161
KC (binario, p=0,5%)	0,1117	0,1677	0,0015	9,2622e-04	0,6054	0,0015	0,2370	0,5260	0,0219
KC (binario, p=1%)	0,0640	0,0432	5,9617e-04	5,7440e-05	0,0343	5,9617e-04	0,8968	0,9702	0,0060
KC (binario, p=2%)	0,0453	0,6303	1,5290e-04	3,0452e-04	0,2237	1,5290e-04	0,3599	0,5758	0,0508

KC (ternario, p=0%)	**0,4655**	**0,6533**	**0,0064**	**1,2507e-04**	**0,1133**	**0,0064**	**0,7618**	**0,0065**	**0,0842**
KC (ternario, p=0,5%)	**0,3399**	**0,6077**	**0,5854**	**1,9633e-04**	**0,2501**	**0,5210**	**0,8968**	**0,0571**	**0,1335**
KC (ternario, p=1%)	**0,0414**	**0,3045**	**0,6533**	**0,0037**	**0,7769**	**0,7002**	**0,8968**	**0,1917**	**0,0014**
KC (ternario, p=2%)	**0,0042**	**0,0776**	**0,0103**	**0,0059**	**0,7515**	**0,0103**	**0,1011**	**0,7940**	**0,0338**

Hypnogram

Results obtained from the application of the test for the indices relating to the hypnogram unluckily demonstrate a p-value > 0.05, not significant to differentiate our RBD patients.

- Fig 16. *SWS = slow wave sleep; SE = sleep efficiency; WASO = wake after sleep onset.*

Indice	Ipotesi	*p-value*
Sleep Diversity Index	0	0,1503
Awakening Index	0	0,2217
WASO	0	0,7220
SE (%)	0	0,5464
Deep Sleep Efficiency (%)	0	0,6538
Number of stage shifts per hour	0	0,2787
Frequency of sights from SWS to N1 or N2	0	0,8644
Mean duration of REM	0	0,1581
Total duration of REM / Total Sleep Time (%)	0	0,5269
Number of REM / Number of total stages	0	0,6150

SWS = slow wave sleep; SE = sleep efficiency; WASO = wake after sleep onset

Discussion

In our study it was possible to record several statistically significant results for a p-value <0.05 equal to 67 on 319 tests performed (21%). The highest number of statistically significant results, equal to 48 out of 67 (71.6%), was obtained by the complexities of ***Lempel-Ziv and Kolmogorov tests.*** Among 48 results acquired in the context of non-linear complexity indices, 26 (54.2%) were identified or expected in the epochs that included the REM phase.

Almost all the complexity indices with binary coding, and two of those with ternary coding, were statistically significant in the study of *the first epoch of the N2-REM transition*, and the only one excluded (for p = 2%) has a p-value = 0.0508 (statistical significance limit).

In addition, *the REM phase first epoch, preceded by a phase N2*, recorded the highest number of significant results in relation to the number of tests carried out for each phase, equal to 22 out of 29 (75.9%).

The complexity measures that recorded the highest number of statistically significant results (6 out of 48 for Kolmogorov and the same number for Lempel-Ziv) are those in which data were converted into the elements of a binary string and in which p assumed the value of 1% (12.5% for each).

The measure that produced the least number of statistically significant results is the tertiary Kolmogorov complexity with p = 0.5%, which is attributed only one significant result out of 48 (2.1%). In both cases, it is possible to hypothesize that the motivation lies in the choice of the encoding and / or the p-value, but it would be useful to apply the complexity indices on a larger sample.

Favorable outcomes (17) in the context of **linear indices** in the time and frequency domain, the largest number (16) was obtained for the REM phase (94.1%). Furthermore, almost all linear indices in the *time domain* (with the exception of the HR average) and *all indices in the frequency domain*, turned out the rejection of the null hypothesis in the N2-REM transition. This could confirm what we previously observed for the REM phase thanks to non-linear indices.

