Universidade de Lisboa

Faculdade de Medicina de Lisboa

Non REM sleep pathology in REM sleep behavior disorder

Arousal analysis

Rita Cristina Alves Louro Miguel

Orientadora: Dr.ª Ana Rita Fernandes Peralta

Co-orientadora: Dr.ª Carla Cristina Paulo Gabriel Bentes

Dissertação especialmente elaborada para obtenção do grau de Mestre em Neurociências

2017

Universidade de Lisboa

Faculdade de Medicina de Lisboa

Non REM sleep pathology in REM sleep behavior disorder

Arousal analysis

Rita Cristina Alves Louro Miguel

Orientadora: Dr.ª Ana Rita Fernandes Peralta

Co-orientadora: Dr.ª Carla Cristina Paulo Gabriel Bentes

Dissertação especialmente elaborada para obtenção do grau de Mestre em Neurociências

2017

A impressão desta dissertação foi aprovada pelo Conselho Científico da Faculdade de Medicina da Universidade de Lisboa em reunião de 21 de novembro de 2017.

RESUMO

INTRODUÇÃO E HIPÓTESE

A Perturbação do Comportamento do Sono REM (RBD) é uma forma de parassónia caraterizada por perda da normal atonia muscular esquelética durante o sono REM, levando ao aparecimento de atividade motora excessiva associada a sonhos, os quais são frequentemente vívidos e sentidos como desagradáveis. Alguns estudos prévios têm sugerido que, em formas idiopáticas de RBD (RBDi), as alterações da fisiologia de sono podem também envolver o sono não REM (NREM). Tais alterações poderão contribuir para a fragmentação do sono e para o aparecimento de sintomas diurnos. Porém, a expressão clínica e polissonográfica da desregulação do sono NREM em RBDi continua por esclarecer. Tendo em conta o conhecimento atual sobre a assinatura clínica e neuropatológica desta entidade, admitimos que os reguladores do ciclo sono-vigília (circuito *flip-flop*), sediados no tronco cerebral superior e implicados no processo homeostático e ultradiário de regulação do sono, possam ser um possível mecanismo envolvido na desregulação do sono NREM em RBDi. Ao nível da macroestrutura do sono, a distribuição dos períodos de sono NREM e da fase N3 ao longo dos ciclos de sono sucessivos é uma medida da regulação do processo homeostático do sono. Ao nível da microestrutura do sono, os eventos fásicos relacionados com a intrusão de micro-períodos de vigília durante o sono, expressos sob a forma de padrão cíclico alternante e microdespertares, oferecem uma medida da estabilidade do circuito *flip-flop* durante o sono. Um possível mecanismo para o aumento esperado nos parâmetros de fragmentação e instabilidade do sono em doentes com RBDi pode ser posto em evidência através da análise da composição espetral do sinal de EEG no período imediatamente prévio aos microdespertares.

OBJETIVOS

1. Analisar a distribuição do sono NREM, estádio N3 e sono REM ao longo do período sono noturno em doentes com RBDi e controlos;

2. Analisar medidas de instabilidade e de fragmentação durante o sono NREM e REM, incluindo análise do padrão cíclico alternante e microdespertares em doentes com RBDi e controlos;

3. Analisar a composição espetral do EEG no período pré-microdespertar/ despertar, incluindo: (i) o cálculo da potência relativa (PR) de seis bandas de frequência durante o período pré-microdespertar/ despertar e durante um período de *baseline*, (ii) o cálculo da variação do período de *baseline* para o período pré-microdespertar/ despertar e (iii) a comparação da variação das PRs entre doentes com RBDi e controlos para cada banda espetral.

MÉTODOS

Foram analisados retrospetivamente todos os doentes com o diagnóstico de RBDi e controlos que entre Janeiro de 2007 e Dezembro de 2015 realizaram polissonografia (PSG) no Laboratório de EEG/ Sono do Hospital de Santa Maria, Centro Hospitalar Lisboa Ocidental. Foram incluídos 10 doentes com RBDi e 15 participantes controlos, comparáveis no que respeita à distribuição por sexo e idade, sem outras co-morbilidades relacionadas com o sono e sem patologia neuroloógica diagnosyticada.

Para o cumprimento do objetivo 1, as PSGs foram re-estadiadas por dois observadores independentes de forma a atingir um consenso final. As medidas convencionais da arquitetura macroestrutural de sono foram analisadas, incluindo duração e distribuição de cada fase de sono ao longo dos ciclos de sono.

Para o cumprimento do objetivo 2, os eventos fásicos de instabilidade e fragmentação do sono, incluindo o padrão cíclico alternante e os microdespertares isolados (microdespertares não associados a atividade de sincronização do tipo complexos K ou surtos de atividade delta), foram marcados por análise visual e analisados em termos de índice, taxa e duração; as fases A do padrão cíclico alternante foram classificadas em fases A1, A2 e A3 de acordo com a prevalência do componente de sincronização.

Para o cumprimento do objetivo 3, foram selecionados microdespertares ou despertares espontâneos precedidos por uma período mínimo de 120 segundos de

estabilidade no EEG. A potência relativa (PR) de 6 bandas de frequência do EEG foi calculada, segundo a segundo, a partir da Transformada de *Fourrier* no período de 30 segundos prévio a cada evento selecionado e num período convencionada de *baseline.* A média das PRs no período de *baseline* foi normalizada para o valor 1. A média da variação das PRs, nas diferentes frequências espetrais, no período pré-microdespertar/ despertar foi calculado em relação ao período de *baseline.* A média da variação das PRs foi comparada entre RBDs e controlos.

O presente trabalho foi aprovado pela Comissão de Ética do Hospital de Santa Maria.

RESULTADOS

A análise macroestrutural foi semelhante nos dois grupos, excepto por uma redução de sono REM no grupo RBDi e alterações na distribuição do estádio N3. Nos controlos, observou-se uma diminuição significativa na duração da fase N3 do primeiro para o último ciclo de sono, enquanto que nos doentes com RBDi esta variação não se verificou.

Na análise dos parâmetros microestruturais de fragmentação e instabilidade do sono, o índice de microdespertares espontâneos, total, durante o sono não REM ou durante o sono REM, foi sempre mais elevado nos doentes com RBDi, em comparação com os controlos. Durante o sono NREM, a percentagem de microdespertares isolados foi também mais elevada em doentes com RBDi, em comparação com os controlos. Os doentes com RBDi apresentaram uma menor duração e menor taxa da fase A1 do CAP, enquanto que as fases A2 e A3 do CAP não diferiram significativamente entre os dois grupos.

Na análise da composição espetral do EEG no período de pré-despertar/ microdespertar, os eventos ocorridos em sono NREM nos controlos foram caraterizados por um aumento significativo da PR da banda delta e pela redução da PR das restantes bandas teta, alfa, sigmas e beta, em relação ao período de *baseline*. Em doente com RBDi, este perfil foi sobreponível, exceto pelo aumento da atividade da banda teta e pela perda da diminuição das bandas sigma observada durante o estádio N2.

Durante o sono REM, em ambos os grupos, os microdespertares/ despertares são precedidos de aumentos da banda delta. No grupo controlo, a atividade das frequências mais altas (alfa, sigma rápido e beta) permaneceu inalterada, enquanto que a atividade do sigma lento diminuiu. Em doentes com RBDi, este padrão modificou-se, tendo sido registada uma diminuição da actividade na banda alfa, enquanto que ambas as bandas sigma permaneceram inalteradas; registou-se ainda uma tendência para a atividade beta (p = 0.09) aumentar no período pré-despertar/ microdespertar.

Analisando despertares e microdespertares separadamente, controlos estiveram associados ao mesmo padrão (aumento da atividade da banda delta e diminuição das restantes bandas espetrais) em ambos os eventos, com exceção para a banda teta, a qual diminuiu no período pré-microdespertar mas permaneceu inalterada no período pré-despertar. Em doentes com RBDi, o aumento da banda delta e a diminuição da banda alfa foi comum a ambos os eventos, enquanto que o comportamento das restantes bandas espetrais foi distinto: os microdespertares estiveram associados a uma diminuição das bandas sigma alto e beta (sigma baixo permaneceu inalterada), enquanto que os despertares estiveram associados a uma diminuição do sigma baixo apenas (tendo as bandas sigma alto e beta permanecido inalteradas).

DISCUSSÃO

A análise polissonográfica do sono NREM em doentes com RBDi revelou alterações a nível da macro e da microestrutura.

A alteração observada na distribuição da duração dos episódios de fase N3 pode sugerir uma disrupção de mecanismos homeostáticos de regulação do sono ou estar relacionada com as alterações microestruturais observadas nos parâmetros de instabilidade e de fragmentação em RBDi. Os microdespertares em doentes com RBDi diferiram em termos de frequência e de morfologia; estas diferenças refletiram-se num aumento no índice de microdespertares espontâneos e numa maior percentagem de microdespertares não associados a atividade eletroencefalográfica do tipo sincronização. Estes dados sugerem que, nestes doentes, os microdespertares ocorrem mais frequentemente isolados, não associados a

mecanismos protetores do sono, sugerindo um nível diferente de sincronização dos neurónios tálamo-corticais durante o sono NREM. Este pode ser um dos mecanismos da maior suscetibilidade destes doentes a fragmentação do sono.

Relativamente à análise espetral no período de pré-despertar/ microdespertar durante o sono NREM, com exceção da atividade sigma, o padrão observado sugere que a ativação cortical evolui no sentido de favorecer mecanismos promotores do sono, tanto em controlos como em doentes com RBDi. Estes dados mostram que, apesar da proporção de microdespertares associados a um componente de sincronização encontrar-se reduzida em doentes com RBDi, a atividade delta é passível de ser evocada no período pré-microdespertar/ despertar numa magnitude comparável à dos controlos. A perda da redução da banda sigma observada nos doentes pode dever-se à diminuição tónica da atividade sigma, presente ao longo de todo o sono e previamente descrita nesta patologia. Tendo em conta o papel que a atividade sigma parece desempenhar como elemento promotor do sono, a sua redução pode fornecer um outro mecanismo responsável pela maior fragmentação e instabilidade do sono encontrada em doentes com RBDi. Avaliando separadamente microdespertares e despertares, a análise das bandas espetrais permite ainda distinguir diferentes ambientes oscilatórios em controlos e RBDi, nomeadamente a presença de uma maior expressão de ritmos ativadores (banda beta) e menor expressão de ritmos protetores (banda sigma), podendo determinar uma maior vulnerabilidade dos doentes para despertares prolongados.

Globalmente, estes dados suportam a hipótese inicialmente colocada do envolvimento patológico do sono NREM em RBDi. Futuramente, será necessário caraterizar a repercussão clínica das alterações observadas e analisar sua evolução ao longo da história natural do RBD.

Palavras-chave: sono não REM, RBD, microdespertar, parassónia, análise espetral.

ABSTRACT

INTRODUCTION AND HYPOTHESIS

There is some evidence suggesting the pathological involvement of non REM (NREM) sleep in REM sleep behavior disorder (RBD). Few studies have looked at arousal system polysomnographic expression during NREM sleep in idiopathic RBD (iRBD). Based on actual knowledge about the clinical and pathological signatures of RBD, we hypothesize that the sleep-wake regulators placed in upper brainstem may be a potential target, early involved in neurodegeneration process in iRBD, leading to widespread sleep changes including NREM sleep.

OBJECTIVES AND METHODS

To address this issue, we performed a macrostructural and microstructural polysomnographic exploration of sleep in iRBD patients and controls, considering the following objectives:

1. Analysis of distribution of NREM sleep, N3 stage and REM sleep across the sleep period;

2. Analysis of instability and fragmentation measures during NREM and REM sleep, including the CAPs and arousals;

3. Analysis of spectral composition of EEG during the pre-arousal/awakening period, including the variation of the relative power (PR) of six EEG frequencies during the pre-arousal/ awakening relative to a baseline periods and comparison of PRs variations between iRBD patients and controls, within each spectral band, sleep stage and type of arousal (arousal/ awakening).

RESULTS

Ten iRBD patients (with a median disease duration of 3.5 years) and 15 healthy control subjects were included. In control group, the stage N3 length declines across the night, with significant differences even in the first to second sleep cycles. In iRBD group, the

stage N3 length distribution is significantly different across the first four sleep cycles and the decline from cycle I to cycle II was practically imperceptible in this group. iRBD patients also show less REM sleep.

iRBD registered a higher spontaneous arousals index (total, during NREM and REM sleep) and a lower subtype A1 rate and length, while phase A2 and A3 subtypes parameters did not differ between the two groups. In NREM sleep, the percentage of arousals non-associated with synchronization (K-complexes or delta bursts) was higher in iRBD.

Concerning the spectral analysis, during NREM sleep controls presented a delta activity increase and a theta, alpha, sigma and beta decrease from baseline to pre-arousal/ awakening period. iRBD patients presented a similar profile, except for theta increase and loss of sigma reduction during stage N2. During REM sleep, sigma activity remained unchanged and alpha activity decreased in pre-arousal/awakening period in iRBD patients. When analysing arousals and awakenings separately, iRBD patients differed from controls by the lack of higher sigma and beta decrease and a greater low sigma decrease during the pre-awakening period.

DISCUSSION

iRBD patients showed a less stringent decrease of stage N3 length across the night, which may reflect a homeostatic and circadian sleep system dysregulation. iRBD patients were associated with higher expression of instability and fragmentation parameters, specifically in spontaneous arousals occurring outside the protective framework of delta waves during NREM sleep, which may reflect a lower level of synchronization of thalamo-cortical neurons in iRBD. This may be a possible mechanism for the sleep fragmentation increase.

In both groups, pre-arousal/ awakening spectral changes during NREM sleep were directed toward sleep maintenance (higher delta power), except for the sigma band activity which seemed to be impaired in iRBD patients. Considering the sleep-promoting influence of sigma activity, the tonic decrease of sigma activity may predispose the cortex cerebral to greater fragmentation and instability during sleep. iRBD patients also differed in awakening spectral analysis, by the higher expression

of activation rhythms (higher beta) and lower expression of sleep promoting rhythms (lower sigma).

Collectively, these results support our initial hypothesis, suggesting that iRBD is a more generalized sleep disorder, also affecting NREM sleep, namely through the arousal system dysregulation. Future studies may explore the evolution of these microstructural and macrostructural parameters along the natural history of iRBD and characterize the clinical expression of NREM sleep involvement in iRBD, namely correlating the excessive diurnal somnolence with these parameters.

