POLITECNICO DI TORINO

Master's Degree course in Mechatronic Engineering

Master's Degree Thesis

Dissociation Index (DI) for the evaluation of REM Sleep Behaviour Disorder in ALS patients

Supervisors
Prof. Gabriella Olmo
Prof. Irene Rechichi

Candidate
Emanuela Volpicella

Academic Year 2022-2023

To my beloved parents,
for always being a guiding light in my darkest moments.

Ai miei genitori,
per essere sempre il mio faro nei momenti piu' bui.

Abstract

This thesis work aims at developing a Machine Learning (ML) algorithm able to recognize and predict the progression of amyotrophic lateral sclerosis (ALS), which is commonly known as Lou Gehrig's disease. There is evidence that rapid eye movement (REM) sleep behaviour disorder (RBD) may serve as a precursor or early indicator of ALS, owing to the shared motor system dysfunction between the two conditions. Genetic or environmental factors might also contribute to the development of both diseases.
Hence, this thesis examines several studies about RBD and REM sleep without atonia (RSWA), as they have been associated with various neurodegenerative pathologies, primarily those in the alpha-synucleinopathies group, such as Parkinson's disease and Dementia with Lewy bodies.

For this purpose, comprehensive datasets on ALS and RBD patients' sleep patterns, motor abilities, and health records have been collected and analyzed. The study could help to develop a precise prediction system for ALS using non-invasive measures such as polysomnography, which is a sleep study that records brain waves, eye movements, muscle activity and other indicators to reveal the presence of sleep disorders.

Unfortunately, acquiring and then analysing polysomnographies has some drawbacks: patients need to spend at least one night in the hospital and this significantly affects the quality of their sleeping and sleep technicians manually score eight hour long (or even more) records of sleeping for each patient. This requires the technicians to be extremely accurate and expert in the field of sleep scoring. Therefore, a ML approach could improve and facilitate the task, thus enhancing its speed and precision.

As a preliminary step, manual evaluation of the RBD and its state of the art were analyzed, focusing on how the RAI, SINBAR and Montreal indices are usually computed and estimated. In a second step those results were compared to those obtained by the Dissociation Index showing the goodness of such an algorithm.
Data were acquired from a set of patients of the Regional Centre for Sleep Medicine at the Molinette Hospital in Turin in three different stages: at the first check-up and later on, at the 6- and 12-month follow-ups.
Since ALS is a rapid-course disease with a very low life expectancy, some patients in the final stage were not able to attend follow-ups and unfortunately, others had already passed away.
Research shows that RBD symptoms may appear several years in advance with respect to those of affected by ALS; in this scenario, such a prediction model can significantly enhance the ALS diagnosis and treatment, potentially paving the way for novel therapies and improving *patients'* quality of life.

Contents

Chapter 1

Introduction

Rapid advances in medical research have unveiled intricate connections between seemingly disparate diseases, igniting fresh insights into the mechanisms underlying their development. One such captivating interplay has emerged between the enigmatic world of amyotrophic lateral sclerosis (ALS) and the intriguing realm of REM sleep behaviour disorder (RBD). ALS, often referred to as Lou $Gehrig's$ disease, is a progressive neurodegenerative disorder that leads to the selective deterioration of motor neurons, resulting in muscle weakness, paralysis, and ultimately, fatal respiratory failure. In contrast, RBD is a sleep disorder characterized by the loss of the typical muscle atonia during REM sleep, leading to vivid and often violent dream enactments.

While ALS and RBD might appear unrelated at first glance, growing evidence suggests a remarkable association that has caught the attention of researchers and clinicians alike. Recent studies have demonstrated a compelling link between the two conditions, suggesting that RBD may act as an early biomarker or even a potential risk factor for the subsequent development of ALS. This connection has set the stage for a deeper exploration of shared pathophysiological mechanisms and genetic underpinnings that could shed light on both disorders.

The convergence between ALS and RBD becomes even more intriguing when considering the potential implications for early diagnosis and intervention strategies. Identifying RBD in individuals who have not yet manifested the full spectrum of ALS symptoms could offer a unique window of opportunity for therapeutic interventions that might slow down or even prevent the progression of ALS. Furthermore, deciphering the intricate neurobiological interactions between these disorders could provide crucial insights into the mechanisms of neurodegeneration, potentially unlocking novel avenues for the development of targeted therapies.

Through the pages that follow, we will delve into these two fields, looking for the connection between them and providing a new solution for interpreting differently the results of $patients'$ examinations.

In the first chapter, we can dig into the topic: we will start by analyzing the clinical

background and separately the two diseases understanding their mechanisms of action and development. We will also provide a differentiation between two clinical conditions: RBD and RSWA according to what has been stated until now by medical literature.

To create a machine learning algorithm, having a suitable dataset is essential. This dataset serves as the foundation upon which the algorithm is trained and tested: for this purpose, the second chapter has been drawn up. As it was previously blinding, the disease progresses exceedingly fast. In light of this, I deemed it valuable to allocate a section to assess patient survival rates.

The core essence of this dissertation can be found in the third chapter: here, we will dive into the methods used to develop the algorithm. Firstly, it has been crucial to understand how the RBD can be estimated and evaluated. For this reason, we will analyze the gold standard of the manual evaluation of RBD represented by RAI, Montreal and SINBAR indices. Once this evaluation has been completed, it is possible to compute it for the set of patients involved in this work: this will serve as our benchmark for assessing the effectiveness of the developed machine-learning algorithm explained in the last section of this chapter and in the following one.

The final chapters are devoted to discussing the outcomes obtained. In the fourth chapter, a detailed exposition of the research methodology is provided, elucidating the various techniques, tools, and procedures used in the course of the investigation. This chapter not only sheds light on the theoretical framework underpinning the study but also delves into the practical aspects of data collection, analysis, and experimentation. Additionally, Chapter Four serves as a repository for the numerical results acquired during the research process. It offers a meticulous presentation of empirical data, showcasing the outcomes of experiments, simulations, and quantitative analyses conducted. These numerical results serve as a vital component in substantiating the $research's$ claims, enabling readers to assess the validity and reliability of the $study's$ findings.

A crucial checkpoint is set for future implementations and enhancements of the research approach in the fifth chapter. It provides guidance for researchers, practitioners, and stakeholders interested in building upon the $study's$ foundations. These conclusions encapsulate the core contributions and implications of the research, offering a clear and concise summary of its key takeaways. By identifying areas for further exploration, potential refinements, and avenues for future research, this chapter paves the way for ongoing innovation and development in the field.

1.1 Clinical and technical background

REM sleep plays a key-role in overall sleep quality and cognitive functioning, making it a subject of intense study, particularly in the context of sleep disorders.

Before delving specifically into REM sleep and its role in sleep disorders, it is essential to understand the sleep cycle. Sleep is not a monotonous state; instead, it includes several stages, typically categorized into non-REM (NREM) and REM sleep.

To better understand these features, researchers often utilize a tool called *hypnogram*,

which is a graphical representation of an *individual's* sleep cycle throughout the night. It displays the transitions between different sleep stages, including REM and non-REM (NREM) sleep and provides a visual representation of the sleep architecture. Thanks to the analysis of the hypnogram, physiologists can identify anomalies in sleep patterns, such as prolonged periods of wakefulness or abnormal REM sleep occurrences, investigating sleep disorders or mental health-related impairments. Many psychiatric conditions, as a matter of fact, including bipolar disorder and schizophrenia, are associated with irregularities in sleep patterns and disturbances in the sleep cycle. This visual representation plays a crucial role in diagnosing and studying sleeping issues, helping healthcare professionals adapt treatment plans for individuals experiencing sleep disturbances in the context of mental health disorders and neurodegenerative diseases.

As said before, a sleep cycle is usually an alternation of NREM and REM sleep:

- **NREM Stage 1 (N1)**: N1 is a transitional stage marked by the onset of sleep, it usually lasts for few minutes. Brainwave patterns shift from alpha to theta waves, indicating a reduction in mental activity. It is a light sleep stage, and individuals may experience hypnagogic hallucinations or sudden muscle twitches.

- **NREM Stage 2 (N2):** N2 is a deeper stage of NREM sleep. It is a more stable stage of sleep and represents a significant portion of the sleep cycle. Brain activity continues to slow down, spontaneous muscle activity decreases further, and sleep spindles (bursts of rhythmic brainwave activity) and K-complexes (sharp waveforms) become prominent. Sleep spindles are thought to play a role in memory consolidation, while K-complexes may serve as a protective mechanism against sleep disruptions.

- **NREM Stage 3 (N3):** Also known as slow-wave sleep (SWS) or deep sleep, N3 is characterized by the slowest brain waves of the sleep cycle. Since muscle activity is at its lowest, during this stage it may be challenging to awaken the sleeper: it is believed to play a crucial role in physical restoration and rejuvenation, muscle repair, growth, and maintenance. At this stage, the growth hormone release is at its highest, helping to complete the regeneration of the entire human body. N3, often referred to as slow-wave sleep (SWS) or deep sleep, is characterized by the presence of delta waves on electroencephalogram (EEG) recordings, indicating a state of profound physical and mental rest, with slow and steady breathing and heart rate. Furthermore, it is involved in memory consolidation, particularly for declarative and procedural memories, helping organize and store information acquired during wakefulness.