Among the *frequency domain* measurements, the one that recorded the highest number of significant results (3 out of 6, 50%) belongs to the very low frequency (VLF) signal band. This suggests, in agreement with the literature, that the ***adrenergic component*** may be the one most compromised in the autonomic degeneration process.

Only in REM phase preceded by N2 stage it was possible to record two results with p-value <0.05 in the field of *fetal heart variability indices*, therefore it can be said that they were not particularly effective to distinguish the two populations in examination. This is maybe for the difficulty of adapting an index used for fetal epochs to the adult study and the choice to downsample the NN series in order to adapt it to the research needs of this thesis. The 48 tests carried out on *the longest epoch of the N2 and N3* sleep stages are also included on the totality of the 319 tests performed.

When discussing the results, it can be noted that some of the indices analyzed have been more successful in detecting statistically significant differences between the two populations examined; while others, unfortunately, did not highlight any kind of distinction about heart rate variability.

Only the analysis of *N2 and N3 phase longest epoch*, and the *first epoch of the N3-N2 phase transition*, did not lead to the rejection of the null hypothesis for at least one of the analyzed indices. In the first case, it could come from choice of the longest epoch as the period on which to conduct our analyzes because, even if methodologically correct, it is impossible to obtain an identical standard for all, so the results will be strongly depending on which epoch it worked longer and at what time of sleep it was. In fact, when the analysis was conducted on the first epoch, the number of results with a p-value <0.05 was higher. In the second case, it is not possible to exclude that an arousal during transition N3-N2 stage, unlike the other transitions in which sleep goes deeper, may have influenced the results. So, it would be useful to have the data from the analysis of each phase combination.

REM phase showed the most interesting results in the field of application of heart rate variability indices: among 67 results, 44 (65.7%) were identified in the phase of sleep characterized by rapid eye movements, the same stage affected primarily during REM sleep behavior disorder and secondarily during Parkinson's disease.

Through the ***clinical-demographic analysis*** it was possible to find a statistically significant difference ($p = 0.0284$) in the duration (in years) of the clinical history of REM Sleep behavior disorder. In agreement with the literature and with the mean ± SD and median data (RBD: mean 5.65

± 5.04, median 4.5; PD: 11.23 ± 8.77, median 9), which identify in the group PDRBD a longer duration of the disease, this finding would confirm the importance of behavioral disturbance in REM sleep as a marker of the prodromal phase of Parkinson's disease. In fact, if it is true that the behavioral disorder in REM sleep precedes the onset of an alpha-synucleinopathy with a temporal latency ranging from years to decades, it is probable that almost all patients belonging to the group IRBD should be relocated within the PDRBD group when it's been a long time since diagnosis.

Further significant results come from the analysis of ***polysomnographic parameters***, in particular the number of periodic leg movements during sleep ($p = 0.0297$), the PLMS index ($p = 0.0229$) and the AHI index ($p = 0.0487$) maybe that's because of the greater degree of motor and functional impairment present in Parkinson's disease, which would attenuate the manifestations of any motor activity recorded during sleep, including the sympathetic-mediated response to any obstructive nocturnal apnea.

Considering these results, it can be concluded that in REM phase it is possible to find the greatest number of differences in terms of heart rate variability, probably because of its structural instability and, in particular, cardiovascular activity. In fact, it is possible to suppose that a lowering in heart rate variability can emerge more easily in a phase of sleep normally characterized by rapid fluctuations in this parameter. It would be interesting to understand, in a possible future extension of this study, whether these differences are maintained for each stage of Parkinson's disease severity.

On the role of the encodings used for data processing and on the choice of the p value, it is complicated to draw general conclusions, as all the complexity indices have proved effective in leading to the rejection of the null hypothesis, with few substantial differences.

Linear measurements in the time and frequency domain would appear less effective overall in detecting significant differences between the two groups examined, but it is not excluded that this may be due to the lack of a valid standard for all registrations.