Kew words: NREM sleep, RBD, arousal, parasomnia, spectral EEG.

INDEX

ABBREVIATIONS

AASM– American Association of Sleep Medicine

CAP – Cyclic alternating pattern

EEG – Electroencephalogram

EOG – Electrooculogram

EMG – Electromyogram

EDS – Excessive diurnal somnolence

FFT – Fast Fourrier Transform

Hz – Hertz

iRBD – idiopathic RBD

LDT – Laterodorsal tegmental nuclei

NREM sleep– Non REM sleep

OSAS – Obstructive sleep apnea syndrome

PD – Parkinson disease

PPT – Pedunculopontine tegmental nuclei

PSG –Polysomnography

RAS – Reticular activation system

REM sleep – Rapid eye movements sleep

RBD – REM sleep behavior disorder

RSWA - REM sleep without atonia

LIST OF TABLES

LIST OF FIGURES

1. Introduction

Sleep disruption is a major and sometimes inaugural manifestation of neurodegenerative diseases, especially for alpha-synucleinopathies.[1] The evaluation of sleep disturbance may provide a detailed window into the underlying pathophysiologic mechanism of these disorders, while the daytime functioning is still unchanged.

In this chapter, the author makes a brief review on the physiological and functional characteristics underlying the normal sleep and describes the state-of-the-art regarding the REM sleep behavior disorder, highlighting the most pertinent aspects to the present work.

1.1 NORMAL SLEEP PHYSIOLOGY

In the sleep laboratory, sleep is defined by measuring the electrical activity of cortical neurons and muscle cells. Scalp electrodes record the electroencephalogram (EEG), electrodes placed on skeletal muscles record the electromyogram (EMG), and electrodes placed near the ocular globe record the electrooculogram (EOG). Collectively, these electrophysiological techniques are called polysomnography (PSG).

EEG signal results from the extracellular flow of current associated with summated excitatory and inhibitory postsynaptic potentials. The recorded electrical activity is characterized by co-occurrence of interdigitate EEG rhythms, traditionally divided into frequency bands. Desynchronized low-amplitude high-frequency activity (including alpha and beta bands) is the typical expression of cortical cell activation and is implicated in brain functions as arousal and activating systems during sleep; this activity is mediated by afferents coming from the brainstem and basal forebrain. On the contrary, synchronized high-amplitude low-frequency activity (delta band) seems to reflect the intensity of recuperative functions of sleep and to play a pivotal role in homeostatic organization of brain activity across sleep period[2,3]; this activity is mediated by thalamocortical neuronal circuitry, which block or attenuate the depolarizing influence of ascending signals, thus modulating the susceptibility of cerebral cortex to activating stimuli.[2,3]

Beyond these elementary sleep rhythms, different vigilance states - wakefulness and sleep states – are identified by visual analysis of polysomnographic recordings, based on a confluence of electrophysiological and behavioral patterns: in the normal adult, wakefulness is defined by a desynchronized, low-amplitude high-frequency EEG activity (predominantly, beta and alpha rhythms); non-rapid-eye-movement (NREM) sleep is indicated by a progressive increase of synchronized, high-amplitude low-frequency EEG activity (predominantly, theta and delta rhythms) and by a muscle tone decline; and REM sleep is characterized by a desynchronized low-amplitude higher-frequency EEG activity (predominantly, theta range activity), coupled with a complete loss of muscle tone (REM sleep muscle atonia). In the course of NREM sleep, the EEG pattern evolves from the stage N1 (with a predominant theta activity) to the stage N2 (characterized by the appearance of thalamocortical spindles and K-complexes in

EEG) and the stage N3 (characterized by prominent delta waves). NREM and REM sleep cycles (so-called ultradian sleep cycles) are repeated 4-6 times per night, approximately every 90 minutes, and shape the macrostructure of sleep, graphically represented by a sleep hypnogram.[2] See Appendix 8.1. Generally, a healthy adult sleeper will have a sleep efficiency ≥ 85% (that is, ≤15% of the total time in bed will be spent wake), NREM sleep accounts for 75-80% of total sleep time (TST, 1-5% of stage N1, 40-55% of stage N2 and 15-25% of stage N3) while REM sleep accounts for 20-25% of TST.[3]

Several regulatory mechanisms influence the sleep process. The propensity for sleep results from combined effects of the circadian system (termed process C, that is entrained by environmental factors such as light or temperature and mediated by suprachiastamic nucleus in anterior hypothalamus, causing a greater or lesser sleep propensity at specific times of the day) and the homeostatic system (termed process S, that provides the drive for sleep in response to prior wakefulness – sleep pressure).[7] A reliable electrophysiological measure of homeostatic integrity of sleep can be obtained through the differential distribution of stage N3 and delta frequency power throughout the sleep period: in physiological conditions, the degree of pre-sleep homeostatic sleep pressure increases gradually with increasing time spent wake, which results in initial elevation of time spent in stage N3 during the first sleep cycles, followed by progressive decline, as the sleep pressure declines across the night; furthermore, after sleep deprivation, increased sleep drive results in increase in stage N3 time and delta power, suggesting a distinct physiological role in the homeostatic control of sleep.[3]

Circadian and homeostatic inputs change slowly over the course of the day. However, the normal transition from the wake state to NREM and REM sleep take place over just seconds to minutes. The ultradian system (alternation between REM-NREM sleep) is a third regulator, which mediates the level of arousability and EEG synchronization and allows the rapid transition between the three states of vigilance. This activity occurs because the reciprocal antagonism between wake- and sleep-promoting influences, originating in brainstem and hypothalamic neuronal networks.

The reticular activation system (RAS), at the rostral pontine-caudal midbrain, promotes the cortical desynchronization, typical of wake or REM sleep states, through two main ascending pathways:[4,5]

I. Dorsal pathway or thalamocortical projection system: mainly comes from cholinergic pedunculopontine and laterodorsal tegmental nuclei (PPT-LDT), which innervate the thalamic reticular and relay nuclei thus opening the way for thalamocortical transmission and cortical activation;

II. Ventral pathway: mainly contains monoaminergic neurons of the locus coeruleus, raphe nuclei, parabrachial nucleus, periaqueductal gray matter and tuberomammillary nucleus, which send axons, through the lateral hypothalamus, diffusely to the basal forebrain and cerebral cortex.

All of these circuits within the brainstem, forebrain ad hypothalamus are required for shifting the cortical electrical activity from the production of high-amplitude low-frequency activity (predominant during the NREM sleep), to the production of low-amplitude high-frequency activity (predominant during the wakefulness and REM sleep). The main biochemical difference between wake and REM sleep is that during REM sleep only the cholinergic ascending arousal system (dorsal pathway) is activated, which is responsible for regulating the cortical components of REM sleep. This system also sends descending projections, via ventral medulla, to the alpha motor neurons, which hyperpolarizes the spinal motor neurons and produces REM sleep atonia pattern.[4,5]

At sleep onset, the RAS inputs diminish under the influence of circadian and homeostatic signals. Furthermore, RAS is actively inhibited by the GABA-ergic and galaninergic inhibitory afferents, originating in the ventrolateral preoptic nuclei (VLPO), localized in posterolateral hypothalamus.[4,5] The ascending activation decrease allows synchronized thalamocortical rhythms to emerge and prevail across the NREM sleep. Later, such thalamocortical rhythms are abolished by the renewal of the RAS dorsal pathway in REM sleep, and of both RAS pathways in waking. This mutual antagonism between the wake-promoting RAS system and the sleep-promoting VLPO system, in which each half strongly inhibits the other, provides the substrate of the flip-flop switch that ensures rapid and complete transitions between wake-NREM-REM sleep states and prevents intermediate states.

Despite this macrostructural architecture, physiological sleep does not exclude the occurrence of phasic events of transient cortical activation. The term arousal refers to brief intrusion of wakefulness into sleep; it is defined as an abrupt shift of the ongoing EEG activity toward desynchronized activity, including alpha, theta or beta frequencies but not spindles that, contrarily to awakening, lasts up to 15 seconds.[8] See Appendix 8.1.

During NREM sleep, arousal phenomena may be associated to different morphological features: besides the above mentioned desynchronization-type arousal, up to 90% of arousals are preceded by synchronized EEG activity bursts (delta activity or K-complexes) which, in a paradoxically way, reflect the mobilisation of anti-arousal and sleep-promoting swings that attenuate the cerebral cortex susceptibility to depolarizing influence of arousal system.[3,9] In physiological conditions, during NREM sleep, synchronization and desynchronization components of arousal response are structurally embedded in a spontaneous pseudo-periodic biphasic EEG rhythm called cyclic alternating pattern (CAP).[10,11] CAP is characterized by a cyclic alternation of cerebral activation sequences (phase A) followed by deactivation periods of return to background theta-delta activity (phase B). Phase A is further classified into three different subtypes (A1, A2 and A3) considering the synchronization component prevalence (A1 more than 80%, A2 from 50% to 80% and A3 less than 50%). See Appendix 8.1. The differences among these arousal-related events indicate the dynamic balance between rhythms predominantly wake-promoting (subtype A2 and A3, and isolated arousals not preceded by synchronization activity) and rhythms predominantly sleep-promoting (subtype A1). In physiological conditions, the stage progression across the NREM sleep is influenced by the differential distribution of arousal-related phasic events across the sleep cycles:[3,11,12] subtype A1 appears mainly during periods of high homeostatic pressure (first sleep cycles and at the onset of each sleep cycle, where they accompany the progressive transition from N1 stage to N2 and N3 deeper sleep stages), thus promoting the process of build-up and maintenance of EEG synchronization; on the contrary, subtypes A2 and A3 and isolated arousals occur mainly when the homeostatic pressure is low (last sleep cycles and close of the end of each sleep cycle, where they prepare the progressive transition to desynchronized EEG background), thus promoting the onset of REM sleep or final wake. Therefore, the arousal phenomena during sleep are not limited to a single

pattern and are not randomly scattered across the sleep but appear structurally distributed within, reflecting the relationship between state arousability and the tendency of state shifts according to its position within the course of sleep. Evidence suggests that this reciprocal relationship between wake- and sleep-promoting arousal-related events would be similar to that seen with the so-called flip-flop circuitry, implicated on macrostructural regulation of sleep (ultradian NREM-REM sleep cyclicity).[13,14] As at macrostructural level, the thalamocortical circuitry influence the arousal system and the cortical activation state: more specifically, thalamocortical neuronal circuitry generate burst firing in the delta frequency range (K-complexes and delta burst) and sleep spindles in response to incoming arousing stimuli, synchronizing diencephalon and cerebral cortex, thus attenuating the cortex cerebral susceptibility to depolarizing cholinergic influence of activating inputs.[2,3,15] In contrast, a transient reduction of this oscillatory thalamic activity could provide a time window for sensorial transmission through the thalamic relay. This may be one of the mechanisms by which the ascending cholinergic pathways are able to induce desynchronization and arousal. Moreover, experimental evidence has shown that thalamic activity depends not only on ascending cholinergic influences but also on non-cholinergic preoptic anterior hypothalamic and basal forebrain regions, which could also act directly on the cortex influencing the arousal response.[16]

Beyond the information that sleep stage scoring and arousals analysis provides, the activity of certain frequency bands provides also reliable information about the quality of the sleep. Spectral analysis of sleep EEG microstructure in the time-frequency domain has showed how much the different rhythms coexist at all time in the sleep. Delta band (<4 Hz) has been the most intensively investigated band; its role in homeostatic and ultradian process of sleep was above described. Sigma activity (12-15 Hz, produced by sleep spindles mainly during stage N2 of NREM sleep) seems to be the expression of the thalamic filter to the passage of sensory input to the cortex, which may serve to promote sleep continuity. Spindles might also have a role in neuronal plasticity, learning and memory consolidation during the sleep.[3] Higher frequency bands, such as alpha and beta, reflects within-sleep arousal level.[3,62]

As already discussed, spontaneous arousal-related events are a physiological phenomenon acting as an intrinsic microstructural component of sleep organization, particularly in NREM sleep, occurring often within CAP structure and in close temporal

relation with stage shifts. Nevertheless, arousal-related events may also be elicited in response to sleep-disturbing factors. When pathological arousals occur in excess they may impair the sleep continuity. Sleep fragmentation reduces sleep restorative properties and may determine diurnal symptoms such as excessive daytime somnolence. In general, pathologic arousal response differ according to the specific mechanism of sleep disorders.[3] In some disorders, like breathing-related sleep disorders, exogenous or endogenous stimuli promoting arousal may be identified, however in other cases arousals may be only associated to oscillations of the arousal level. Regarding neurodegenerative disorders, despite the cause of sleep fragmentation may be attributed to several factors (including comorbid conditions, mediation and age-related modifications), the neurodegenerative process itself may play a central role. A typical example is the alpha-synucleinopathies, in which sleep dysregulation is a manifestation with instrumental value for its diagnosis.

1.2 REM SLEEP BEHAVIOR DISORDER

REM sleep behavior disorder (RBD) is a distinct parasomnia, firstly described in humans by Schenck *et al* in 1986.[17] It is characterized by a partial or complete loss of normal skeletal muscle atonia during REM sleep, which allow the emergence of violent nocturnal dream enactment behaviors that are potentially injurious to the patient or to the patient´s bedpartner.[17] In the sleep laboratory, such activity is correlated with an enhanced EMG activity during REM sleep, called REM sleep without atonia (RSWA). The RBD prevalence in the general population is 0.5%[18] and it is more common in people over 50 years of age.

Clinical and pathological evidence has shown that idiopathic iRBD (iRBD) precede, in 80% of cases, the later development of neurodegenerative diseases (symptomatic RBD), in particular alpha-synucleinopathies (such as Parkinson disease, dementia with Lewy bodies and multiple system atrophy) by several decades.[19] These data suggest that iRBD may represent an early clinical manifestation of an evolving neurodegenerative disorder, primarily impairing the dorsolateral pons and rostral medulla, implicated areas in the REM sleep muscle atonia, and further impairing other upper structures progressively, including midbrain and neocortex.[20] The fact that iRBD

often heralds future Parkinsonism and/or dementia makes this entity a potential therapeutic target for future preventive treatments.