- **REM sleep:** REM sleep is the most distinct stage of the sleep cycle. As the name suggests, REM sleep is marked by rapid horizontal movements of the eyes beneath closed eyelids. This phase is strictly related to vivid, emotionally charged dreaming with intricate, bizarre, or even surreal content. Another crucial feature of this sleeping stage is muscle atonia: a state of temporary paralysis that ensures dream

experiences remain in the realm of the mind and do not lead to physical movements. Despite the *body's* paralysis, brain activity during REM sleep is high and resembles the one of wakefulness. EEG recordings show rapid, irregular, and desynchronized brainwave patterns. This stage is associated with irregular breathing, increased heart rate, and fluctuations in blood pressure. These changes, coupled with muscle paralysis, contribute to the unique characteristics of REM sleep. Furthermore, it is believed to play a vital role in memory consolidation, particularly in processing emotionally charged or procedural memories. The brain processes and integrates information gathered throughout the day, aiding learning. It is associated with increased creativity and problem-solving abilities. Dreams often involve novel ideas and scenarios, potentially contributing to creative thinking. It may help regulate emotions and emotional experiences. Dreams during this stage often feature emotionally charged content, providing an opportunity for the brain to process and make sense of emotional events. Adequate REM sleep is thought to reduce stress and emotional reactivity. Disruptions in REM sleep may lead to heightened stress levels and emotional instability. Typically, it occurs approximately 90 minutes after falling asleep, and each subsequent cycle includes longer and more prominent REM periods. As the night advances, REM sleep duration increases.

These characteristics are essential for understanding its functions and its potential role in sleep disorders.

1.2 REM sleep Behaviour Disorder (RBD)

REM sleep behaviour disorder (RBD) is a sleep disorder characterized by abnormal movements and behaviours during rapid eye movement (REM) sleep. It is considered a parasomnia, which involves unwanted events or experiences occurring while sleeping. It is believed to be caused by a disruption in the normal inhibition of muscle activity during REM sleep, leading to the acting out of dreams.

The symptoms of RBD primarily involve the execution of complex motor behaviours during sleep. Individuals affected by RBD may exhibit actions such as punching, kicking, jumping, or shouting while asleep. These behaviours can be violent and potentially dangerous, both for the individual itself and for their sleep partner. RBD is typically more common in males and has been associated with the degeneration of dopaminergic neurons in the brain. [3], [2] Diagnosing RBD involves a comprehensive evaluation of the *patient's* medical history, sleep history, and a polysomnography study. The latter is a sleep study that measures various physiological parameters during sleep, including brain activity, muscle tone, and eye movements. An absence of muscle atonia, the normal paralysis of muscles during REM sleep, is a defining characteristic of RBD.

Treatment options for RBD mainly involve managing the symptoms and reducing the risk of injury. Medications such as clonazepam, a benzodiazepine, have been found to be effective in controlling the motor behaviours associated with RBD. [11] However, long-term use of these medications can lead to side effects and may not be suitable

for everyone. Furthermore, it is essential to address any underlying neurodegenerative conditions that may be present or developing. It has been showed that RBD is often a precursor to neurodegenerative disorders such as $Parkinson's$ disease and Dementia with Lewy bodies. This disorder has been associated with various neurodegenerative diseases, not only alpha-synucleinopathies (PD, DLB). Recent studies show a connection also to Amyotrophic Lateral Sclerosis (ALS). [16] Both RBD and ALS are complex neurological conditions that have sparked much interest and research in recent years. The prevalence of RBD in these conditions suggests that there may be a shared underlying mechanism or pathology. According to some different studies, this raises the possibility of using RBD as a potential biomarker for the early detection of these disorders.

One study published in the Scientific Reports [15] found that over 20% of ALS patients also exhibited symptoms of RBD. This highlights the potential for RBD to serve as a clinical marker for the early detection of ALS.

Understanding the relationship between RBD and ALS is crucial for early diagnosis and intervention. Identifying RBD in ALS patients can help medical professionals initiate appropriate treatment strategies and potentially slow the progression of the disease. Furthermore, studying RBD in ALS patients may provide valuable insights into the underlying mechanisms of both conditions. As the field of neuroscience continues to advance, studies like this contribute to a broader understanding of these conditions and pave the way for improved patient care and outcomes.

In the study of RBD, Electromyographic (EMG) recordings are a crucial tool in revealing different patterns of muscle activation during REM sleep. It is essential to consider how these patterns have a significant impact on the patients in order to fully comprehend the fundamentals of RBD and its associated motor manifestations.

Essentially, it is possible to distinguish between tonic activity and phasic activity.

Tonic activity is characterized by a sustained and continuous level of muscle activity during REM sleep. Within the context of RBD, this continuous muscular engagement throughout REM sleep is deemed abnormal. Typically, during healthy REM sleep, muscles remain relaxed (atonic) as a protective mechanism to prevent physical movements while individuals vividly dream. In individuals with RBD, electromyographic (EMG) recordings unveil the presence of tonic activity as an enduring, elevated muscle activity during REM sleep. This observation signifies that the muscles do not reach the desired state of atonia, leaving room for sustained or prolonged motor behaviours during this sleep stage. The clinical significance of tonic activity lies in its association with RBD. The persistence of muscle activity throughout REM sleep reveals the loss of the normal muscle atonia seen in healthy sleep. Hence, individuals with RBD are capable of physically acting out their dreams, often leading to complex behaviours such as punching, kicking, or other intricate movements.

Phasic activity, in stark contrast to tonic activity, is characterized by short bursts or episodes of muscle activity during REM sleep. These bursts are typically brief and sporadic, occurring intermittently throughout REM sleep. In the context of RBD, phasic activity is also discernible in EMG recordings during REM sleep. Unlike the continuous nature of tonic activity, phasic activity manifests as fleeting bursts of muscle contractions. Remarkably, these bursts of muscle activity are often linked to the content of dreams,

aligning closely with the moments when individuals enact their dreams. Consequently, they are responsible for the defining dream-enacting behaviours observed in RBD. The clinical significance of phasic activity in RBD is profound, as it directly corresponds to the dream content and the subsequent behaviours exhibited during REM sleep. These bursts of muscle activity are synchronized with the occurrences of dream enactment, and the nature of these dreams can sometimes be characterized by violent or disruptive actions.

1.2.1 REM sleep without atonia (RSWA)

On the other hand, REM Sleep Without Atonia (RSWA) is characterized by the absence of muscle atonia during REM sleep with no physical acting out of dreams, presenting a distinctive clinical profile. Indeed, it is defined as excessive or intermittent chin EMG activity during REM sleep, thus manifesting as tonic or phasic activity, and, due to this reason, is considered the polysomnographic hallmark of RBD, though it lacks the intense, aggressive, and violent behaviours seen in RBD, making it a comparatively milder disorder in terms of physical manifestations.

The underlying mechanisms of RSWA remain a subject of ongoing research, but several factors have been suggested. Neurologically, RSWA has been associated with certain conditions, such as $Parkinson's$ disease and multiple system atrophy, which affect the brainstem structures responsible for regulating muscle atonia during REM sleep. Additionally, neurotransmitter imbalances, particularly related to serotonin and noradrenaline, have been proposed as contributors to RSWA. These neurotransmitters play pivotal roles in the modulation of REM sleep and muscle tone. [8]

The clinical diagnosis of RSWA necessarily involves a combination of clinical evaluation and objective assessment through polysomnography (PSG). During PSG, increased muscle activity during REM sleep is observed, indicating the absence of normal muscle atonia. This objective measurement, coupled with a clinical evaluation, aids in diagnosing RSWA.

Some studies [4, 14] have suggested that the misfolding and aggregation of proteins, while different in ALS (e.g., TDP-43, SOD1) compared to alpha-synucleinopathies, could potentially intersect or influence one another. This phenomenon, known as "cross-seeding," implies that misfolded proteins from one neurodegenerative disorder could potentially impact the aggregation of proteins linked to another disorder. This intersection of protein misfolding may contribute to the observed clinical associations. In addition, neuroinflammation and neurodegeneration are common features across various neurodegenerative diseases. These processes can affect different regions of the brain, potentially leading to diverse clinical manifestations. Shared neuroinflammatory pathways might play a role in the co-occurrence of RBD/RSWA and ALS.

1.3 Amyotrophic Lateral Sclerosis (ALS) in the context of neurodegenerative pathologies

Amyotrophic lateral sclerosis (ALS), often referred to as Lou *Gehrig's* disease from the famous baseball player who passed away in 1941 because of this disease, is a devastating neurodegenerative disorder that has confused the medical community for centuries. Unfortunately, it remains an enigmatic condition with no known cure yet. In recent years, researchers have made significant improvements in understanding the pathophysiology of ALS, shedding light on its complex nature.