It would be desirable, in a future extension of the study, to obtain more easily comparable results through recordings starting at the same time, equal duration and collected with the same sampling frequency. In fact, it is not possible to exclude that having resampled the frequencies to 512 Hz may have influenced the expected results.

Furthermore, because of the strong correlation between the calculation of the time domain indices and the duration of the recordings, in the future, it could be possible to use recordings lasting 24 hours to observe a more faithful representation of the fluctuations of the parameter over a whole day.

Finally, it can be observed that no test led to the rejection of the null hypothesis referring to the *hypnogram indices*. Among these, the indices that reported the lowest p value are Sleep Diversity Index ($p = 0.1503$), or the entropy associated with the hypnogram, and the average duration of the REM phase ($p = 0.1518$).

It is possible to hypothesize that if the sample was sufficiently large and the index was calculated on a hypnogram without cuts, also reviewing and validating the unstaged, the result could fall below the established significance value.

Conclusions

Studies and researches on Rem Behavior Sleep disorder aims to clarify its etiopathogenesis and relationship with alpha-synucleinopathies, in particular with Parkinson's disease.

So long as both diseases seem to belong to a single pathological continuum, it is useful to understand if there are differences, within the RBD population, between patients suffering from idiopathic form of the disorder and the secondary form of Parkinson's disease patients. These differences can be highlighted using some biomarkers capable of identify patients at high risk of conversion to the pathology or an unfavorable course of the disease. Moreover, REM sleep behavior disorder itself is the main marker of alpha-synucleinopathy.

As part of this thesis, it was decided to use heart rate variability indices as biomarkers of autonomic dysfunction proving to be a useful tool in distinguishing patients with idiopathic REM sleep behavior disorder or secondary to Parkinson's disease.

The REM sleep phase allowed to record the greatest number of significant differences both when the first and the longest epochs were studied; while the measures that proved most useful in pursuing the objective of the thesis were the complexities of Lempel-Ziv and Kolmogorov, with no particular differences regarding the coding or p-value chosen for data processing.

Therefore, the *non-linear indices of complexity Lempel-Ziv and Kolmogorov* could represent markers of the *severity* of the disease, allowing to identify a greater degree of autonomic dysfunction in patients with REM sleep behavior disorder secondary to Parkinson's disease.

However, our study has limitations that cannot be overlooked. It would be desirable, for the purpose of a future extension of the work, to recruit a greater number of participants, from which to obtain enough data to conduct a complete analysis.

Furthermore, having available a larger sample of patients, it would be possible to introduce new study variables, such as the gender of the participants or the degree of severity of the disease, in order to understand whether there may be differences between the different subpopulations.

Moreover, it would be useful to change methods and materials implemented in the work by defining a standard, which allows to obtain easily comparable results.

The recordings were obtained in a period of 8-10 hours but it might be thought to prepare recordings lasting 24 hours (able to provide a more faithful representation of the heart rate variability), starting all at the same time, equal in duration and collected with the same sampling frequency.

Unfortunately, it was not possible to highlight differences between the two groups on the basis of the indices referring to the hypnogram, although this was also part of the objectives of the thesis. Again, it would be useful to have a fully validated staging report on which to conduct the analysis of the indices referring to the hypnogram, to which measures similar to the Sleep Diversity Index could be added. The aim is to observe whether, through an uncut hypnogram, the two indices that showed the lower p-value, could overcome the significance limit we have choosen.

Polysomnography Heart Rate Variability analysis could represent an easy and non-invasive tool to identify, among patients suffering from REM Behavior Sleep Disorder, idiopathic form or secondary to Parkinson's disease. It could be even useful when an early diagnosis of alfa-synucleinopathy is needed, when it is necessary monitoring a probable conversion from idiopathic RBD form to the seconday one, or evaluate the effectiveness of RBD therapies.

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