RBD is believed to arise from the degeneration of pontomedullary structures that mediate the REM sleep atonia.[21] During normal REM sleep, the complete muscle atonia of somatic musculature results mainly from the active GABA/ glycininergic inhibition of the lower motor neuron via glutaminergic neurons of the subcoeruleus (or sublaterodorsal) nucleus localized in the pons. This neuronal mechanism was primarily demonstrated in cats by Michel Jouvet in 1959[22,23] and only further demonstrated in humans with acute vascular and inflammatory lesions housed in pons dorsal tegmentum and ventral part of medulla.[24,25,26] Lesions in the subcoeruleus nucleus and ventromedial medulla release the tonic inhibition on spinal motoneurons leading to RSWA. Others pontine nuclei are known to influence the REM circuitry, including the noradrenergic locus coeruleus and the cholinergic, pedunculopontine and laterodorsal tegmental nuclei, however the exact contribution of these structures to the iRBD phenotype remains under discussion.[27]

REM sleep dysfunction remains the hallmark of the iRBD pathophysiology. However, currently, several lines of evidence have suggested that an additional component of NREM sleep dysregulation may also be implicated in iRBD phenotype. Nevertheless, the underlying mechanisms and characterization of a presumptive NREM sleep involvement remains to be elucidated. In the following section, it will be described the actual knowledge supporting the involvement of NREM sleep in iRBD.

1.2.1 NREM SLEEP PATHOLOGY IN iRBD

A. CLINICAL DATA

The association of idiopathic or symptomatic RBD with NREM sleep comorbidities, including periodic limb movement of sleep[28,29] and NREM sleep parasomnia,[30,31,32] suggests an additional contribution of NREM sleep dysregulation in iRBD patients. More recently, Miguel R. and Arnulf I.[33] described the first case showing the intrusion of typical REM sleep phasic activity into NREM sleep, including iRBD-like dream-enactment behaviors, in a patient with symptomatic RBD and Parkinson disease. The

authors concluded that this case may illustrate a tendency of iRBD patients to exhibit a more generalized sleep motor dysregulation over the disease course.

B. PHYSIOLOGICAL DATA

Changes have been reported in relation to NREM sleep EEG abnormalities in iRBD. Two studies demonstrated that sleep spindles activity during all stages of NREM sleep is impaired in iRBD when compared to controls.[34,35] The authors suggest that the sleep spindles activity reduction in these patients may reflect the degeneration of pre-thalamic afferent fibers arising from brainstem, which is present at very early stages of alpha-synucleinopathies.

C. PATHOLOGICAL DATA

In neurodegenerative diseases in which iRBD is frequent, neuronal cell loss was early observed in structures modulating the REM sleep (including the subcoeruleus nucleus, ventromedial medulla and PPT/ LDT nucleus) and amygdala.[27] Descending fibers of the subcoeruleus are specifically responsible for RWA in iRBD. Thus, brainstem structures responsible for the pathogenesis of iRBD are also implicated in the above described cortical activation system, which modulate the sleep homeostasis and ultradian cyclicity.

To sum up, currently there is some evidence suggesting the pathological involvement of NREM sleep in iRBD. Based on this actual knowledge about the clinical and pathological signatures of iRBD, we hypothesize that the wake-promoting structures, placed in upper brainstem and implicated in ultradian and homeostatic system, may be a potential target, early involved in neurodegenerative process in iRBD.The co-localization of the structures mediating the wake cholinergic system and the clinical iRBD expression, both localized in the ponto-mesencephalic area, can provide the anatomic basis for the combined dysfunction of these two systems in iRBD patients. Few studies have looked at the arousal system polysomnographic expression during

NREM sleep in iRBD. The study of arousal-related events in iRBD constitutes a particularly relevant avenue to attain this question. CAPs and isolated arousals are a fertile ground for sleep disorders manifestations that are related to an unstable sleep condition. The quantitative analysis of sleep EEG may offer a better understanding of arousals and CAPs by providing more sensitive measures for the arousal phenomena study.

2. Hypothesis, Objectives and Expected Results

2.1 HYPOTHESIS

We hypothesize that physiological changes that occur in iRBD also affect NREM sleep.

Wake-promoting system dysregulation may be a potential mechanism of NREM sleep impairment in iRBD.

Arousal-related events, expressed as CAP and isolated arousals and composing the microstructural matrix of NREM sleep, provide a sensitive and accurate measure of wake-sleep promoting system instability.

The spectral components of the EEG signal before arousals or awakening would differ between controls and iRBD patients, providing a possible cause for the presumed increase of sleep instability and fragmentation parameters in iRBD patients.

2.2 OBJECTIVES

2.2.1 OBJECTIVE 1

To analyze the sleep macrostructural architecture, namely the distribution of NREM sleep and N3 stage across the sleep period.

2.2.2 OBJECTIVE 2

To analyze instability and fragmentation measures during NREM and REM sleep, namely CAP and arousals analysis.

2.2.3 OBJECTIVE 3

To analyze the spectral composition of the EEG during the pre-arousal/ awakening episodes, in comparison to the baseline period.

2.3 EXPECTED RESULTS

Based on the hypothesis and objectives stated above, we expect to find NREM sleep changes in iRBD patients, expressed in changes in macrostructural parameters. We further expect to find an increase of sleep instability and fragmentation parameters (namely, arousals and CAPs measures during NREM sleep) in iRBD patients, when compared to controls. We also expected to find a decreased cortical activation of anti-arousal phenomena during the pre-arousal/ awakening period (as demonstrated by a lower delta and/ or sigma range reactivity), thus suggesting this might be the underlying mechanism for the presumed greater instability achieved in iRBD.

3. Methods

3.1 EXPERIMENTAL SETUP

3.1.1 PARTICIPANTS

All patients with diagnosis of iRBD between January 2007 and December 2015 were retrospectively screened for this study from the Sleep Laboratory data base from the Department of Neurosciences and Mental Health, Hospital Santa Maria (Centro Hospitalar Lisboa Ocidental). All iRBD patients have been previously referred to a neurology or sleep disorders clinic due to a history of nocturnal motor agitation. Eligible patients for this study had a PSG-confirmed iRBD diagnosis, according to the actual International Classification of Sleep Disorders criteria.[36] See Appendix 8.2. The age of iRBD symptoms onset, repertoire of nocturnal behaviors in iRBD patients, current medication, presence of excessive diurnal somnolence or other sleep symptoms suggesting sleep comorbidities were recorded in an interview conducted by a physician with specialized training in sleep disorders at the PSG time. All patients had a baseline neurologic examination excluding the presence of any neurodegenerative disease clinical evidence, according to UK brain bank criteria for Parkinsonism[37] and DSM-IV criteria for dementia.[38]

Healthy controls subjects were selected from the Sleep Laboratory database by the main investigator (R.M.). All controls have been previously observed in a neurology or sleep disorders outpatient clinic. The reasons for performing a PSG were: snoring, reevaluation of continuous positive airway pressure (CPAP) therapy indication in obstructive sleep apnea syndrome (OSAS) patients after ponderal loss and restless legs syndrome. Furthermore, the presence of RBD symptoms, or other symptoms suggesting any other uncontrolled sleep disorder, was ruled out by a structured sleep questionnaire administered to all subjects and/or to his/her bedpartner at the PSG time. In order to avoid selection bias and to increase randomness, we chose the first consecutive subjects within the same age and sex range.

Non-eligibility criteria for iRBD patients and controls inclusion were: (1) clinical or polysomnographic diagnosis of other sleep disorder (namely, apnea-hypopnea index (IAH) > 5 per hour of total sleep, respiratory disturbance index (RDI) > 10 per hour of total sleep, periodic limb movements index (PLMI) > 15 per hour, NREM sleep parasomnia, central disorders of hypersomnolence, nocturnal painful syndromes, insomnia or other condition associated with increases arousal), (2) suggestive history

of psychiatric or other neurological disorders (including epilepsy, brain tumors or stroke), and (3) absence of sufficient information on clinical records.
This study was approved by the Ethics Committee of the Hospital de Santa Maria (see Appendix 8.5).

3.1.2 NOCTURNAL SLEEP RECORDING

Controls and patients underwent in-laboratory or ambulatory PSG, in accordance with American Academy of Sleep Medicine (AASM) 2007 standards, which included six EEG channels (scalp electrodes located using the International 10-20 system, placed at C3, C4, O1, O2 and, in most patients, F3 and F4, all referred to the contralateral mastoid), right and left electrooculogram, bipolar submental and anterior *tibialis* electromyograms, electrocardiogram, oronasal air-flux, chest and abdominal respiratory effort via piezo-electric belts, and pulse oximetry. All iRBD patients performed one in-laboratory video-PSG, which included a synchronized video and audio monitoring. In both groups, the EEG signals were amplified, conditioned by filters (high-pass filter at 0.2 Hz; low-pass filter at 60 Hz), sampled with 256 Hz, digitally filtered and stored with a resolution of 125 Hz using DOMINO^{TM} software. Some subjects were submitted to PSG over two consecutive nights; however, in order to equalize the impact of an eventual first-night effect, in these cases it was only considered the first night PSG.

3.2 DATA ANALYSIS

3.2.1 SLEEP STAGE SCORING

Sleep staging was scored manually at 30-sec intervals based on the American Academy of Sleep Medicine (AASM) classification[39,40] by two independent examiners with specialized training in sleep and polysomnography (one of them was R.M.) in order to ensure a concordance final. The epochs with REM without atonia, respiratory events and periodic limb movements were marked manually on PSGs. Audio and video recordings were examined to detect motor-behavioral activity during REM sleep. Conventional PSG measures included: time in bed, total sleep time (time from sleep onset to the end of the final sleep epoch minus nocturnal time awake), sleep latency (time from lights out to sleep onset), REM latency (time from sleep onset to the first

REM sleep epoch), wake after sleep onset, sleep efficiency (ratio between total sleep time and time in bed), percentage of total sleep time spent in stage 1 (N1%), stage 2 (N2%), stage 3 (N3%) and REM sleep (REM%). Each PSG recording was subdivided into sleep cycles. All sleep cycles started with the first epoch of the NREM sleep and ended with the last epoch of the included REM episode. Cycles without REM sleep were not considered (the cycle was incorporated in the next one, in order to have REM sleep). The NREM sleep periods preceding the last awakening and not followed by REM epochs were not included in the cycle calculation. For each sleep cycle, NREM and REM sleep length were analyzed. In iRBD patients, REM sleep without atonia was calculated as % of 30-sec REM epochs with more than 50% of any EMG activity (phasic and tonic) with an amplitude more than twice the background EMG (EMG activity was scored for the chin muscle).

3.2.2 SLEEP INSTABILITY AND FRAGMENTATION PARAMETERS

Cortical arousal and CAP was visually scored by the main investigator (R.M.).
Cortical arousals were defined according to AASM criteria.[40] In NREM sleep, arousals not preceded by (or mixed with) synchronized activity (K-complexes or delta bursts) were quantified separately. See Appendix 8.1. Considering only the spontaneous arousal, arousal index (number of arousals per hour of sleep) was calculated in total sleep time, NREM and REM sleep, including isolated and arousal incorporate in CAP events.
CAPs was visually scored following the criteria published by Terzano *et al*.[41] that define CAP as a spontaneous rhythm during NREM sleep composed of an EEG transient pattern (phase A) separated by intervals of EEG background activity (phase B). Three phase A subtypes (A1, A2 and A3) were described according to the prevalence of the synchronization component. See Appendix 8.1. CAP data was dichotomized into A1 *versus* A2+A3 phases CAPs. For each phase A subtype, phase A index (number of a given phase A subtype per hour of sleep), rate (percentage ratio of phase A time to NREM sleep time) and duration (the mean duration of each phase A subtype) were calculated. See Appendix 8.1.

3.2.3 PRE-AROUSAL/ AWAKENING SPECTRAL EEG ACTIVITY

It was selected the two first spontaneous arousals or awakenings for each sleep phase, and for each descending and ascending sleep slope in each sleep cycle. It was included just the ones with a minimum window of 120 seconds of stable sleep preceding their onset; the criteria of sleep stability was defined by the absence of phasic events (autonomic or cortical arousals), respiratory events, sleep phase transitions or any EEG artefacts during this period. Arousals or awakenings occurring during N1 sleep stage were not analyzed because this is considered a transitory and unstable sleep stage. The 30 seconds period immediately preceding the selected arousals/awakenings onset were selected for the further EEG spectral analysis (in relation to arousals embedded in CAP A2 or A3 phases, the beginning of event was assumed be at the exact onset of the synchronization component, determined by visual inspection). Fast Fourier Transform (FFT) analysis[42] was performed on the left derivation C3-A2 in all patients in order to reduce the potential EOG artefacts and to maximize the amplitude of synchronized patterns, as some patients did not have frontal derivation. This spectral analysis method mathematically decomposes the original EEG oscillatory activity into its pure sinusoidal components, each of a different frequency, thus allowing to quantify the EEG activity oscillations that were not visually discernible, despite being sensitive to contamination of EEG signals by even small artefacts. The EEG spectral powers were calculated for 1-sec non-overlapping epochs using the DOMINO™ software. Six frequency bands were defined: delta (0.2-4.0 Hz), theta (4-8 Hz), alpha (8-12 Hz), low sigma (12-14 Hz), high sigma (14-16 Hz) and beta (>16 Hz) bands. For each band, the absolute power (μV^2/Hz) within each 1 sec window was calculated along the 30-sec pre-arousal/wake period. To standardize the absolute power across subjects, relative power or RP (the proportion of power in one spectral band vis-à-vis all other bands) was used in the statistical analysis. In order to avoid any interindividual variability, the RPs at each second of the pre-arousal/wake period were compared with the RPs mean during the previous baseline period according to the following equation:

$$(RP\ ratio)_t = \left(RP_{pre-arousal\ or\ wakening}\right)_t \Big/ \left(\frac{\sum_{t=1}^{30}(RP_{baseline})_t}{30}\right)$$

where *RP* indicates the relative power, *t* indicates each second along the 30-second pre-arousal/ awakening period *(t1*, *t2*, *t3* … up to *t30*), and $\frac{\sum_{t=1}^{30}(RP_{baseline})_t}{30}$ indicates the

mean of RP during the baseline period. It was assumed that the baseline period corresponded to first 30-sec of the 120-sec sleep stable period previously defined for each selected arousal or awakening. Using this equation, the mean baseline RPs were normalized to the value 1 and eventual oscillations during the 30-sec pre-arousal/wake period were compared to the value 1, within each frequency band.