Finding successful treatments and interventions requires an absolute understanding of the pathophysiology of ALS. Motor neurons in the brain and spinal cord are the main targets of ALS, which results in progressive muscle atrophy and weakening. Some of the most significant hallmarks of this pathology are:

- **Motor Neuron Degeneration**: these neurons are responsible for transmitting signals from the brain to muscles, enabling voluntary movements. This will lead to progressive muscle weakness, typically starting in the limbs, which is a hallmark of ALS. Patients may exhibit difficulty walking, climbing stairs, or lifting objects or difficulties swallowing (dysphagia) due to the involvement of the muscles responsible for swallowing. Also, respiratory muscles can weaken, leading to breathing difficulties. Many ALS patients, in fact, eventually require ventilatory support.

- **Glutamate Excitotoxicity**: an imbalance in neurotransmitters, particularly excess glutamate, leads to overstimulation of motor neurons, causing cell damage and death.

- **Genetic Factors**: approximately 10% of ALS cases are familial, with specific gene mutations identified as causative factors. Mutations in genes have been linked to both familial and sporadic ALS.

- **Oxidative Stress and Inflammation**: oxidative stress and inflammation in the central nervous system contribute to motor neuron damage in ALS.

- **Protein Misfolding**: an accumulation of misfolded proteins in motor neurons is a prominent pathological feature in many ALS cases.

- **Astrocyte and Microglial Involvement**: non-neuronal cells, particularly astrocytes and microglia, play a role in ALS pathogenesis, contributing to neuroinflammation and motor neuron death.

Other common clinical manifestations are **spasticity** and increased muscle tone leading to stiffness and reduced range of motion; **cognitive changes**, ranging from mild cognitive impairment to frontotemporal dementia (FTD); **bulbar symptoms**, affecting speech and facial muscles. Given the heterogeneity of ALS symptoms, accurate and early diagnosis is crucial. Unfortunately, there is no single definitive test for ALS yet, making it a diagnosis of exclusion and relying on clinical criteria.

Chapter 2

Introduction to the observational clinical study

This chapter aims to discuss the design of the retrospective study in light of the characteristics of the patient data-set, the input features, and the assessed endpoints.

2.1 Population study

The *study's* participant population was constructed in the context of a Longitudinal Study of the Regional Centre for Sleep Medicine at Molinette Hospital in Turin, which encompassed data collection between December 2021 and April 2023, spanning three distinct time points: initially at the baseline examination, followed by assessments at the 6-month and 12-month follow-up intervals. The clinical trial is currently underway with an active recruiting process for new volunteer participants. The aim of this longitudinal study is to assess the effect of Melatonin-based treatment on the extent of RSWA, over a 18-month total time period. It is relevant to highlight that all statistical analyses presented henceforth pertain to the entire group of patients enrolled in this research.

However, it is noteworthy that our focus shifts to a well-defined subgroup within the original cohort for the evaluation of specific parameters essential to the computation of various indices, encompassing sleep parameters, REM sleep Atonia Index (RAI) [4, 6], Montreal and SINBAR scoring [13], and the Dissociation Index (DI) [10]. This deliberate subdivision facilitates a more detailed examination of the targeted parameters and their relevance within the context of our *study's* objectives.

In the following tables, demographic and clinical data of the cohort are presented and classified according to the availability of medical records. The first one exclusively encompasses patients for whom we had access only to the initial examination data.

PatientID	PatientSEX	PatientAGE	follow-up at 6months	follow-up at 12months
3371	M	60	N	N
3331	F	64	N	N
3300	M	62	N	N
3453	M	64	N	N
3458	M	71	N	N
3491	M	68	N	N
3510	M	73	N	N
3515	F	54	N	N
3529	M	82	N	N
3551	M	66	N	N
3695	M	79	N	N
3749	F	56	N	N
3753	M	57	N	N
3792	M	77	N	N
3847	M	70	N	N
3901	F	68	N	N
3929	M	74	N	N
3944	M	71	N	N
3950	M	71	N	N
3958	M	77	N	N
3979	F	76	N	N
3991	F	63	N	N
4000	F	74	N	N
4022	M	46	N	N
4115	M	57	N	N
4121	M	70	N	N
4159	F	-	N	N
4211	F	73	N	N

Table 2.1. Population study acquired at the Regional Centre for Sleep Medicine, Molinette Hospital, Turin, Italy.

.

Conversely, the second table consists of all patients for whom we not only had access to the initial examination records but also to at least one of the two subsequent follow-up records.

PatientID	PatientSEX	PatientAGE	follow-up at 6months	follow-up at 12months
3683	M	69	Y	N
3196	M	73	Y	N
3245	M	67	Y	N
3306	M	70	Y	N
3336	F	56	Y	N
3378	F	70	Y	N
3379	M	74	Y	N
3459	F	82	Y	N
3461	F	58	Y	N
3462	F	59	Y	N
3528	M	62	Y	N
3532	M	63	Y	N
3533	M	77	Y	N
3616	M	49	Y	N
3620	M	63	Y	N
3626	F	54	Y	N
3631	M	61	Y	N
3701	M	61	Y	N
3777	M	69	Y	N
3820	F	61	Y	N
3823	M	58	Y	N
3172	F	77	Y	Y
3170	M	43	Y	Y
3368	F	80	Y	Y
3181	M	47	Y	Y
3330	F	60	Y	Y
3340	M	58	Y	Y
3514	F	77	Y	Y
3655	F	54	Y	Y
3698	M	67	Y	Y

Table 2.2. Population study acquired at the Regional Centre for Sleep Medicine, Molinette Hospital, Turin, Italy.

.

As illustrated by the pie chart below, the original cohort of patients obtained for this study exhibits a notable preponderance of male individuals in comparison to their female counterparts. This finding aligns with broader epidemiological data indicating a higher incidence of neurodegenerative diseases among males as compared to females, with estimates often ranging from about 60%-70% of cases occurring in males and 30%-40% in females. [12]
However, specific sex ratios for neurodegenerative diseases may vary by region, population, and through time. Additionally, research is ongoing to better understand the factors contributing to these gender differences in disease incidence. These percentages can vary, and it is relevant to consult up-to-date epidemiological studies or global health organizations for the most accurate and region-specific statistics on the ALS sex ratio.

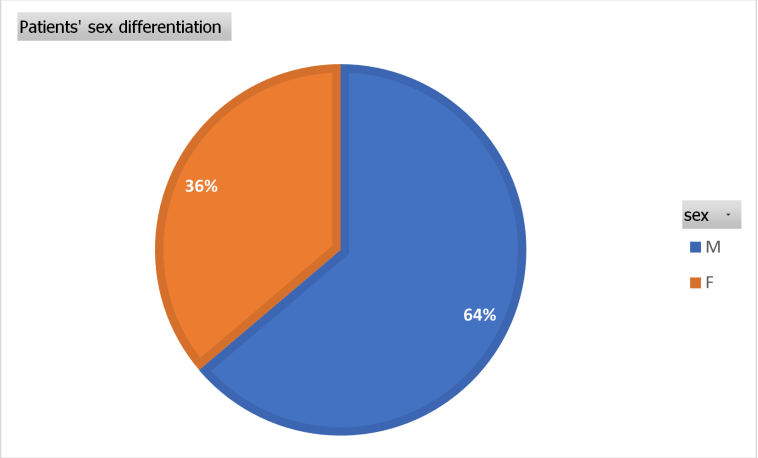

Figure 2.1. Sex Differentiation.

2.2 Survival Outcomes

The survival outcomes of Amyotrophic Lateral Sclerosis (ALS) patients one year after diagnosis can vary widely depending on several factors, including the age of onset, the rate of disease progression, and the effectiveness of medical interventions and support. Generally, ALS is a progressive and often fatal neurodegenerative disease, and one-year survival rates reflect this challenging prognosis. It is important to remember that ALS is a highly variable disease, and individual experiences can deviate from the averages.

From the histogram below, the survival outcomes can be highlighted.
The original cohort involved in this study is composed of 58 patients (21 women, 37 men). Only 30 of them attended the first follow-up after 6 months from the first examination, representing slightly more than half of the total.
After one year from the first acquisition, only nine patients have repeated the examination, representing only slightly more than 15%. Many of them had worse health conditions, having difficulties in undertaking the exam, others instead had already passed away.

These findings support the existing statistical data, confirming that Amyotrophic Lateral Sclerosis (ALS) is a disease defined by rapid disease development, eventually resulting in dramatically reduced life expectancy.

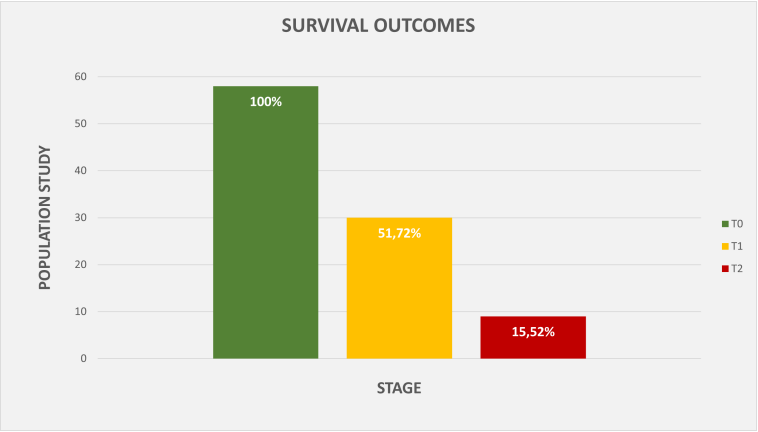

Figure 2.2. Survival Outcomes

Chapter 3

Material and Methods

3.1 Manual evaluation of RBD

Clinical assessment plays a pivotal role in the evaluation of REM Behaviour Disorder. Physicians employ a combination of clinical interviews, patient history, and specialized tests to arrive at a diagnosis. Furthermore, as said before, it is essential to differentiate RBD from other sleep disorders, particularly because it can sometimes be a precursor to neurodegenerative conditions.