.

To test the hypothesis that the magnitude of potential changes of pre-arousals/wake spectral power differ between iRBD and controls, the arithmetic mean of RP ratios within each spectral band was calculated in each study group and compared between them. Appendix 8.4 is an illustrative example of that.

Assuming the continuum of arousal response hypothesis, in which arousal and awakening probably represent neurophysiological events with different activation level, these two events were analyzed separately.

3.3 STATISTICAL ANALYSIS

Data were analyzed using descriptive statistics and exploratory data analysis. Continuous variables were expressed as median ± standard deviation. Categorical variables were expressed as frequencies (percentages).

Demographic, clinical, sleep fragmentation and instability variables distributions were compared using the non-parametric Mann-Whitney U test (2 samples) and Kruskal-Wallis test (more than two samples). The results of statistical inference were presented as *p*-value.

Concerning the FFT spectral analysis, two comparisons were made:

1. Pre-arousal/awakening period RPs, within each spectral band, were compared with respective baseline period, considering separately iRBD patients and controls, and considering separately each sleep stage (N2, N3 and REM), and arousals or awakenings events. For this analysis, it was used the non-parametric Mann-Whitney U test and results were presented as *p*-value.

2. The magnitude of changes of pre-arousals/awakening spectral power was compared between iRBD and controls using the arithmetic mean of RP ratios within each spectral band, considering also separately each sleep stage and arousals or awakenings events. Because each iRBD patient and control subject was associated to several selected arousals or awakening events, the mean RP ratios were compared

between two groups using GLM (generalized linear model) procedure of repeated-measures (ANOVA ONE WAY with repeated measures). As the number of arousals or awakenings was different for each subject, Model with Sum of Squares Type III were chosen. The results of statistical inference were presented as *p*-value and *observed power* (credibility of correctly rejecting the null hypothesis) or 1-*observed power* (credibility of correctly no rejecting the null hypothesis). Significance was taken as a *p-value* ≤ 0.05.

Statistical analysis were performed under collaboration of Teresa Marques, professor in Laboratório de Biomatemática from Faculdade de Medicina da Universidade de Lisboa. It was used the IBM® SPSS® Statistics program, version 23.0.

4. Results

4.1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

The iRBD group consisted of 10 patients, 60% men, with a median age at symptoms onset of 62 years (range 41-80 years), a median disease duration of 3.5 years (range 1-10 years) and a median age at the time of PSG of 65 years (range 50-77 years). Three iRBD patients (30%) were under iRBD-specific medication at the time of PSG (clonazepam and melatonin). The control group consisted of 15 healthy control subjects, 40% men, with a median age at the time of PSG of 60 years (range 51-77 years). There was no difference in age at PSG time or gender between the iRBD and the control group. See Table 4.1.

Table 4.1 Demographic and clinical characteristics.

	iRBD (n = 10)	*Controls (n = 15)*	*P value*
Age at the time of PSG, years	65.0 ± 8.3	60.0 ± 7.3	0.091
Gender, male/female	6/4	6/9	0.428
Reported iRBD onset, years	62.0 ± 11.3	N/A	N/A
iRBD duration, years	3.5 ± 3.5	N/A	N/A
Concurrent medication			
Clonazepam (0.5-1 mg), n (%)	2 (20%)	0	N/A
Melatonin (2 mg), n (%)	1 (10%)	0	N/A

Values are expressed as median ± standard deviation, numbers and frequencies.
CI, confidence interval; iRBD, idiopathic rapid eye movement sleep behavior disorder; N/A, not applicable.

In iRBD group, the median percentage of epochs of REM sleep without atonia was 46.65 ± 19.1% (range, 17.9-75.3%); in subgroup of naïve patient for iRBD treatment, the median percentage of of epochs of REM sleep without atonia was 32.20 ± 15.8% (range, 17.9-57.7%). All iRBD patients reported nocturnal motor-behavioral episodes suggesting dream enactment behaviors. According to patient´s bedpartner report or video-PSG, jerky movements affected at least 60% of patients, purposeful motor episodes were present in 100% of patients (including facial expressions, gesturing, punching, kicking, bicycling and jump out of bed) and vocalizations were identified in 60% of patients (including intelligible sleeptalking and crying). The results are summarized in Table 4.2.

Only one iRBD patient presented symptoms of excessive diurnal somnolence requiring wake-promoting treatment (Modafinil 100 mg per day). This patient did not have a narcoleptic-like phenotype on Multiple Sleep Latencies Test (mean sleep

latency of 10.5 minutes across 5 naps, without sleep onset REM episodes). Due to this low frequency, it was not possible to correlate the sleepiness with fragmentation and instability polysomnographic parameters.

Table 4.2 Behavioral and polysomnographic characteristics of iRBD.

REM sleep without atonia*, %		46.65 ± 19.1
Reported motor-behavioural episodes		
1. Jerky movements, n (%)		6 (60%)
2. Purposeful motor episodes, n (%)	Facial expressions (laughing)	2 (20%)
	Gesturing (pointing, grabbing, objects manipulation, throw away)	4 (40%)
	Punching	4 (40%)
	Kicking	5 (50%)
	Others (bicycling, jump out of bed)	2 (20%)
3. Vocalizations, n (%)	Sleeptalking	5 (50%)
	Crying	2 (20%)

Values are expressed as median ± standard deviation, number and frequencies.
*Calculated as % of 30-sec REM epochs with more than 50% of any EMG activity (phasic and tonic) with an amplitude more than twice the background EMG. EMG activity was scored for the chin muscle.

4.2 MACROSTRUCTURAL SLEEP FEATURES

General sleep scoring parameters are described in Table 4.3. Comparing to reference values, the sleep efficiency was decreased, due to a WASO increase, and the percentage of stage N1 was increased in both groups. All sleep scoring parameters did not differ between the groups, except for lower percentage of REM sleep and higher PLMS index in iRBD.

Table 4.3 Sleep scoring parameters.

	iRBD	*Controls*	*P value*	*Reference*
Total sleep time (min)	407.3 ± 95.8	441.5 ± 43.3	0.226	
Sleep efficiency (min)	75.6 ± 24.1	79.4 ± 18.5	0.688	≥85%
WASO (min)	71.2 ± 36.2	57.8 ± 31.8	0.365	
Stage N1 (%)	15.3 ± 5.3	9.6 ± 5.3	0.111	1-5%
Stage N2 (%)	39.2 ± 14.2	41.7 ± 9.8	0.688	40-55%
Stage N3 (%)	28.7 ± 9.4	25.5 ± 10.1	0.428	15-25%
Stage REM sleep (%)	16.5 ± 5.5	21.0 ± 3.6	0.041	20-25%
Sleep onset-latency (min)	18.0 ± 6.5	11.0 ± 10.9	0.370	<15-20 min
REM sleep latency (min)	66.8 ± 75.2	66.0 ± 54.3	1.000	> 60 min
N3 in cycle 1 (%)	45.9 ± 15.0	46.5 ± 15.5	0.928	
AHI total (n/h)	3.2 ± 3.3	1.1 ± 2.1	0.400	
RDI total (n/h)	5.1 ± 3.6	2.6 ± 2.0	0.089	
PLMS index at AT (n/h)	8.4 ± 5.6	0.7 ± 4.7	0.015	

Values are expressed as median ± standard deviation, number and frequencies.
AIH, apnea-hypopnea index; AT, anterior tibialis; iRBD, idiopathic rapid eye movement sleep behavior disorder; PLMS, periodic leg movements during sleep; RDI, respiratory disturbance index; WASO, wake after sleep onset.

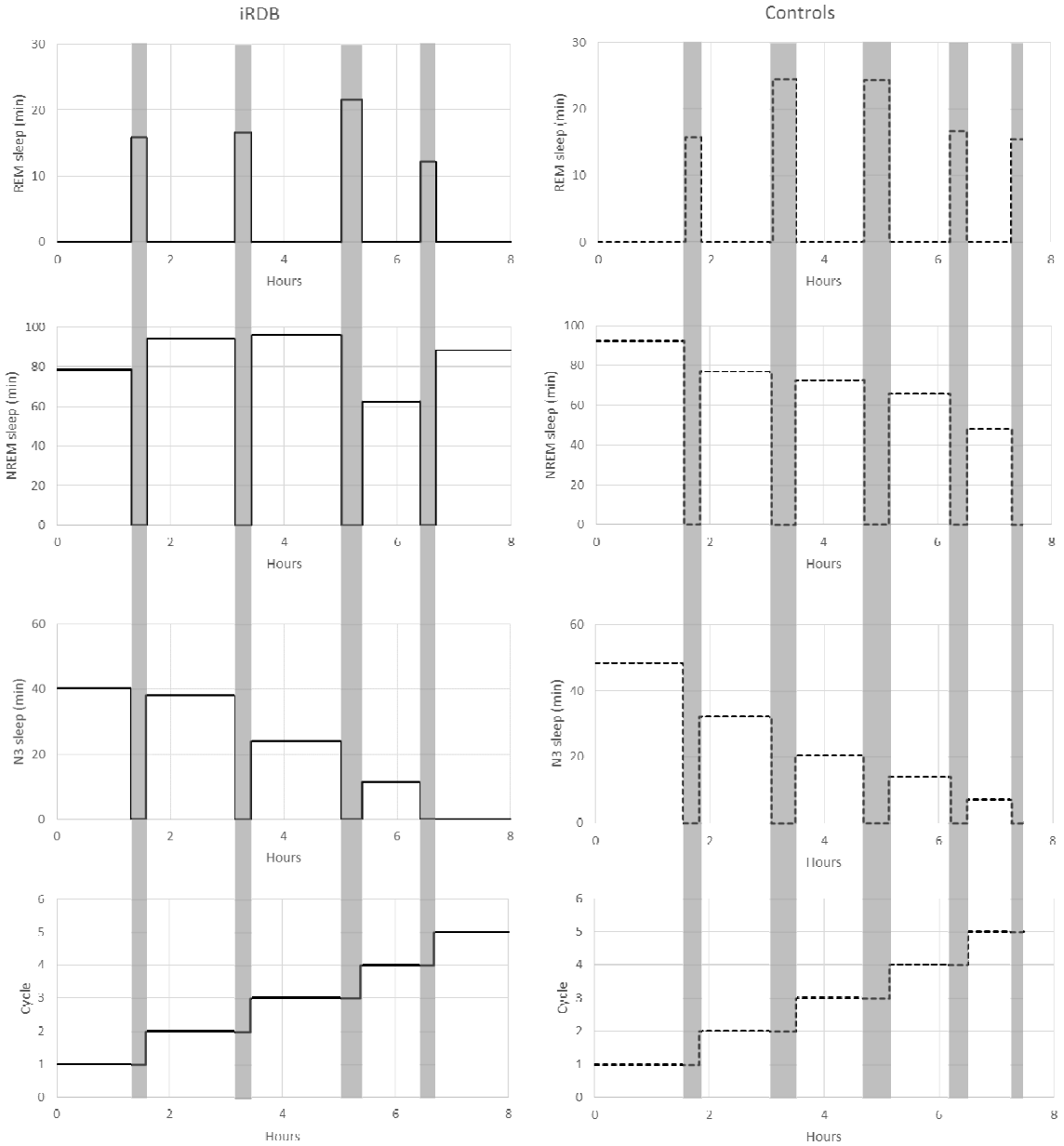
In iRBD and controls subjects, sleep was organized into up to five complete sleep cycles. The Table 4.4 and Figure 4.1 present the macrostructural parameters of each sleep cycle. For the statistical comparison of NREM sleep and stage N3 length across the sleep cycles, the 5th cycle data was excluded in both groups, because it was present in only few patients from each group. In the control group, the stage N3 length declines across the night (p< 0.05). In iRBD group, the stage N3 length declines also across the night (p< 0.05) but no statically differences were observed in the post hoc analysis (p> 0.05). The decline from cycle I to cycle II was practically imperceptible in this group (see Figure 4.1).

Table 4.4 PSG parameters in the sleep cycles.

	I cycle	*II cycle*	*III cycle*	*IV cycle*	*V cycle*	*Comparing within each group*	
Group: iRBD						*P value*	*Bonferroni*
Subjects No.	10	10	10	8	1		
TST (min)	89.5 ± 39.0	110.8 ± 53.5	117.6 ± 23.5	74.4 ± 23.0	109.5	0.070	-
NREM (min)	78.6 ± 33.3	94.2 ± 50.2	96.0 ± 22.6	62.2 ± 22.0	88.5	0.097	-
N3 (min)	38.3 ± 16.4	38.1 ± 42.8	24.1 ± 12.1	11.4 ± 13.7	0	0.034	All p> 0.05
REM (min)	15.8 ± 11.1	16.6 ± 9.0	21.6 ± 14.3	12.1 ± 5.1	21	0.561	-
Group: Controls							
Subjects No.	15	15	15	12	7		
TST (min)	108.7 ± 46.8	101.1 ± 25.7	98.5 ± 26.5	82.1 ± 8.7	63.7 ± 16.7	0.379	-
NREM (min)	92.4 ± 44.0	76.9 ± 17.6	72.6 ± 21.5	65.9 ± 5.3	48.2 ± 19.3	0.451	-
N3 (min)	48.4 ± 25	32.2 ± 18	20.5 ± 16	14 ± 14	7.2 ± 8.6	0.001	C1 > C3: p=0.001 C1 > C4: p=0.000
REM (min)	15.7 ± 7.6	24.5 ± 16.4	27.3 ± 11.2	19.9 ± 8.83	17.6 ± 2.90	0.247	-

Values are expressed as median ± standard deviation and number. NA, not possible. In iRBD control, we excluded V cycle for comparison sleep cycle length within each group.

Figure 4.1 Distribution of REM, NREM sleep and N3 stage across the 5 completed sleep cycles. The values express the mean length of NREM, REM sleep and stage N3 across the sleep cycles. Notice the correspondence between each sleep cycle and the respective REM, NREM sleep and stage N3 length. The grey bars represent the REM sleep length, while the not filled bars represent the NREM sleep and stage N3 length in each sleep cycle. The last graph contains the mean time of each sleep cycle.



4.3 FRAGMENTATION AND INSTABILITY PARAMETERS

iRBD registered higher spontaneous arousals index, including total, during NREM and REM sleep ($p < 0.05$). In NREM sleep, the percentage of arousals non-associated with synchronization (K-complexes or delta) was also significantly higher in iRBD ($p < 0.05$). CAP parameters analysis showed that A1 phase subtype was significantly lower in iRBD patients in terms of rate and length ($p < 0.05$), while phase A2 and A3 subtypes parameters did not differ between the two groups. These arousals and CAP parameters results did not change when considering only the treatment-naïve iRBD patients. The results are summarized in Table 4.5.

Table 4.5 Sleep fragmentation and instability parameters.

	iRBD	*Controls*	*P value*
Arousals (n/h)			
Total	12.5 ± 5.7	8.3 ± 3.3	0.031
NREM sleep	12.5 ± 6.2	7.9 ± 3.3	0.026
REM sleep	11.9 ± 7.7	4.5 ± 4.1	0.005
Arousals in NREM sleep without K-complexes or delta (n/h)	2.7 ± 1.2	0.9 ± 1.2	0.002
Arousals in NREM sleep without K-complexes or delta (%)	23.7 ± 12.1	9.9 ± 5	0.006
Phase A index (n/h)			
Total	57.9 ± 2.93	62.6 ± 19.8	0.607
A1	44 ± 17.8	46.4 ± 9.8	0.892
A2	3 ± 2.5	2.6 ± 1	0.605
A3	5.4 ± 2.7	4.5 ± 2.2	0.367
A2 + A3	10.8 ± 3.7	7.4 ± 2.5	0.115
Phase A rate (%)			
Total	9.87 ± 4.7	10.8 ± 4.95	0.661
A1	7.6 ± 2.8	11.8 ± 1.9	0.007
A2	1.6 ± 1.2	1.4 ± 0.5	0.428
A3	2 ± 0.9	1.5 ± 0.8	0.367
A2 + A3	4.7 ± 1.7	2.9 ± 1	0.285
Phase A length (sec)			
A1	5 ± 0.8	6 ± 0.7	0.010
A2	14 ± 4.1	12 ± 2.4	0.397
A3	10 ± 1.4	9 ± 1.3	0.765
A2 + A3	11.8 ± 2.5	11 ± 1.6	0.495

Values are expressed as median ± standard deviation, number and frequencies.
iRBD, idiopathic rapid eye movement sleep behavior disorder; NREM, non REM sleep; Phase A rate and percentage ratio of phase A time to NREM sleep.

4.4 PRE-AROUSAL/ AWAKENING PERIOD SPECTRAL ANALYSIS

To obtain insight into the mechanisms that might have contributed to the altered fragmentation and instability measures, the spectral composition of the EEG signal along the 30 seconds preceding the arousals and awakenings events was evaluated and compared with a previous baseline period. Using the selection criteria above mentioned, 143 events (arousals and awakenings) from iRBD group and 245 from controls group were included in this analysis. The distribution over the sleep cycles and sleep phases is presented in Appendix 8.3.

Table 4.6 shows the oscillations of spectral composition of EEG signal occurred in different sleep stages along the 30 seconds before arousal or awakening onset, relative to the baseline period. We analysed the sleep stages (NREM and REM sleep) and the arousal-related events (arousals and awakenings) separately. This analysis was repeated for each frequency band. All these results did not changed when considering only the treatment-naïve iRBD patients.

4.4.1 NREM SLEEP

Considering the patterns occurring during NREM sleep in controls, stages N2 and N3 were characterized by a significant increase of delta activity, accompanied by a decrease of theta, alpha, and sigma frequencies during the pre-arousal/awakening period, relative to the baseline period; beta activity decreased during stage N2 but remained unchanged during the stage N3.

iRBD showed a similar pattern, with delta power increase and alpha and beta power decrease in the pre-arousal/awakening period. However, the other spectral bands showed slightly different results: theta and sigma activity decreases occurred only during stage N3; in stage N2, theta activity registered even an increase and sigma activity remained unchanged.

Comparing the PR oscillations magnitude between controls and iRBD during NREM sleep, the two groups differed in the theta activity during stage N2, which decreased in controls and increased in iRBD patients. The variation magnitude within the other spectral bands was not statistically different between controls and iRBD.

4.4.2 REM SLEEP

Considering the patterns occurring during REM sleep, controls showed a similar pattern to NREM sleep with an increase in delta activity and a decrease in theta and sigma low activities. The main differences concerned alpha, sigma high and beta activities, which did not change during the pre-arousal/awakening period in this sleep stage.

iRBD patients showed a similar profile regarding to the delta increase and theta decrease; beta and high sigma activities remained unchanged. Contrary to controls, however, this group showed a decrease in alpha activity and unchanged sigma activity prior to arousal/ awakening.

Despite the different pre-arousal pattern, there were no differences between controls and iRBD patients when comparing the PR oscillations magnitude within each frequency band.

4.4.3 AROUSALS *VS.* AWAKENINGS

In controls, both arousals and awakenings showed increased delta and decreased alpha, sigma and beta. Theta activity decreased only during pre-arousal and remained unchanged during pre-awakening period.

For iRBD, similar trends were obtained for delta and alpha bands. The other bands had subtle changes, the most significant being that higher frequencies (beta and high sigma) did not decrease and low sigma decreased further than in controls in the pre-awakening period - the magnitude of these changes in relation to controls was significant.

Table 4.6/ Figure 4.2 Spectral composition of EEG during the pre-arousal/ awakening periods. The comparison between the pre-arousal/awakening period and respective baseline period is presented within each group; the comparison of RP ratio between two groups is presented at right side.

Table 4.6.1 Delta

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	35.191	1.046	<0.001	36.488	1.056	<0.001	0.190
Phase							
N2	32.627	1.055	<0.001	33.911	1.064	<0.001	0.698
N3	44.041	1.037	<0.001	45.145	1.061	<0.001	0.758
REM	28.303	1.039	<0.001	30.579	1.034	<0.001	0.865
Type of arousal							
Arousals	35.058	1.045	<0.001	36.234	1.057	<0.001	0.149
Awakenings	36.058	1.050	<0.001	37.256	1.053	<0.001	0.422

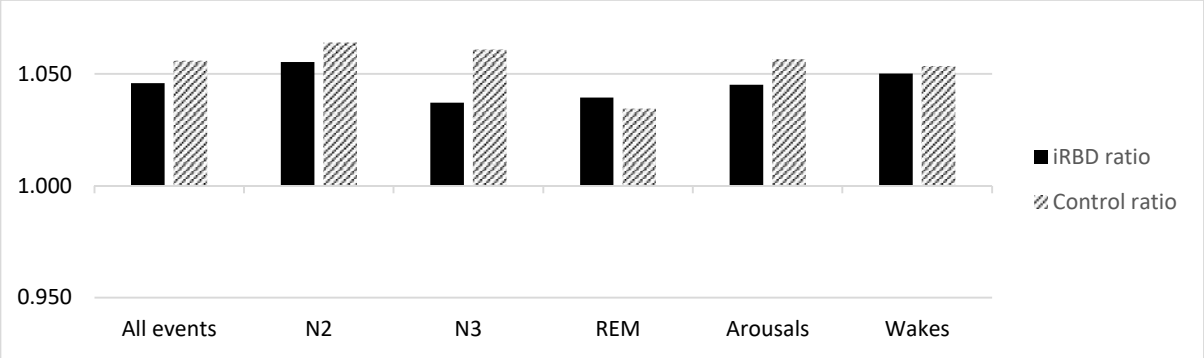


Table 4.6.2 Theta

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	20.664	0.998	0.557	20.093	0.983	0.000	0.304
Phase							
N2	21.346	1.020	<0.001	20.832	0.991	0.002	0.025
N3	19.813	0.982	0.001	19.546	0.972	<0.001	0.645
REM	20.503	0.980	<0.001	19.399	0.983	<0.001	0.673
Type of arousal							
Arousals	20.684	0.998	0.616	20.267	0.979	<0.001	0.233
Awakenings	20.530	0.999	0.880	19.567	0.997	0.511	0.523

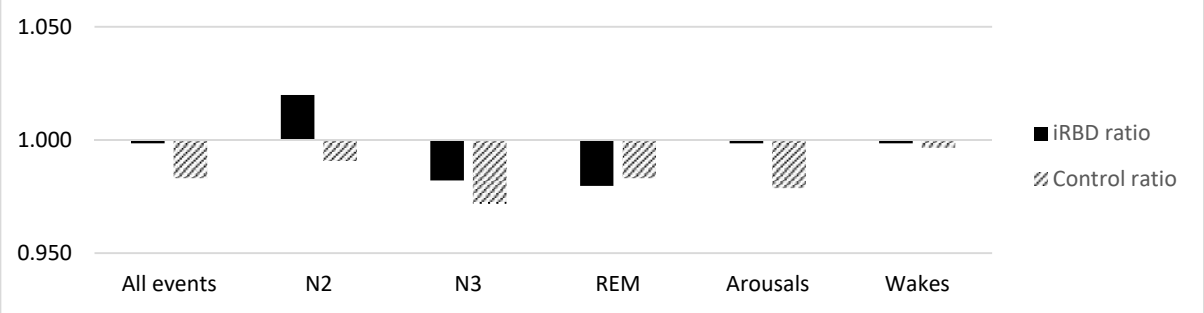


Table 4.6.3 Alpha

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	15.276	0.982	<0.001	15.438	0.969	<0.001	0.758
Phase							
N2	16.166	0.982	0.003	16.301	0.982	<0.001	0.256
N3	12.756	0.982	0.003	14.643	0.923	<0.001	0.149
REM	16.938	0.980	0.005	14.821	1.002	0.598	0.852
Type of arousal							
Arousals	15.331	0.987	0.006	15.499	0.966	<0.001	0.748
Awakenings	14.920	0.947	<0.001	15.253	0.977	<0.001	0.359

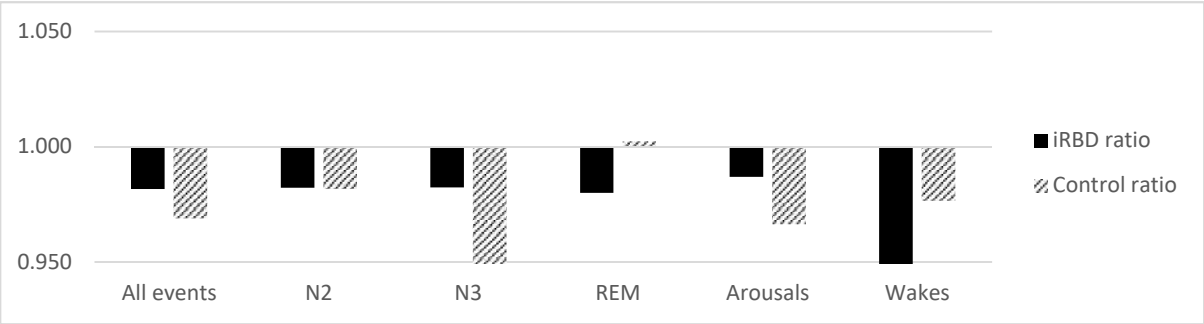


Table 4.6.4 Lower Sigma

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	5.740	0.987	0.007	6.012	0.958	<0.001	0.608
Phase							
N2	6.161	1.000	0.965	6.560	0.961	<0.001	0.540
N3	4.925	0.960	<0.001	5.005	0.941	<0.001	0.504
REM	6.026	0.999	0.883	6.238	0.974	0.001	0.952
Type of arousal							
Arousals	5.699	0.995	0.311	6.016	0.956	<0.001	0.588
Awakenings	6.011	0.935	<0.001	6.000	0.963	<0.001	0.001

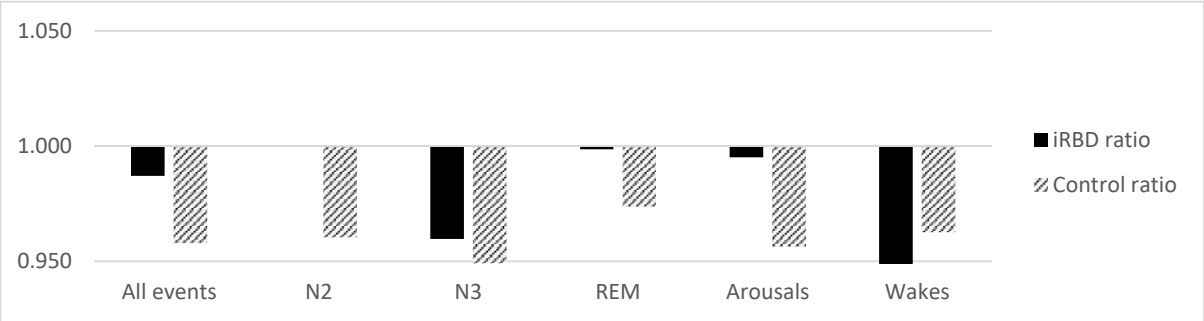


Table 4.6.5 Higher Sigma

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	4.500	0.993	0.095	4.879	0.967	<0.001	0.682
Phase							
N2	4.786	1.005	0.351	5.265	0.966	<0.001	0.417
N3	3.637	0.974	0.004	3.817	0.948	<0.001	0.845
REM	5.102	0.997	0.605	5.474	0.991	0.272	0.909
Type of arousal							
Arousals	4.552	0.991	0.046	4.903	0.971	<0.001	0.564
Awakenings	4.156	1.009	0.337	4.806	0.953	<0.001	0.069