When evaluating a patient suspected of having RBD, clinicians often begin with a comprehensive clinical interview. Patients are asked about their sleep patterns, dream experiences, and any unusual behaviours during sleep. Close attention is paid to descriptions of dream enactment, including any violent or aggressive actions. Accurate diagnosis of RBD relies on specific criteria outlined in the International Classification of Sleep Disorders (ICSD) and the Diagnostic and Statistical Manual of Mental Disorders (DSM). These criteria help distinguish RBD from other sleep disorders. The ICSD-3 (International Classification of Sleep Disorders, Third Edition) criteria provide a standardized framework for diagnosing RBD. These criteria emphasize the presence of specific clinical and polysomnographic features.

- Presence of REM Sleep without Atonia: One of the hallmark features of RBD is the presence of REM sleep without atonia. Atonia refers to the normal paralysis of skeletal muscles that occurs during REM sleep, preventing individuals from physically acting out their dreams. In RBD, this atonia is disrupted, allowing individuals to enact their dreams physically.

- Clinical History of Dream-Enacting Behaviours: To meet the ICSD-3 criteria, patients must have a documented clinical history of dream-enacting behaviours during REM sleep. These behaviours can vary widely and may include actions such as talking, yelling, punching, kicking, or even getting out of bed during dream episodes. Importantly, these actions are not consciously initiated but are instead a manifestation of the dream content.

- Exclusion of Other Potential Causes: The ICSD-3 criteria also emphasize the importance of ruling out other potential causes or disorders that could explain the observed behaviours. This step is crucial in ensuring that the diagnosed condition is indeed RBD and not another sleep disorder or medical condition.

These ICSD-3 criteria provide a structured approach to diagnosing RBD and serve as a valuable tool for healthcare professionals, ensuring consistency and accuracy in the diagnosis of this sleep disorder.

The DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) also includes diagnostic criteria for RBD. These criteria align closely with the ICSD-3 criteria and provide additional guidance for clinicians in identifying and diagnosing RBD. The DSM-5 criteria for RBD include:

- Recurrent episodes of arousal during sleep associated with vocalization and/or complex motor behaviours: This criterion focuses on the recurrent nature of the episodes, emphasizing that RBD is not a one-time occurrence but a repetitive pattern of sleep-related behaviours.

- Partial or complete amnesia for the dream: Individuals with RBD often have partial or complete amnesia for the content of the dreams that lead to the observed behaviours. This amnesia is a distinguishing feature from other sleep-related disorders.

- The sleep disturbance is not better explained by another sleep disorder, medical condition, medication, or substance use: like the ICSD-3 criteria, the DSM-5 criteria emphasize the importance of excluding other potential causes for the observed sleep disturbances. This ensures that the diagnosis of RBD is accurate and not confounded by other factors.

The DSM-5 criteria complement the ICSD-3 criteria and are particularly relevant for mental health professionals and clinicians who use the DSM-5 as a diagnostic reference.

In addition to the established diagnostic criteria outlined in the ICSD-3 and DSM-5, there are several specialized indices and assessment tools that further aid in the manual evaluation of REM Behaviour Disorder (RBD). These indices provide valuable insights and quantifiable measures to enhance the understanding and diagnosis of RBD. Among these, the RAI (REM Atonia Index), Montreal Criteria, and SINBAR (Sleep Innsbruck Barcelona) criteria stand out as noteworthy contributions to the assessment of RBD.

3.1.1 REM Atonia Index (RAI)

Recent research has suggested that the REM Atonia Index (RAI) can serve as a valuable adjunct to the diagnostic criteria outlined in the ICSD-3 and DSM-5. It is a continuous index defined in the range [0, 1] [7], where 0 represents total loss of physiological chin atonia during REM sleep. Between these extremes, RAI values provide a continuum,

with values less than 0.8 strongly indicative of altered (reduced) chin EMG atonia during REM sleep. Values falling between 0.8 and 0.9 suggest a less pronounced alteration of atonia, while values above 0.9 are characteristic of normal recordings [13], [7]

Hence, lower RAI values – i.e., below 0.7 – can provide additional objective evidence of disrupted REM sleep muscle atonia, and may be used to support the diagnosis of RBD, serving as a fast, first-line tool for screening [13]. Incorporating the RAI into the manual evaluation of RBD enhances diagnostic accuracy and aids in distinguishing it from other sleep disorders with similar clinical presentations. The RAI functions very well as a first-line screening tool for RSWA; on the other hand, monitoring the overall progression of RBD and its potential association with neurodegenerative diseases (such as $Parkinson's$ disease and Dementia with Lewy bodies) is a challenging task, and longitudinal assessments should be based not only on the evaluation of EMG tone dissociation, but also on other parameters. This may help to provide insights into disease severity and evolution.

The RAI is derived from EMG recordings during REM sleep. The computation of such measure involves first meticulous scoring of sleep stages, done manually by trained sleep technologists, at times aided by automated scoring systems. Once REM sleep has been identified, the index quantification is performed automatically by implementing the method presented by Ferri et al., 2018 [5] This relies on analysing the amplitude chin EMG during REM Sleep, thus providing an objective measure of muscle tone during REM sleep. Specifically, the algorithm works as follows:

1. Filter the EMG signal in the range 10–100 Hz, allowing for high-frequency noise reduction and minimisation of slow drifts,

2. Rectify the EMG signal,

3. Segment the EMG signal in 1-second epochs, to be employed for further computation,

4. Count the number of 1-second epochs with amplitude lower than 1 , amplitude in the range 1–2 and amplitude higher than 2 .

Then, the RAI value is obtained as follows:

$$RAI = \frac{amp \leq 1}{100 - 1 \leq amp \leq 2} \tag{3.1}$$

RAI values below the threshold of 0.7 are a strong indicator of REM Sleep Without Atonia, and may suggest a higher risk for RBD. Indeed, the cited study also demonstrated that RBD patients typically exhibit lower RAI values compared to individuals without RBD. An improved version of the RAI accounts for the background noise surrounding each 1-second epoch [7]; this thesis work implements this new version.

Tracking changes or fluctuations in the RAI can help clinicians and researchers identify individuals who may benefit from closer monitoring and early intervention.

The quantification and analysis of chin EMG activity during sleep are critical aspects of sleep research, offering valuable insights into the neuromuscular dynamics that underlie various sleep stages. Other studies by Figorilly et al. [13] explore in detail the

automated scoring algorithm utilized for the assessment of chin EMG activity, employing the HypnoLab software. This sophisticated software, along with its digital signal processing techniques, plays a pivotal role in the computer-aided diagnosis (CAD) of sleep disorders. Indeed, the use of an automated scoring system not only reduces the inter-rater variability which necessarily affects manual scoring, but also allows for the analysis of large datasets with greater efficiency and consistency. [13]

The Hypnolab software is also able to define 20 amplitude classes, each representing a distinct range of amplitude values, such as amp$\leq$ 1, 1$\leq$ amp $\leq$2, and so forth, up to amp$\geq$19, where each number represents the value in of EMG activity. These amplitude classes serve as a basis for characterizing the intensity and patterns of EMG activity. Indeed, these amplitude classes may hold significant clinical relevance. Muscle atonia, the state characterized by reduced muscle activity or complete inactivity, is indicated by high values in the first class (amp$\leq$1). In contrast, both phasic and tonic activations are expected to result in higher values in the remaining amplitude classes (e.g., 1$\leq$ amp$\leq$2 and beyond). This classification scheme provides a framework for distinguishing between different types and levels of muscle activity during sleep.

In conclusion, the automated quantification of chin EMG activity during sleep represents a significant advancement in sleep research methodology. The utilization of the HypnoLab software, with its advanced signal processing techniques and robust algorithm, offers a comprehensive and objective means of assessing muscular activity patterns during sleep. As sleep research continues to evolve, other automated scoring algorithms are provided [1], allowing for the faster, computer-aided scoring of RSWA.

Although computerised versions of such metrics are effective instruments for assessing RSWA, they come with some limitations. First, the scoring of EMG activity can vary between sleep laboratories and scorers. Therefore, standardization of scoring methods and inter-rater reliability assessments are necessary to ensure consistency in RAI values across different settings. In addition, the interpretation of such atonia metrics should always be considered in the clinical context; indeed, they do not provide information about the underlying cause of RBD, which can be multifactorial.

The following subsections present other metrics currently employed in the RSWA evaluation.