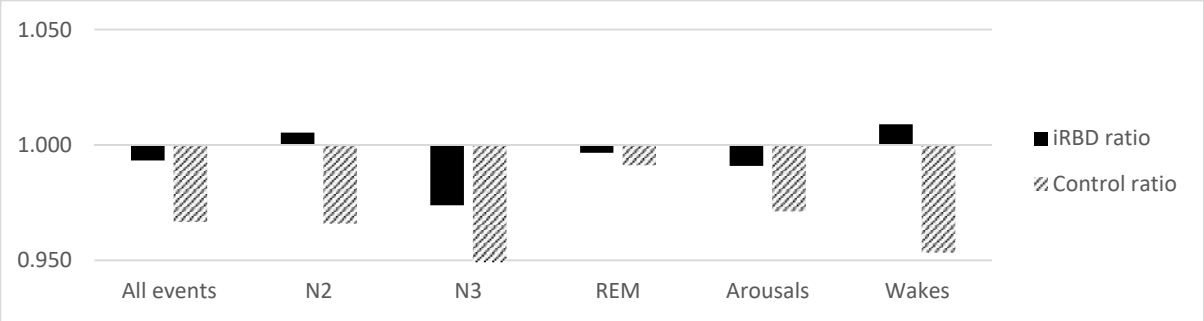
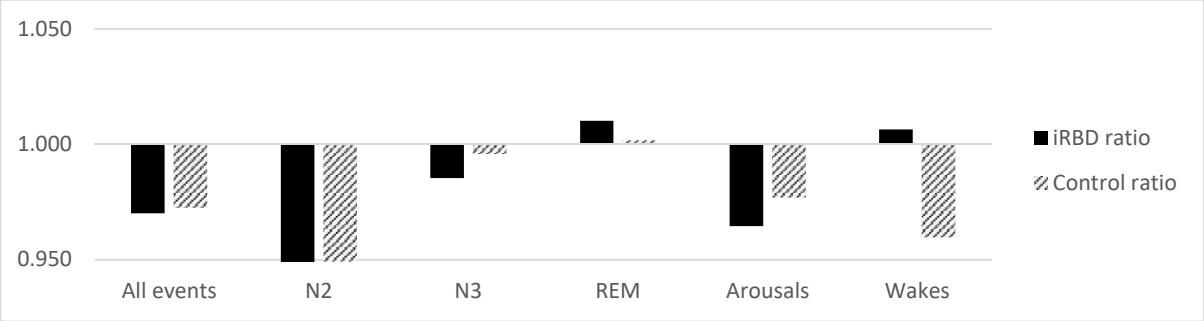


Table 4.6.6 Beta

	iRBD			Control			*Comparing iRBD and controls*
	Baseline average	*RP ratio*	*p value*	*Baseline average*	*RP ratio*	*p value*	*p value*
All events	18.630	0.970	<0.001	17.093	0.973	<0.001	0.968
Phase							
N2	18.914	0.938	<0.001	17.135	0.941	<0.001	0.793
N3	14.828	0.985	0.002	11.844	0.996	0.524	0.456
REM	23.128	1.010	0.099	23.488	1.002	0.758	0.964
Type of arousal							
Arousals	18.677	0.965	<0.001	17.084	0.977	<0.001	0.565
Awakenings	18.322	1.006	0.535	17.118	0.960	<0.001	0.035



5. Discussion

In our study, iRBD patients presented (i) a changed stage N3 distribution across the nocturnal sleep, (ii) an increase in instability and fragmentation parameters and (iii) a changed spectral composition during the pre-arousal/awakening period.

5.1 MACROSTRUCTURAL SLEEP FEATURES

Both groups, controls and iRBD patients, showed a decrease in sleep efficiency and an increase in the percentage of stage N1. Given that subjects with other sleep pathology were excluded from the study, these results may reflect a partial first-night effect, more evident among iRBD patients, since all these subjects performed PSG in laboratorial conditions. However, it has shown that a significant first-night effect does not exist for arousals or other arousal-related events and the data may be comparable.[63]

Our iRBD patients showed less REM sleep than controls. This as also been found in other studies of iRBD patients. It probably reflects the neurodegenerative changes of REM sleep mechanisms in the brainstem, coupled with increased fragmentation due to RBD behaviors.

Despite the similar global length of each NREM sleep stage in iRBD and controls subjects, the sleep cycles composition during the night was slightly different. Specifically, the progressive length decline of stage N3 episodes, observed in controls, was not present in iRBD patients, particularly in the beginning of the sleep period.

Christensen *at al*[43] was the first to analyse the REM and NREM sleep stability in 23 patients with iRBD (with a mean disease duration of 5 years) and 27 patients with Parkinson disease (nineteen with symptomatic RBD), based on the frequencies of wake-sleep and REM-NREM sleep shifts. When comparing with controls, iRBD and Parkinson disease (PD) patients showed a trend toward higher frequency of REM-NREM sleep transitions, despite this trend was only statistically significant in PD patients. The authors conclude that sleep regulating mechanism are affected in iRBD and PD, thus iRBD may represent an intermediate stage between normal sleeper and PD patients, which is in accordance with Braak staging of α-synucleinopathies.[20] Our study partially support their conclusion. However, this study did not analyse changes in stage N3 and the homeostatic drive throughout the night.

In the present study, NREM sleep consolidation impairment in iRBD may reflect an attenuation of the sleep homeostatic drive. Sleep homeostasis is considered to reflect the accumulation of sleep homeostatic factors during wakening, which inhibit the reticular activating system and thereby increase the propensity for sleep and facilitate the slow oscillations typical of NREM sleep.[2] Stage N3, often measured as slow wave activity (delta range activity with > 75μV and < 4Hz), has been viewed as a homeostatic drive biomarker, reflecting the intensity of recuperative functions of sleep.[44] In physiological conditions, nocturnal stage N3 distribution follows a homeostatic pattern: at the beginning of the night, the firsts stage N3 episodes length increases in proportion to the prior wakefulness period, and it diminishes progressively along the night as the sleep pressure declines. The selectivity of iRBD pathology for brainstem nuclei that play a role in sleep homeostatic system (including the cholinergic pedunculopontine and laterodorsal tegmental nuclei) provide a possible anatomic subtract for the decline of stage N3 consolidation in this condition.

One of the possible clinical correlates of changes in sleep macrostruture and homeostatic drive is excessive daytime somnolence. To the best of our knowledge, there is only a previous study that analysed the excessive diurnal somnolence in idiopathic iRBD.[45] In that study, somnolence was more severe in iRBD than in controls and predicted a more rapid phenoconversion of iRBD to Parkinsonism and dementia. The authors conclude that sleepiness is an early marker of iRBD, probably reflecting the degeneration of brainstem flip-flop circuitry. Other source of evidence for the involvement of homeostatic and ultradian systems in iRBD pathology comes from the pharmacological approach of this entity. Clonazepam is the current first-line treatment for iRBD.[46] Despite its mechanism of action remains unknown, some evidence suggests that clonazepam effect might be mediated through the enhancement of GABAergic inhibition, thus leading to flip-flop circuitry stabilization.[47]

Another possible explanation for the changes in sleep macrostruture observed in iRBD patients is a circadian drive disruption. One previous study analysed the clock-dependent REM sleep characteristics in 10 iRBD patients (the duration on disease is not mentioned) and 10 controls.[48] Among all sleep stages, REM sleep is the most circadian-dependent. The authors showed a loss of the physiological increase in REM sleep duration from the first to the fourth sleep cycle. NREM sleep distribution across the night was not studied. These results suggest that an alteration in the circadian

system is present in iRBD. Other source of evidence for the involvement of circadian system in iRBD comes from the melatonin use in the RBD treatment. Melatonin is a hormone secreted by the pineal gland involved in the endogenous synchronization of circadian rhythms. It though that the efficacy of melatonin on RBD symptoms is due to its chronobiotic proprieties by stabilizing the central biological rhythms timing.[49]

5.2 FRAGMENTATION AND INSTABILITY PARAMETERS

On the other hand, the decline of NREM sleep consolidation in iRBD can reflect an increased sleep fragmentation and instability rather than a primary change in the dynamic of homeostatic or circadian control of sleep.

Sleep fragmentation was a remarkable aspect in iRBD patients in the current study. Arousal index was increased in this group during both NREM and REM sleep. Arousals have a correlate in CAP phenomena. Namely, CAP A2 and A3, due to the preponderance of high frequencies, usually incorporate most arousals. Only arousals not preceded by synchronized delta activity (slow waves and K-complexes) are excluded from CAP structure. In our cohort, the CAP A2 and A3 indexes and rates did not differ between the two groups. The arousal index increase, observed in iRBD, was due to a greater occurrence of arousals not preceded by synchronized EEG activity. Moreover, the CAP A1 rate and length were significantly lower in iRBD patients, despite a similar CAP index. This possibly indicates that in our sample, iRBD patients have shorter duration of delta bursts, despite a similar number of events. The mechanism for this selective change in the length of the delta bursts remains to be elucidated.

These results, showing significant differences in fragmentation and instability measures between iRBD and control group, partially support our initial hypothesis. The absence of sleep co-morbidities in our cohort suggests that NREM sleep fragmentation/ instability may be explained by the neurodegenerative process itself implicated in iRBD. As far as we know, there is only one previous study evaluating CAP parameters in 31 iRBD patients.[50] In this study, iRBD was associated to lower phase A1 index and higher phase A2 and A3 indexes, comparing with controls. Contrary to our study, the phase A subtypes were analysed only in term of index (and

not in terms of rate which would provide a more accurate data on the time spent in phase A) and the arousals not preceded by synchronized activity were not analysed; in addition, some iRBD data was not mentioned (such as the mean disease duration at the time of PSG and the RWA cutoff to consider the diagnosis of RBD), and iRBD patients with PLMI > 15 per hour were not excluded from that study – these aspects may limit direct comparisons between the two cohorts. However, both studies coincide in indicating a decrease in synchronized EEG responses and an increase in predominantly desynchronized arousals.

The increase of arousals frequency not associated with EEG synchronization patterns suggest that changes in the arousal mechanisms may occur in iRBD. Our study specifically suggests that arousals occur mainly outside the protective framework of delta waves, which may reflect a lower level of synchronization of thalamo-cortical neurons in iRBD.

Several lines of evidence support the contention that delta EEG activity elicited by the arousing response prevents or attenuates the depolarizing and wake-promoting influence of cholinergic innervation of reticular ascending system[3,51] thus protecting the sleep continuity and its restoring effect. The impairment of this sleep-protective anti-arousal mechanism makes sleep vulnerable to develop a pathological increase of arousals. The laterodorsal tegmental nucleus, which plays a crucial role in the pathogenesis of iRBD, is also implicated in the modulation of arousal response through the ascending cholinergic activation of intralaminar thalamus (representing the ventral pathway of RAS). Taking into account the ascending neurodegenerative process proposed by Braak *et al*,[20] before the phenoconversion of iRBD to Parkinsonism and dementia (Braak stage 1 and 2) the neurodegeneration probably did not yet involve the thalamus but involves these prethalamic afferent fibers arising from upper brainstem. The neurodegeneration of pre-thalamic fibers would induce changes in the thalamic–induced EEG activity such as K-complexes and delta activity. Previous studies have evoked the same reason to explain the decrease of sleep spindles density (another hallmark of NREM sleep) during NREM sleep in iRBD comparing with controls.[52] The disruption of arousal system in iRBD, via degeneration of ventral pathway, may firstly compromise the microstructural phasic event embedded in arousal response (CAP and arousal phenomena) and later on affect the macrostructural sleep architecture. This might explain the fact that microstructural

markers were changed in iRBD, despite the global preservation of length of each sleep stage. In iRBD patients, arousal stimulus may be, on some instances, unable to generate the protective delta power, therefore making the arousal possible. This is further re-enforced by the changes encountered in CAP A1 phases.

5.3 PRE-AROUSAL/ AWAKENING PERIOD SPECTRAL ANALYSIS

Changes in spectral EEG frequencies during pre-arousal/awakening period may be understood as a manifestation of different mechanisms: 1) changes that occur in the gradual process of awakening (assuming that this is a gradual process and not a sudden change in state); 2) mechanisms the brain puts forward to prevent sleep discontinuity; 3) a specific context of brain activity that makes sensory input and brain activation possible.

In this study, arousals and awakenings were preceded by significant changes in spectral composition of EEG signal. Spectral composition changes of tonic EEG activity anticipated by tenths of seconds the phasic activation of cerebral cortex during cortical arousal.

5.3.1 NREM SLEEP

In general, controls subjects showed a variation pattern of EEG signal toward a relative increase in delta activity in relation to all other EEG spectral bands, from the baseline to the pre-arousal/wake period. Except for the sigma frequency behavior, this profile suggests that the cortical activation during pre-arousal period was directed toward sleep maintenance. In iRBD, this profile was also observed, with the exception of theta activity increase, which differed significantly from controls, and the absence of changes in sigma bands in stage N2.

The more consistent change, observed in all NREM sleep stages, arousals and awakening, concerned the delta activity increase, which was similar in both controls and iRBD. These results indicate that delta activity increase, preceding arousals/ awakening, is a feature present in both physiological and pathological sleep. Despite the differences previously observed in the synchronization component of arousals,

both iRBD and controls were able to activate sleep-promoting rhythms during the pre-arousal period, reducing the cortical sensitivity to incoming activating stimuli at the time of arousal. Although an early delta activity increase preceding arousals has been previously reported in NREM sleep among healthy sleepers,[53,54] as far as we know this is the first study to analyse the pre-arousal EEG spectral profile in iRBD patients. It important to re-enforce that the procedural definition for arousal used in our study included the burst of synchronized activity in the arousal beginning. This means that this increase in delta power occurs before the visible burst of delta that is incorporated in CAP A2 and A3 types of arousal.

Sigma activity, expressed in the form of sleep spindles during NREM sleep, has also been a subject of research. Two types of spindles differ in frequency and topography: slower spindles (13-14 Hz, predominant in frontocentral area), and faster spindles (15-16 Hz, predominant in centro-parietal area).[55] In the present study, controls showed sigma activity decrease preceding arousal events during NREM sleep. As far as we know, only a single previous study addressed this issue;[53] contrary to our results, these authors found an increase of sigma activity since 21 seconds of pre-arousal period among middle-aged women and no significant changes among the elderly. These discrepancies might be explained by differences in operational concepts: 1) The age of our control and iRBD groups, that renders them close the to the elderly group of this study; 2) The arousal definition - in this study, the beginning of arousal was assumed to be at the exact onset of the desynchronization component; thus the synchronization activity was excluded, even for arousals embedded in CAP A2 and A3); 3) The baseline period definition also differed - in this study, the interval 36-21 sec before the arousals was designated as the baseline, which may have minimized the sigma activity during the baseline period and maximized eventual oscillation from baseline to pre-arousal period; alternatively, it is also possible that there are dynamic oscillations in the sigma band throughout this 30 seconds pre-arousal period that explain this different results – for instance, it is possible that sigma activity is at first reduced, allowing for incoming sensory stimuli to initiate the arousal phenomena and recruit sleep promoting mechanisms like spindles. This may lead to different sigma band behaviors dependent on the baseline period selected.

In iRBD patients, no significant changes of sigma activity were observed from the baseline to the pre-arousal period, except for events occurring in stage N3, where

sigma activity decreases comparing to the baseline period. It is widely accepted that sleep spindles are generated in the thalamic reticular nucleus; as for delta activity, sleep spindles seem to play an important role as arousal inhibitors,[56,57] inducing a sustained hyperpolarized state of the thalamocortical relay cells, thus reducing the transmission of sensory information through the thalamus. In the reverse, in physiological sleep it can be assumed that sigma and spindle activity inhibition, immediately previous to cortical arousal, could allow a temporarily improved sensory inflow, which is presumed to play an important evolutionary role (i.e. scanning the environment for possible threats). In iRBD patients, the non-reactivity of sigma band during pre-arousal period may suggest that this dynamic is changed. Indeed, previous studies have demonstrated that all-night sleep spindle activity is pathologically decreased in iRBD patients, possibly due to degeneration of pre-thalamic fibers originated from the ascending cholinergic system;[52] this may attenuate the phasic reactivity of sigma frequency at the time of arousal. Therefore, it is possible that the absence of changes pre-arousal reflect a floor effect - this frequency band is already less active throughout sleep, namely in the baseline period, leading to an inability to detect subtle changes during the pre-arousal period. In addition, the theta activity increase during the stage N2 present in iRBD may be related to a relative increase in the baseline theta frequency due to the absent reactivity of sigma.

5.3.2 REM SLEEP

In REM sleep the pre-arousal/awakening period was similar in terms of the increased delta activity and decreased theta and lower sigma. However, in control subjects, higher frequencies (alpha, high sigma and beta) remained unchanged relative to baseline. No study previously analysed the spectral EEG behavior pre-arousal in REM sleep in healthy sleepers. In the study driven by Bruce *at al*[53] and previously mentioned, REM sleep analyses were not made. The absence of changes of the higher frequencies (alpha, beta, high sigma) in REM sleep prior to arousals probably reflect the fact that this frequencies are typical REM sleep oscillatory activities not necessarily related to arousal or cortical activation through sensory stimuli. In fact, previous studies have shown that in normal adults, arousals and awakenings are more common in REM sleep and may constitute a normal phenomena, such that a sleep

cycle may in fact be NREM-REM sleep-Awake. Within such a framework, arousals from REM sleep come naturally within the more activated cortical environment of this sleep state and this may be reflected in lesser spectral activity changes in high frequencies in this sleep period.[16]

However, in iRBD patients, frequencies within the 8-14 Hz band behaved differently from controls. Low sigma did not change (instead of decreasing), as occurred in NREM sleep. Beta activity also remained unchanged. The most striking difference was that alpha activity showed a significant decrease prior to arousal (instead of no change). Previous studies have analysed the EEG spectral activity in REM sleep in RBD patients. Drug-naïve iRBD patients were shown to have lesser frequencies below 15 Hz in REM sleep and higher frequencies within the beta range in this sleep stage.[64] Other study found that PD-RBD patients, in REM sleep, subthalamic nucleus beta activity is higher than in NREM sleep, reaching levels similar to the wake state. They further showed that in these patients RBD-related movements had a different profile from wake-related movement, with beta synchronization instead of desynchronization, reflecting a presumably different motor pathway.[58,59] Taken together these studies mostly focus on the relevance of beta activity changes in iRBD, in close parallel to the importance of this exaggerated basal ganglia activity in Parkinson's disease (PD).[65] Our results show that this band, in scalp EEG recordings, does not seem to be involved in REM sleep fragmentation. These results do not seem unexpected, as we have focused on arousals not related to RBD abnormal movements and this band as mostly been implicated in the abnormal motor pathway of PD patients.

The relevance of the alpha frequency change remains to be elucidated. In healthy sleepers, during REM sleep, and contrary to wakefulness, alpha power increases in response to external stimuli, presumably reflecting sensory processing.[66] Therefore, this decrease in alpha power prior to wakefulness is probably not related to increased sensory stimuli. The decrease we found in the alpha band in iRBD patients may be related to the fact that we study segments of non-RBD like phenomena. In fact, RBD like phenomena are associated with phasic REM periods, namely alpha bursts.[67] It is therefore possible that we selected more tonic REM periods, giving rise to a spurious alpha band decrease. However, previous studies have shown that there are different alpha oscillations in REM sleep, in terms of topography and reactitivity to phasic REM

stimuli.[68] Therefore, further studies, specifically analysing different REM sleep stages and alpha band characteristics are necessary to understand this finding.

5.3.3 AROUSALS *VERSUS* AWAKENINGS

We aimed at analysing arousals and awakenings based on the assumption that these two phenomena represent a continuum of brain activation. From a clinical perspective, it is reasonable to assume that awakenings represent a more relevant phenomena, leading to more pronounced sleep fragmentation. In this framework, it is interesting to acknowledge that the changes between iRBD and controls were only statistically significant during awakenings. The most relevant change in iRBD patient is that beta activity did not decrease prior to awakening and low sigma decreased further than in controls.

As previously mentioned, iRBD patients seem to have impaired spindle activity. It may be hypothesized that the higher decrease in spindles in these patients during a brief period, together with increased high frequencies, may render the brain more susceptible to react to environmental or intrinsic stimuli leading to more prolonged awakening. To the best of our knowledge, only Bruce *at al*[53] looked at the spectral differences preceding brief *vs*. prolonged arousals. These authors specifically evaluated the differences in delta band and concluded that long arousals lacked the increase in delta power that short arousals heralded, suggesting that this less de-activated state made longer arousals possible. These changes did not occur in the elderly, known to have more pronounced NREM sleep awakenings.[60] These authors did not report changes in other frequency bands. Their study, however, like ours, also suggests that analysing the brain spectral environment prior to prolonged and brief sleep-wake transitions may be a useful model to disclose possible physiopatological mechanisms involved in sleep fragmentation in specific disorders.

6. Conclusions and study limitations

In conclusion, we have shown that iRBD patients have significant macro and microstructural NREM sleep changes, specifically associated with higher expression of instability and fragmentation parameters, with spontaneous arousals occurring more frequently outside the protective framework of delta waves during NREM sleep. Despite the differences observed in synchronization component of arousals, both iRBD and controls were able to activate sleep-promoting rhythms (increase of delta activity) during the pre-arousal period, presumably reducing the cortical sensitivity to incoming activating stimuli at the time of arousal. The pre-arousal/awakening changes during NREM sleep were directed toward sleep maintenance, except for the sigma band activity which seemed to be impaired in iRBD patients. The decrease of long delta bursts (A1 duration and rate) or K-complex associated with arousals and decrease of sigma band re-activity during the pre-arousal/awakening period may reflect a lower level of synchronization of thalamo-cortical neurons in iRBD, which may be due to pre-thalamic fibers degeneration originated from the ascending cholinergic system. When comparing arousals *vs.* awakening in iRBD and controls, the lack of beta activity reduction and the greater reduction of low sigma activity prior to awakening observed in iRBD may contribute to longer arousals.

In addition to sleep microstructural changes, iRBD also showed macrostructural changes, namely in homeostatic distribution of stage N3 across the night-time.

Collectively, these results support our initial hypothesis, suggesting that iRBD is a more generalized sleep disorder, also affecting the NREM sleep, namely through the arousal system dysregulation.

This study presents important limitations, namely the limited number of participants and the retrospective design (despite all the analysed parameters have been re-analysed by the main investigator at the time of study). FFT is the most widely method to quantify the frequency content of EEG signal, however this method assumes that signal is stationary and does not provide any information in time domain, which may reduce the ability to detect more transient EEG activities (such as brief K-complexes and spindles). In addition, spectral analysis was performed only on the derivation C3-A2 which limits the expression and topographical understanding of these phenomena. Furthermore, it is also important to acknowledge that the control group used a convenience sample of patients that were referred for PSG. Despite the absence of

clear cut sleep and neurological disorders, this group had sleep complaints that made the PSG referral clinical relevant. Further studies with normal controls should be performed to confirm our results.

Despite that, our data seem to provide information not available before and useful for the understanding of the arousal system disruption mechanism and the NREM sleep involvement in iRBD patients.

Future studies may explore the evolution of these microstructural and macrostructural parameters along the natural history of iRBD and characterize the clinical expression of NREM sleep involvement in iRBD, namely to correlate the excessive diurnal somnolence with these parameters.

7. Bibliography

1. Iranzo A. Sleep in Neurodegenerative Diseases. Sleep Med Clin. 2016 Mar;11(1):1-18.

2. Brown RE, Basheer R, McKenna JT, et al. Control of sleep and wakefulness. Physiol Rev. 2012 Jul; 92(3): 1087-187.

3. Halász P, Terzano M, Parrino L, et al. The nature of arousal in sleep. J Sleep Res. 2004 Mar; 13 (1): 1-23.

4. Lu J, Sherman D, Devor M, et al. A putative flip-flop switch for control of REM sleep. Nature. 2006 Jun; 441(7093): 589-94.

5. Saper CB, Fuller PM, Pedersen NP, et al. Sleep state switching. Neuron. 2010 Dec; 68(6): 1023-42.

6. Saper CB. The neurobiology of sleep. Continuum 2013 Feb; 19(1): 19-31.

7. Borbély AA, Daan S, Wirz-Justice A, et al. The two-process model of sleep regulation: a reappraisal. J Sleep Res. 2016 Apr; 25(2): 131-43.

8. Berry RB, Brooks R, Gamaldo CE, et al for the American Academy of Sleep Medicine. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications, 2nd ed. Westchester: American Academy of Sleep Medicine, 2012.

9. Hirshkowitz M. Arousals and anti-arousals. Sleep Med. 2002 May; 3: 203–4.

10. Terzano MG, Mancia D, Salati MR, et al. The cyclic alternating pattern as a physiologic component of normal NREM sleep. Sleep. 1985; 8(2):137-45.

11. Terzano MG, Parrino L. Origin and Significance of the Cyclic Alternating Pattern (CAP). Sleep Med Rev. 2000 Feb; 4(1): 101-23.

12. Terzano MG, Parrino L, Smerieri A, et al. CAP and arousals are involved in the homeostatic and ultradian sleep processes. J Sleep Res 2005 Dec; 14(4): 359-68.

13. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci. 2001 Dec; 24(12): 726–31.

14. Halász P, Bodizs R. Dynamic Structure of NREM Sleep. 1st ed. London: Springer-Verlag; 2013.

15. David F, Schmiedt JT, Taylor HL, et al. Essential thalamic contribution to slow waves of natural sleep. J Neurosci. 2013 Dec; 33(50): 19599-610.

16. Akerstedt T, Billiard M, Bonnet M, et al. Awakening from sleep. Sleep Med Rev. 2002 Aug;6(4):267-86.

17. Schenck CH, Bundlie SR, Ettinger MG, et al. Chronic behavioral disorders of human REM sleep: a new category of parasomnia. Sleep. 1986 Jun; 9:293–308.

18. Ohayon MM, Caulet M, Priest RG. Violent behavior during sleep. J Clin Psychiatry. 1997 Aug; 58: 369–76.

19. Iranzo A, Tolosa E, Gelpi E, et al. Neurodegenerative disease status and post-mortem pathology in idiopathic rapid-eye-movement sleep behaviour disorder: an observational cohort study. Lancet Neurol. 2013 May; 12(5): 443-53.

20. Braak H, Del Tredici K. Neuroanatomy and pathology of sporadic Parkinson's disease. Adv Anat Embryol Cell Biol. 2009; 201: 1-119.

21. Peever J, Luppi PH, Montplaisir J. Breakdown in REM sleep circuitry underlies REM sleep behavior disorder. Trends Neurosci. 2014 May; 37(5): 279-88.

22. Jouvet M, Michel F. Corrélations électromyographiques du sommeil chez le chat de cortiqué et mésencéphalique chronique. C.R. Soc. Biol. 1959; 153: 422-25.

23. Jouvet M, Mounier D. Effect of lesions of the pontile reticular formation on sleep in the cat. C.R. Soc. Biol. 1960; 154: 2301-05.

24. Limousin N, Dehais C, Gout O, et al. A brainstem inflammatory lesion causing REM sleep behavior disorder and sleepwalking (parasomnia overlap disorder). Sleep Med. 2009 Oct; 10(9): 1059-62.

25. Kimura K, Tachibana N, Kohyama J, et al. A discrete pontine ischemic lesion could cause REM sleep behavior disorder. Neurology. 2000 Sep 26; 55(6): 894-5.

26. Xi Z, Luning W. REM sleep behavior disorder in a patient with pontine stroke. Sleep Med. 2009 Jan; 10(1): 143-6.

27. Boeve BF, Silber MH, Saper CB, et al. Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. Brain. 2007 Nov; 130(Pt 11): 2770-88.

28. Fantini ML, Michaud M, Gosselin N, et al. Periodic leg movements in REM sleep behavior disorder and related autonomic and EEG activation. Neurology. 2002 Dec 24; 59(12): 1889-94.

29. Sasai T, Inoue Y, Matsuura M. Clinical significance of periodic leg movements during sleep in rapid eye movement sleep behavior disorder. J Neurol. 2011 Nov; 258(11): 1971-8.

30. Di Fabio N, Poryazova R, Oberholzer M, et al. REM sleep behaviour disorder and overlap parasomnia in patients with Parkinson´s disease. Eur Neurol 2013; 70(5-6): 297-303.

31. Schenck CH, Boyd JL, Mahowald MW. A parasomnia overlap disorder involving sleepwalking, sleep terrors, and REM sleep behavior disorder in 33 polysomnographically confirmed cases. Sleep 1997; 20(11): 972-81.

32. Dumitrascu O, Schenck CH, Applebee G, et al. Parasomnia overlap disorder: a distinct pathophysiologic entity or a variant of rapid eye movement sleep behavior disorder? A case series. Sleep Med 2013; 14(11): 1217-20.

33. Miguel R, Arnulf I. Phasic activity during non REM sleep. Sleep Med. 2017 Jan; 29:37-40.

34. Christensen JA, Kempfner J, Zoetmulder M, et al. Decreased sleep spindle density in patients with idiopathic REM sleep behavior disorder and patients with Parkinson's disease. Clin Neurophysiol. 2014 Mar; 125(3): 512-9.

35. O'Reilly C, Godin I, Montplaisir J, et al. REM sleep behaviour disorder is associated with lower fast and higher slow sleep spindle densities. J Sleep Res. 2015 Dec; 24(6): 593-601.