3.1.2 Montreal Scoring Criteria

The Montreal Scoring System represent a set of expert consensus guidelines developed to assist in the clinical diagnosis of RBD. These criteria build upon the diagnostic criteria outlined in the ICSD-3 and the DSM-5 and provide additional clinical insights into RBD evaluation. The Montreal Criteria emphasize several key aspects of RBD assessment, including:

- The presence of dream-enacting behaviours during REM sleep
- The exclusion of other potential causes of parasomnia, such as other sleep disorders or medication side effects
- The importance of a detailed clinical history to identify RBD-related symptoms and behaviours

These criteria serve as a practical tool for clinicians and researchers to systematically evaluate patients suspected of having RBD. The incorporation of the Montreal Criteria into the manual evaluation process enhances the clinical accuracy of RBD diagnosis and ensures a thorough assessment of relevant clinical features. As it has been described by Figorilli et al. [13] this method, adapted to 30-second epochs, employs a sophisticated approach to classify EMG activity as either "tonic" or "atonic." Furthermore, it takes into account the density of phasic chin EMG activity during sleep, shedding light on the complexities of sleep patterns and potential abnormalities. The procedure is a refinement of previous techniques, adapted to 30-second epochs and each of them is meticulously evaluated, and classified as "tonic" when there is increased sustained EMG activity present in more than 50% of the *epoch's* duration. This sustained activity is characterized by an amplitude at least twice that of the background EMG muscle tone or more than 10 μV. Conversely, epochs that do not meet these criteria are scored as "atonic". This binary classification provides a clear and objective means of assessing muscular activity during sleep.

One of the key parameters introduced by this method is the concept of "tonic EMG density". Tonic EMG density is defined as the percentage of 30-second epochs that are scored as tonic. This metric serves as a quantitative measure of sustained EMG activity during sleep and can be a valuable tool for identifying patterns and trends in muscular activity.

Phasic chin EMG activity is also evaluated, by dividing each 30-second epoch into 2-second mini-epochs, allowing for a more detailed examination of short bursts of EMG activity. These bursts, referred to as "phasic EMG events", are characterized by their duration, with a range of 0.1 to 10 seconds, and their amplitude, which must exceed four times the amplitude of background EMG activity. Phasic chin EMG density, another crucial parameter, is defined as the percentage of 2-second mini-epochs that contain EMG events meeting the specified criteria. This metric provides insights into the frequency and intensity of phasic chin EMG activity during sleep.

The study by Figorilli et al. [13] establishes criteria for abnormal chin EMG activity during REM sleep. Specifically, REM sleep chin EMG activity is considered abnormal when tonic chin EMG density is equal to or exceeds 30%, and/or phasic chin EMG density is equal to or exceeds 15%. These thresholds serve as diagnostic markers for potential sleep disorders where excessive muscular activity during sleep can disrupt the normal sleep architecture and lead to sleep disturbances.

3.1.3 SINBAR Scoring Criteria

The SINBAR (Sleep INnsbruck BARcelona) criteria are another set of diagnostic criteria and scoring systems designed to aid in the evaluation of RBD, and the only criteria included by the ICSD-3. These criteria take into account clinical, polysomnographic, and video-based observations to assess RBD-related behaviours. Key components of the SINBAR criteria include:

- Clinical features such as the presence of dream-enacting behaviours
- Polysomnographic evidence of increased muscle activity during REM sleep

- Video recordings of sleep to capture and analyze abnormal behaviours during REM sleep

The SINBAR criteria enhanced the diagnostic accuracy of RSWA. The inclusion of video recordings allows for the direct observation and documentation of dream enactment, further supporting the diagnosis.

The SINBAR methodology includes the analysis of chin EMG activity and flexor digitorum superficialis (FDS), left and right, EMG activity. These sources are employed to compute tonic and phasic activity, as well as the *any* activity, that encompasses both tonic and phasic components, which is crucial for understanding sleep patterns.

Each 30-second epoch of sleep is scored, and categorized as "tonic" when sustained EMG activity is detected in more than 50% of its duration. This sustained activity must exhibit an amplitude that is at least twice that of the background EMG muscle tone or exceeds 10μV. Phasic EMG activity, on the other hand, is evaluated at a finer temporal scale. Each 30-second epoch is divided into 3-second mini-epochs to capture short bursts of EMG activity. Phasic EMG activity is defined as any burst of muscle activity lasting from 0.1 to 5 seconds with an amplitude exceeding twice the background EMG activity. Importantly, the SINBAR *group's* methodology accounts for the complex interplay between tonic and phasic activity. For a phasic chin EMG burst to be scored within a 3-second mini-epoch, it must exhibit at least twice the amplitude of the background tonic EMG activity occurring in the same mini-epoch. This approach enables the detection and quantification of both tonic and phasic components within the same temporal window, providing a more detailed picture of neuromuscular activity during sleep. Finally, to allow for the computation of the SINBAR Index, in each 3-second mini-epoch, the presence of either tonic or phasic EMG activity is recorded as "any" activity. This classification is significant because it allows for the inclusion of EMG activity lasting from 5 to 15 seconds, which was not measured by previous methods.

However, it is essential to establish standardized parameters and thresholds for identifying abnormal EMG activity during sleep. This analysis has led to the identification of specific cutoff values that optimize the balance between sensitivity and specificity in detecting abnormal EMG activity during REM (rapid eye movement) sleep. Specifically, the SINBAR group has determined the optimal cut-off values for achieving high specificity and sensitivity in identifying abnormal EMG activity during REM sleep. [1, 10, 13]

These cutoff values have significant clinical implications, as they serve as diagnostic markers for sleep disorders characterized by abnormal EMG activity. Disorders such as REM sleep behaviour disorder (RBD) are associated with increased muscular activity during REM sleep, and the SINBAR *group's* methodology provides a valuable tool for diagnosing and monitoring such conditions.

CUT-OFF VALUE	IDENTIFIED IN
>16.3%	3-second mini-epochs with phasic chin EMG activity
>18%	3-second mini-epochs with any chin EMG activity
>32%	3-second mini-epochs with any chin EMG activity combined with bilateral phasic EMG activity in the flexor digitorum superficialis (FDS) muscle
>27%	30-second epochs with any chin EMG activity combined with bilateral phasic EMG activity in the flexor digitorum superficialis (FDS) muscle

Table 3.1. Threshold Values identified by the SINBAR guidelines in [1]

.

3.2 Dissociation Index

State-of-the-art and clinically employed methods for RSWA scoring focus on the manual inspection of EMG *signal's* amplitude characteristics during REM sleep (the duration of muscular tone and twitches, in the time domain).

In accordance with the preceding paragraphs, the Montreal and SINBAR techniques, which evaluate the EMG *signal's* amplitude and burst duration during REM sleep in 2(3)-second epochs, respectively, require visual scoring, although the SINBAR criteria were recently implemented in a commercial PSG system [9]
In the process of automating these scoring methods, Machine Learning (ML) approaches have played a fundamental role in the advancement of autonomous sleep analysis. Using polysomnographic (PSG), electromyography (EMG), and electrooculogram (EOG) data, automatic categorization of sleep disorders and RBD diagnosis were attempted. However, the suggested classification technique turns out to be complex since it involves a variety of signals and several characteristics. A method for blind, automated detection of RSWA in polysomnographic data based on spectrum analysis of EMG records during REM sleep in *Parkinson's* disease patients has been presented in [10].

In this dissertation work, those ML methods have been implemented to classify a subject as affected by RSWA or not in ALS patients, adapting the algorithm to their specific features.
The Dissociation Index (DI) is a continuous metric that assesses the degree of impairment of the subject using Euclidean Distance (ED) measures in suitable vector spaces [10]. Indeed, RSWA involves a state separation of mind and body; in fact, although the EEG indicates that the person is in REM, the motor neurons are active and excitable. The ability to describe the degree of dissociation is useful in therapeutic practice and can help with longitudinal examinations. Despite the fact that it requires further validation, the DI might serve as a test bed for a finer evaluation and monitoring of RSWA and its development to neurodegeneration.

In this regard, to automatically identify RSWA patients, supervised learning approaches were used. These methods are fundamental machine learning techniques in which a model is trained on a labelled dataset, called training dataset. The principal aim of supervised learning is to let the model understand the underlying patterns and correlations between the input data and the target labels so that it is possible to make correct predictions or classifications on new, previously unseen data. The model updates its parameters iteratively in order to minimize the gap between its predictions and the actual labels in the training data, often using a specified loss function and optimization method. To avoid performance bias, the analysis was performed using manually (rather than automatically) graded PSG data. The two ML models used were the K-Nearest Neighbour (K-NN) and the Support Vector Machine (SVM). Specifically, those algorithms adopt a binary categorization method to distinguish between RSWA and non-RSWA patients. These aspects will be discussed more in-depth later in the thesis.

As discussed, RBD is considered a precursor to neurodegeneration. Moreover, subjects that present more serious clinical manifestations of RSWA are more likely to develop RBD. In fact, RSWA does not manifest itself at the same dissociation extent for all subjects. In light of these observations, the DI is defined as a distance-based continuous index correlated with the degree of sleep (and atonia) impairment. It acts as a similarity measure, comparing an individual to a reference model of health. This comparison is quantified using the ED, where a zero distance implies identity with the reference (standing for a state of perfect health), while larger distance values signify increasing deviation from this reference. Notably, this study represents a pioneering effort in proposing a distance-based model to assess the level of disease in individuals with sleep-related disorders.
However, it is important to recognize that defining the concept of "neighbourhood" in this context is not straightforward, primarily due to the absence of a clinically validated disease model.

Chapter 4

Experimental results

In the following sections the methodology used will be presented, showing the results produced by the deployment of the developed algorithm.

4.1 Methodology

The data processing and the algorithm software implementation were performed in Matlab 2020b and Python language. First of all, a set of nineteen features to be input to the classification algorithms have been identified. In this study, commonly employed polysomnographic variables [10] are taken into consideration and computationally extracted from the manually annotated hypnogram. They include the Sleep Onset Latency (SOL), Wake After Sleep Onset (WASO), Total Sleep Time (TST), Time in Bed (TIB), Sleep Efficiency (SE), Arousal Index (ARI), Minutes of REM Sleep (MREM), average length and proportion of segments classified as belonging to the same sleep stage, Sleep Transition Index (STI), REM and non-REM (NREM) Fragmentation Indices (RFI and NFI). These latter measure sleep fragmentation patterns, typical of poor sleepers [10].

Other significant features are worked out from EMG data, including RAI and a set of features encompassing information from the spectral analysis of EMG during REM sleep. Following the study from Rechichi et al., 2022 [10], we selected 1-second mini-epochs, to match the RAI computation, and estimated the Power Spectral Density (PSD) using the Welch-modified periodogram with Hamming window. Three features are then obtained: Mean Frequency of the power spectrum estimate, an averaged measure which represents the PSD centroid; Median Frequency (a.k.a. Spectral Edge Frequency at 50%, SEF50) representing the frequency below which 50% of the total power lies; Spectral Edge Frequency at 95% (SEF95), i.e., the frequency below which 95% of the total power lies.

The complete list of features is shown in the table4.1

Type (or Channel)	Feature	Description
Polysomnographic features	Sleep Onset Latency (SOL)	The amount of time required to fall asleep (minutes)
	Wake After Sleep Onset (WASO)	The amount of time the subject is awake during the recording (minutes)
	Total Sleep Time (TST)	Total hours of sleep
	Time in Bed (TIB)	Lights-off to lights-on interval (hours)
	Sleep Efficiency (SE)	The ratio between TST and TIB (%)
	Arousal Index (ARI)	Frequency of occurrence of arousals
	Minutes of REM Sleep (MREM)	Total duration of REM Sleep (minutes)
	Proportion of N1 Sleep (PN1)	N1 sleep per TST (%)
	Proportion of N2 Sleep (PN2)	N2 sleep per TST (%)
	Proportion of SWS Sleep (PN3)	SWS sleep per TST (%)
	Proportion of REM Sleep (PNR)	Proportion of REM sleep per TST (%)
	NREM Fragmentation Index (NFI)	A measure of the number of transitions from NREM to any other NREM stage per hour of NREM sleep
	REM Fragmentation Index (RFI)	A measure of the number of transitions from REM to any other sleep stage per hour of REM
	Wake Proportion (WP)	Awake time during the night (%)
	Sleep Transition Index (STI)	A measure of the number of transitions from REM to NREM (and vice versa) per hours of sleep
	Average Length N1 (ALN1)	Average length of N1 segments (minutes)
	Average Length N2 (ALN2)	Average length of N2 segments (minutes)
	Average Length SWS (ALSWS)	Average length of SWS segments (minutes)
	Average Length REM (ALREM)	Average length of REM segments (minutes)
EMG, time domain	REM Sleep Atonia Index (RAI)	A measure of the amount of atonia during REM Sleep, evaluated on 1-s mini-epochs
EMG, frequency domain	Mean Frequency of REM mini-epochs (MF)	Mean Frequency of EMG signal during REM Sleep, 1-s mini-epochs (Hz)
	Mean Frequency of REM mini-epochs (SEF50)	Median Frequency of EMG signal during REM Sleep, 1-s mini-epochs (Hz)
	Spectral Edge Frequency at 95% of REM mini-epochs (SEF95)	Frequency below which 95% of the total spectral power is found on the EMG signal during REM Sleep, computed on 1-s mini-epochs (Hz)

Figure 4.1. Features implemented in the algorithm [10]

All the features underwent z-score normalization and a two-tailed T-test at the 5% significance level. To perform the analysis, the dataset was partitioned into a training set (70%) and a test set (30%). Performance on the training data was assessed through k-fold Cross-Validation (CV), where k=5. Cross-validation is a method that enables the evaluation of a trained $classifier's$ generalization ability, specifically its capacity to accurately classify new, unseen data. This technique involves randomly dividing the training dataset into k subsets. During each iteration, the model is trained on k-1 subsets and validated on the remaining one.

As far as distance-based algorithms are concerned, in this dissertation work the following two have been exploited:

- K-NN classifies each element by taking the majority vote on the class of its K closest items (i.e., neighbours) [31], where K is a parameter to be optimised.
- SVM aims at finding the hyperplane which effectively separates the elements in the dataset according to their class, by ensuring the maximum distance between the nearest items of each class.

To allow for the computation of the DI, the distance neighbourhood presented in the cited study was employed – i.e., a neighbourhood of R=5.92, and as a reference vector

a healthy model comprising the characteristics of all healthy subjects in the dataset was employed. The DI is computed as:

$$DI_i = \frac{H_i - min}{max - min} \tag{4.1}$$

where H_i represents the ED of the i-th subject, *min* is the minimum admissible distance value (i.e., 0), and *max* is the maximum distance value (i.e., the neighbourhood radius, R2). Values of DI to 0 indicate a strong similarity to a healthy model, and growing values represent increasing dissociation.

4.2 Numerical results and discussion

This section presents the results of the computational analysis performed following the procedures described above.

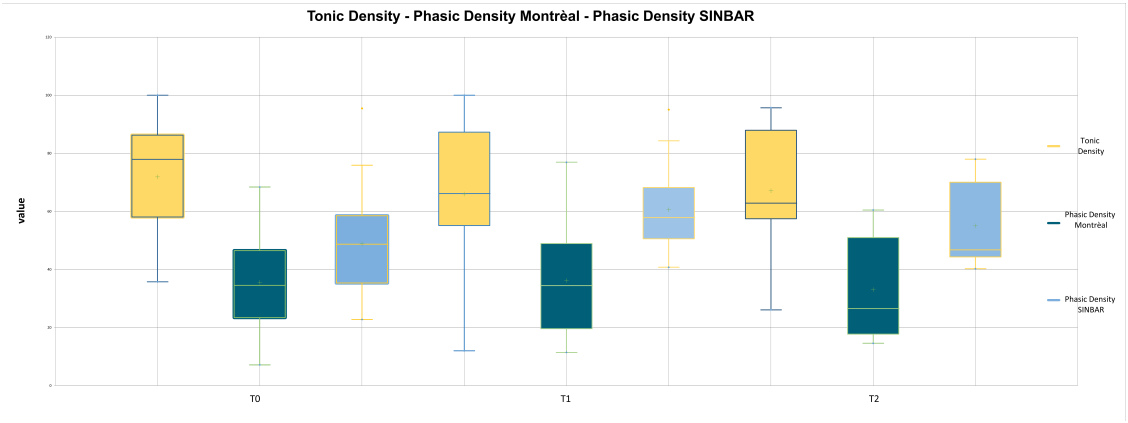

Figure 4.2. Tonic Density, Phasic Density (Montreal method), Phasic Density (SINBAR method)

From the boxplot 4.2 above, we can see that the percentage of tonic density in each stage is much higher than the one of the phasic (regardless of the method employed for the analysis). As it is known, ALS is a neurodegenerative disease that leads to the loss of motor neurons and a progressive reduction in the number of active motor units and a decrease in muscle strength. Consequently, the inability to suppress muscle activity during REM sleep may occur disrupting the inhibitory control and losing the ability to prevent individuals from physically acting out their dreams.

Another important highlight is the higher percentage of phasic activity computed by means of the SINBAR index with respect to the one computed with the Montreal Method. This may be caused by the fact that the SINBAR group differs from Montreal in the way they evaluate phasic density (dividing differently the 30s epochs into shorter mini-epochs) and also because they score as "any" some intermediate activities encompassing both tonic and phasic components, which is relevant for allowing the inclusion of EMG activity lasting from 5 to 15 seconds, which was not measured by previous methods.

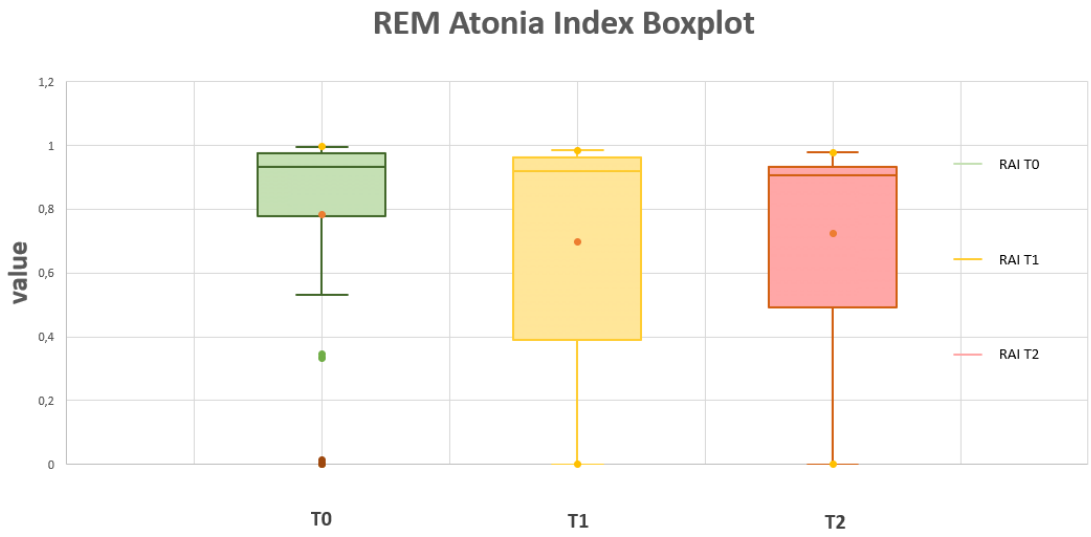

Figure 4.3. REM Atonia Index

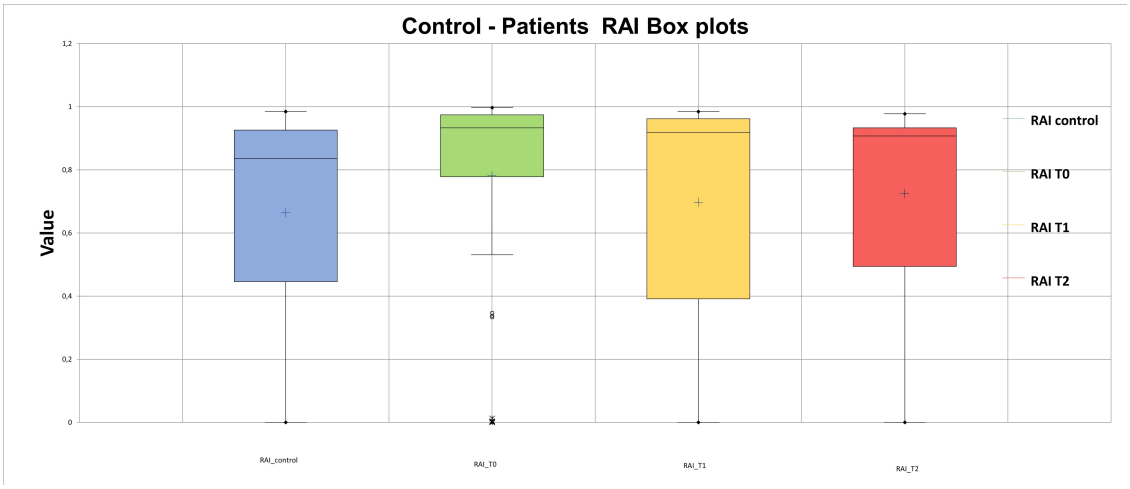

Figure 4.4. Boxplot REM Atonia Index control patients - population study

The boxplot 4.4 above shows four boxes: all of them are RAI evaluations differentiated among control patients and ALS patients in the three stages described in the previous sections. All of them share a median value around 0.8 and slightly over 0.9 showing up a modified muscle activity in most of the patients. Apart from the T0 patients whose data lies mostly in a range between 0.778 (Q1) and 0.974 (Q3), all the other data distributions are negatively skewed showing a strongly altered muscle atonia. The median values of the

boxplots in the three measurements taken at six-month intervals demonstrate that as the disease progresses, the values tend to shift towards lower RAI, indicating an alteration in muscle atonia.
In order to test the algorithm, some control *patients'* sleep parameters have been analyzed. From those features, the same considerations as before have been made, and the computation of RAI for those patients results to be aligned with the parameter intervals. Unfortunately, due to privacy reasons, little to no information was provided on this second cohort (sex differentiation, age, diseases and their relative stages), apart from the fact that they are affected by neurodegenerative diseases different from ALS.

The scatter plot in Figure 4.5 highlights a clear negative correlation between the Dissociation Index (x-axis) and the REM Atonia Index (y-axis). As the value of the DI increases, there is a noticeable decrease in RAI scores. Outliers are minimal and do not significantly affect the overall trend. This suggests that, in general, the points form a clear descending trend.

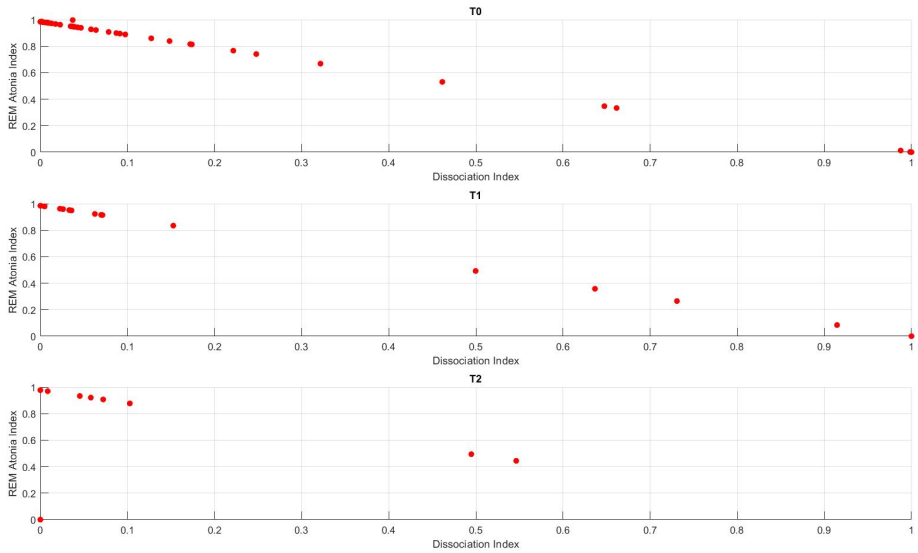

Figure 4.5. Scatter Plot. Dissociation Index - REM Atonia Index

In the boxplot below 4.6 the boxes are positively skewed mainly due to the outliers in the population study, and all of them share very low values of the Dissociation Index. Since the DI is a distance-based index describing the degree of similarity to a healthy model, we have reasons to believe that, regardless of the neurodegenerative diseases affecting the patients, those involved in this cohort mostly belong to Low Tier and Moderate Tier, according to the ranges estimated in [10].

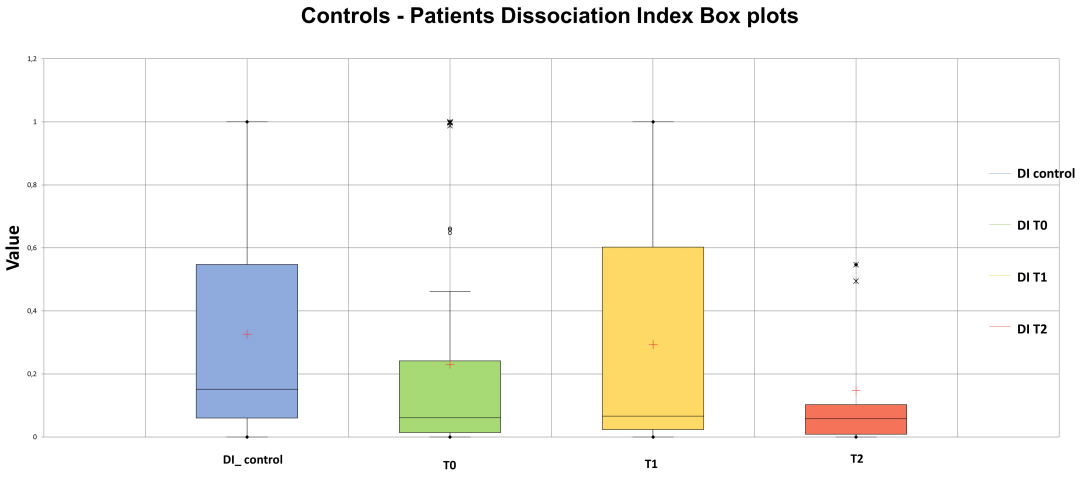

Figure 4.6. Boxplot Dissociation Index control patients - population study

Chapter 5

Conclusion

In conclusion, this dissertation explored the intricate dynamics behind the progression of Amyotrophic Lateral Sclerosis starting from sleep disorders such as REM Sleep Without Atonia and REM sleep Behaviour Disorder.
Through an extensive review of the literature, rigorous data collection and analysis, and a critical examination of various theories and models, we have addressed the central research questions and objectives set out at the beginning of this study.
The literature review highlighted highlighted the ability of Machine Learning algorithms to distinguish between healthy and RBD/RSWA subjects, by leveraging the most significant sleep-related features. In the context of this work, a stratified, longitudinal evaluation of the degree of impairment of ALS subjects has been carried out, through quantitative measures previously presented in research, namely the REM Atonia Index and the Dissociation Index.

Additionally, this research has highlighted limitations and areas for future studies. These include the possibility of having a larger cohort of patients involved in the study and suggest opportunities for further exploration, such as digging deeper into the analysis of the mechanisms behind the progress of REM sleep Without Atonia in ALS patients.
In the broader context, this thesis underscores the importance of continued improvements in sleep research to investigate some relevant peculiarities, such as the way specific pharmacological treatments affect or not the advance of the disease through the passing of time, as we have pointed out for the analysed cohort under melatonin treatments. It is our hope that this work can serve as a foundation for future improvements and validation in the field, providing a springboard for further investigations and advancements.
Finally, this work highlights the importance of having a single parameter in order to evaluate the level of REM dissociation and, therefore, monitor the disease progress and the potential impact it can have on the quality of life and expectancy of patients.

5.1 Future improvements

The association between seemingly unrelated disorders like ALS and RBD provides a unique opportunity to gain insights into the underlying mechanisms of neurodegeneration. By understanding the shared genetic and neurobiological factors that connect these disorders, researchers might uncover fundamental processes that contribute to the development of various neurodegenerative diseases, not just ALS.

Investigating the link between ALS and RBD encourages collaboration between clinicians, neuroscientists, and sleep researchers. This interdisciplinary approach can foster new perspectives and accelerate progress in understanding both disorders, potentially leading to better treatment and quality of patient care.

As already mentioned, RBD itself is an intriguing sleep disorder that is not yet fully understood. Exploring its connection to ALS could shed light on the mechanisms underlying RBD and its relationship to other neurological conditions. This could lead to advancements in sleep medicine and the development of more effective treatments for RBD and other neurodegenerative diseases. In this context, since RBD has been recently acknowledged as an early biomarker for ALS, it could pave the way for more personalized and targeted treatment approaches. Different individuals might have different risk profiles based on their RBD status, allowing for tailored interventions that take into account their specific risk of developing ALS.

On the other hand, considering the connection between ALS and RBD, this could open up novel avenues for therapeutic interventions. Targeting the pathways that are involved in both disorders might lead to the development of treatments that are effective across a spectrum of neurodegenerative conditions, potentially revolutionizing the field of neurology.

Bibliography

[1] Carles Gaig Viola Gschliesser Marc Guaita Verena Raffelseder Laura Ehrmann Nuria Sola Manel Salamero Eduardo Tolosa Werner Poewe Joan Santamaria Birgit HÃ¶gl; SINBAR (Sleep Innsbruck Barcelona) Group Birgit Frauscher, Alex Iranzo. Normative emg values during rem sleep for the diagnosis of rem sleep behavior disorder. *National Library of Medicine*, 2012.

[2] B. F. Boeve, M. H. Silber, C. B. Saper, T. J. Ferman, D. W. Dickson, J. E. Parisi, E. E. Benarroch, J. E. Ahlskog, G. E. Smith, R. C. Caselli, M. Tippman-Peikert, E. J. Olson, S.-C. Lin, T. Young, Z. Wszolek, C. H. Schenck, M. W. Mahowald, P. R. Castillo, K. Del Tredici, and H. Braak. Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. *Brain*, 2007.

[3] Stuart J. McCarter Erik K. St. Louis Bradley F. Boeve. Rem sleep behavior disorder and rem sleep without atonia as an early manifestation of degenerative neurological disease. *Springer Science+Business Media,*, 2012.

[4] Sumit Kumar Chaturvedi, Mohammad Khursheed Siddiqi, Parvez Alam, and Rizwan Hasan Khan. Protein misfolding and aggregation: Mechanism, factors and detection. *Process Biochemistry*.

[5] Raffaele Ferri, Debora Arico', Filomena I I Cosentino, Bartolo Lanuzza, Giacomo Chiaro, and Mauro Manconi. REM sleep without atonia with REM sleep-related motor events: broadening the spectrum of REM sleep behavior disorder. *Sleep*, 2018.

[6] Raffaele Ferri, Christian Franceschini, Marco Zucconi, Stefano Vandi, Francesca Poli, Oliviero Bruni, Carlo Cipolli, Pasquale Montagna, and Giuseppe Plazzi. Searching for a Marker of REM Sleep Behavior Disorder: Submentalis Muscle EMG Amplitude Analysis during Sleep in Patients with Narcolepsy/Cataplexy. *Sleep*, 2008.

[7] Raffaele Ferri, Francesco Rundo, Mauro Manconi, Giuseppe Plazzi, Oliviero Bruni, Alessandro Oldani, Luigi Ferini-Strambi, and Marco Zucconi. Improved computation of the atonia index in normal controls and patients with rem sleep behavior disorder. *Sleep medicine*, 2010.

[8] Michela Figorilli, Giuseppe Lanza, Patrizia Congiu, Rosamaria Lecca, Elisa Casaglia, Maria P. Mogavero, Monica Puligheddu, and Raffaele Ferri. Neurophysiological aspects of rem sleep behavior disorder (rbd): A narrative review. *Brain Sciences*, 2021.

[9] Birgit Frauscher, David Gabelia, Marlene Biermayr, Ambra Stefani, Heinz Hackner, Thomas Mitterling, Werner Poewe, and Birgit Högl. Validation of an integrated software for the detection of rapid eye movement sleep behavior disorder. *Sleep*,

2014.

[10] Maurizio Zibetti Alessandro Cicolin Gabriella Olmo Irene Rechichi, Antonella Iadarola. Assessing rem sleep behaviour disorder: From machine learning classification to the definition of a continuous dissociation index. *International Journal of Environmental Research and Public Health*, 2021.

[11] FÃ©lix Javier JimÃ©nez-JimÃ©nez, Hortensia Alonso-Navarro, Elena GarcÃa-MartÃn, and JosÃ© A. G. AgÃºndez. Current treatment options for rem sleep behaviour disorder. *Journal of Personalized Medicine*, 2021.

[12] Giancarlo Logroscino and Rosanna Tortelli. *Epidemiology of neurodegenerative diseases*. Oxford University Press, 2015.

[13] Maurizio Zibetti Patricia Beudin Monica Puligheddu Leonardo Lopiano Alessandro Cicolin Frank Durif Ana Marques Maria Livia Fantini Michela Figorilli, Raffaele Ferri. Comparison between automatic and visual scorings of rem sleep without atonia for the diagnosis of rem sleep behavior disorder in parkinson's disease. *National Library of Medicine*, 2017.

[14] Chao Peng, John Q. Trojanowski, and Virginia M.-Y. Lee. rotein transmission in neurodegenerative disease. *Nature Reviews Neurology*, 2020.

[15] Medrano-I.H. Collazo S. et al. Segura, T. Symptoms timeline and outcomes in amyotrophic lateral sclerosis using artificial intelligence. *Scientific Reports*, 2023.

[16] Yi-Qun Wang, Wen-Ying Liu, Lei Li, Wei-Min Qu, and Zhi-Li Huang. Neural circuitry underlying rem sleep: A review of the literature and current concepts. *Progress in Neurobiology*, 2021.

List of Tables

List of Figures

Acknowledgements

I would like to express my heartfelt gratitude to my esteemed professor, Gabriella Olmo, and her dedicated PhD student, Irene Rechichi for their invaluable guidance and unwavering support throughout the journey of my dissertation work. Their expertise, patience, and mentorship have been instrumental in shaping this research.
Professor Gabriella Olmo has been an exemplary mentor, providing me with valuable insights, constructive feedback, and the academic rigour needed to excel in this endeavour. Her commitment to my intellectual growth has been truly inspiring. I am equally indebted to Irene Rechichi whose collaboration, enthusiasm, and willingness to share her knowledge have greatly enriched this project.
Their mentorship has been a source of inspiration, and I have learned immensely from their expertise. This dissertation would not have been possible without their support. Thank you for believing in me and allowing me the opportunity to contribute to our field of study.

"Quando la vita mi ha messo davanti
tutte le ragioni
per morire di spavento,
ho notato dentro di me
un infinito coraggio,
non conoscevo, prima del terrore,
la mia audacia.

Quando mi sono trovato dentro
tutte le occasioni
per essere preoccupato,
ho carpito dentro di me
$un'infinita$ fiducia nella vita,
non avevo mai provato, prima $dell'ansia$,
la mia inossidabile fede
per $l'universo$.

Quando la vita mi ha fatto vedere
di che ostacoli é capace,
di quanto un percorso possa essere arduo,
di quanto una difficoltà possa mandare
$all'aria$ un cuore,
ho conosciuto la grinta che mi abitava dentro,
ho frequentato la forza della mia corsa,
non sapevo, prima $dell'avversit$à,
che un uomo
potesse essere in grado
di compiere salti così alti

e ora sono tremendamente convinto
che la vita sia così buona
da farci conoscere la paura
solo per poter presentarci
la nostra parte migliore. "