36. American Academy of Sleep Medicine. International classification of sleep disorders. 2nd ed. Diagnostic and coding manual Westchester (IL): American Academy of Sleep Medicine; 2005.

37. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015 Oct; 30(12): 1591-601.

38. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Association; 2013.

39. Rechtschaffen A, Kales A, editors. A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects. Los Angeles: Brain Information Service/Brain Research Institute, UCLA; 1968.

40. Berry RB, Brooks R, Gamaldo CE, et al for the American Academy of Sleep Medicine. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications, 2nd ed. Westchester: American Academy of Sleep Medicine, 2012.

41. Terzano MG, Parrino L, Smerieri A, et al. Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep. Sleep Med. 2002 Mar; 3(2): 187-99

42. Cooley JW, Tukey OW. An algorithm for the machine calculation of complex Fourier series. Math Compu 1965; 19: 297-301.

43. Christensen JA, Jennum P, Koch H, et al. Sleep stability and transitions in patients with idiopathic REM sleep behavior disorder and patients with Parkinson's disease. Clin Neurophysiol. 2016 Jan; 127(1): 537-43.

44. Benington JH, Heller HC. Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol. 1995 Mar; 45(4): 347-60.

45. Arnulf I, Neutel D, Herlin B, et al. Sleepiness in Idiopathic REM Sleep Behavior Disorder and Parkinson Disease. Sleep. 2015 Oct; 38(10): 1529-35.

46. Schenck CH, Montplaisir JY, Frauscher B, et al. Rapid eye movement sleep behavior disorder: devising controlled active treatment studies for symptomatic and neuroprotective therapy--a consensus statement from the International Rapid Eye Movement Sleep Behavior Disorder Study Group. Sleep Med. 2013 Aug; 14(8): 795-806.

47. Zhong G, Naismith SL, Rogers NL, et al. Sleep-wake disturbances in common neurodegenerative diseases: a closer look at selected aspects of the neural circuitry. J Neurol Sci. 2011 Aug 15; 307(1-2): 9-14.

48. Arnaldi D, Latimier Al, Leu-Semenescu S, et al. Loss of REM sleep features across nightime in REM sleep behavior disorder. Sleep Medicine. 2016 Dec; 17: 134-7.

49. Arendt J, Skene DJ. Melatonin as a chronobiotic. Sleep Med Rev 2005; 9(1): 25–39.

50. Kutlu A, Işeri P, Selekler M, et al. Cyclic alternating pattern analysis in REM sleep behavior disorder. Sleep Breath. 2013 Mar; 17(1): 209-15.

51. Wauquier A, Aloe L, Declerck A. K-complexes: are they signs of arousal or sleep protective? J Sleep Res. 1995 Sep; 4(3): 138-143.

52. Christensen JA, Kempfner J, Zoetmulder M, et al. Decreased sleep spindle density in patients with idiopathic REM sleep behavior disorder and patients with Parkinson's disease. Clin Neurophysiol. 2014 Mar; 125(3): 512-9.

53. Bruce EN, Bruce MC, Ramanand P, et al. Progressive changes in cortical state before and after spontaneous arousals from sleep in elderly and middle-aged women. Neuroscience. 2011 Feb 23; 175: 184-97.

54. Togo F, Cherniack NS, Natelson BH. Electroencephalogram characteristics of autonomic arousals during sleep in healthy men. Clin Neurophysiol. 2006 Dec; 117(12): 2597-603.

55. Anderer P, Klösch G, Gruber G, et al. Low-resolution brain electromagnetic tomography revealed simultaneously active frontal and parietal sleep spindle sources in the human cortex. Neuroscience. 2001; 103(3): 581-92.

56. Kim A, Latchoumane C, Lee S, et al. Optogenetically induced sleep spindle rhythms alter sleep architectures in mice. Proc Natl Acad Sci U S A. 2012 Dec; 109(50): 20673-8.

57. Dang-Vu TT, McKinney SM, Buxton OM, et al. Spontaneous brain rhythms predict sleep stability in the face of noise. Curr Biol. 2010 Aug; 20(15): R626-7. 52.

58. Hackius M, Werth E, Sürücü O, te al. Electrophysiological evidence for alternative motor networks in REM sleep behavior dirsorder. J Neurosci. 2016 Nov; 36(46): 11795-11800.

59. Urresrarazu E, Iriarte J, Alegre M, et al. Beta activity in the subthalamic nucleus during sleep in patients with Parkinson´s disease. Mov Dirsord. 2009 Jan; 24(2): 254-60.

60. Murphy PJ, Rogers NL, Campbell SS. Age differences in the spontaneous termination of sleep. J Sleep Res. 2000 Mar; 9(1): 27-34.

61. Frauscher B, Iranzo A, Gaig C, et al. Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. Sleep. 2012 Jun; 35(6): 835-47.

62. Campbell I. EEG recording and analysis for sleep research. Curr Protoc Neurosci. 2009 Oct; doi: 10.1002/0471142301.ns1002s49.

63. Bonnet JP, Arand DL. Do arousals demonstrate a first night effect? Sleep. 2006; 29:A340.

64. Ferri R, Rundo F, Silvani A, et al. REM sleep EEG instability in REM sleep behavior disorder and clonazepam effects. Sleep, 2017 vol. 40(8).

65. Brown P. Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson´s disease. Mov Disord, 2003 Apr; 18(4): 357-63.

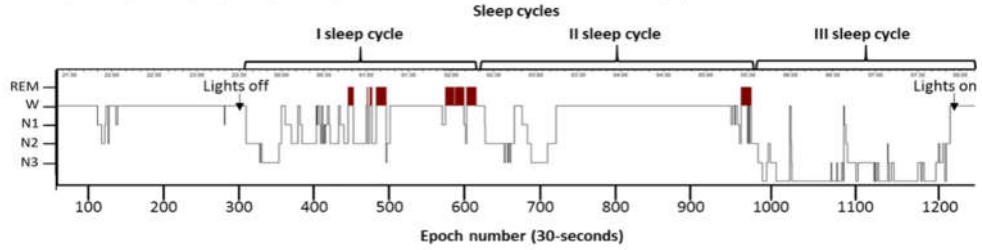
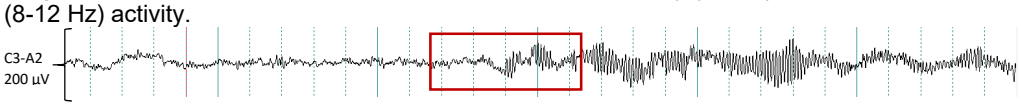
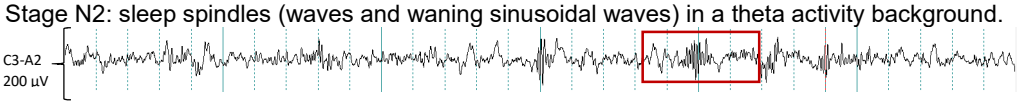
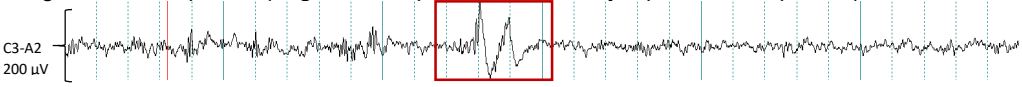
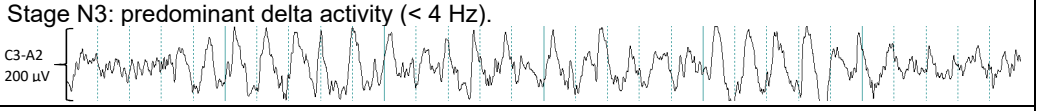
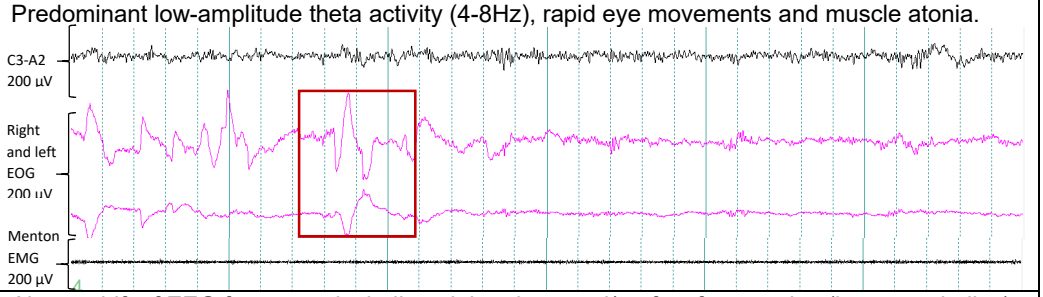
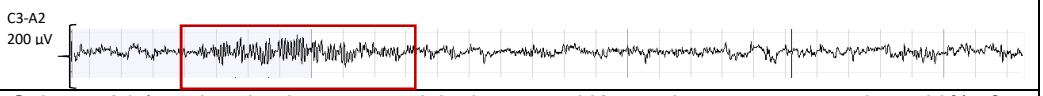
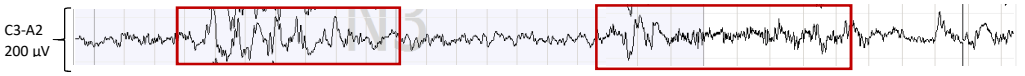
66. Ruby P, Blochet C, Eichenlaub J-B, et al. Alpha reactivity to complex sounds differs during REM sleep and wakefulness. Sterr A, ed. PLoS ONE. 2013; 8(11).

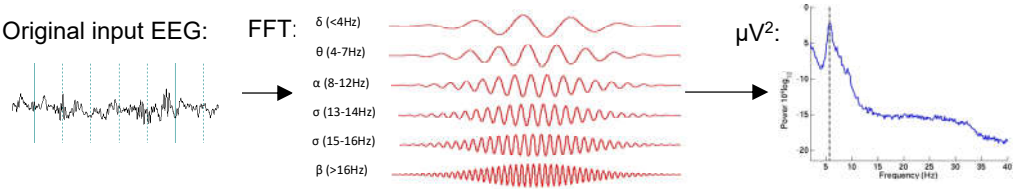
67. Manni R, Terzaghi M, Glorioso M. Motor-behavioral episodes in REM sleep behavior disorder and phasic events during REM sleep. Sleep. 2009; 32(2): 241-245.

68. Cantero JL, Atienza M, Salas RM. Human alpha oscillations in wakefulness, drowsiness period and REM sleep: different electroencephalographic phenomena within the alpha band. Neurophysiol Clin. 2002 Jan; 32(1): 54-71.

7. Appendices and Annexes

8.1 Sleep features expressed in polysomnographic signals.[41]

	Polysomnographic patterns
Sleep macrostructure	Alternation between consecutive NREM and REM sleep periods (sleep hypnogram). **Analysed variables**: total sleep time (TST, time from sleep onset to the end of the final sleep epoch, minus WASO); sleep latency (time from lights out to sleep onset); wake after sleep onset (WASO, the time spent awake between sleep onset and the end of sleep); sleep efficiency (ratio between total sleep time and time in bed); stage N1% (TST percentage spent in stage N1); sleep cycle (a sequence of NREM and REM sleep). 
NREM sleep	Stage N1 and wake: transition from a predominant theta activity (4-8Hz) to a sinusoidal alpha (8-12 Hz) activity.  Stage N2: sleep spindles (waves and waning sinusoidal waves) in a theta activity background.  Stage N2: K-complexes (negative sharp wave followed by a positive component).  Stage N3: predominant delta activity (< 4 Hz). 
REM sleep	Predominant low-amplitude theta activity (4-8Hz), rapid eye movements and muscle atonia. 
Cortical arousals and isolated arousal	Abrupt shift of EEG frequency including alpha, theta and/ or fast frequencies (but not spindles) for a minimum of 3 seconds, with at least 10 seconds of stable sleep preceding the arousal onset. This is an example of isolated arousal (not preceded by synchronization activity). **Analysed variables**: arousal index (number of arousal per hour of sleep); arousal index and percentage in NREM sleep without k-complexes or delta. 
Phase A of cyclic alternating pattern (CAP)	Subtype A1 (synchronized patterns – delta bursts and K-complexes – occupy at least 80% of their length), subtype A2 (synchronized patterns occupy between 50-80% and are followed or mixed with faster rhythms –theta, alpha and beta), and subtype A3 (synchronized patterns occupy less than 50%). This is an example of subtype A1 (left) and A3 (right). **Analysed variables**: phase A index (number of a given phase A subtype per hour of sleep); phase A rate (percentage ratio of total phase A time to NREM sleep time); phase A length (the mean duration of each phase A subtype). 

Fast Fourier Transform	Frequency analysis that decomposes the original and complex EEG oscillatory signal into its pure sinusoidal components. **Analysed variables**: absolute power (or amplitude squared, μV^2) and relative power (RP, % of total power) accumulated over a period of time are calculated for every frequency band. RP ratio (baseline to pre-arousal/awakening RP variation). Original input EEG: FFT: δ (<4Hz) θ (4-7Hz) α (8-12Hz) σ (13-14Hz) σ (15-16Hz) β (>16Hz) μV^2: 

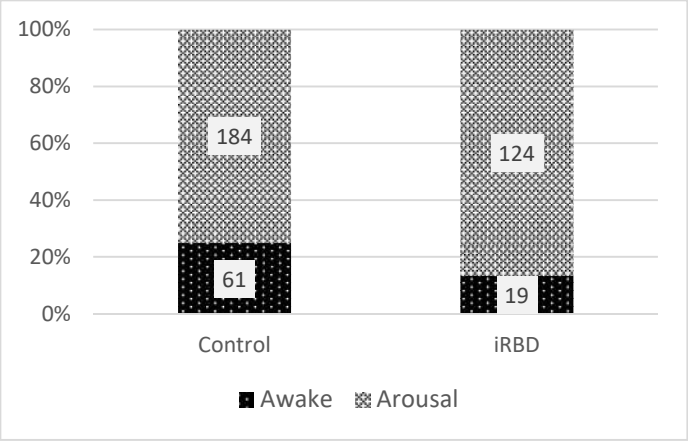
Abbreviations: REM, REM sleep; W, wakefulness; FFT, Fast Fourier Transform.

8.2 Diagnostic criteria for REM sleep behaviour disorders signals.[36]

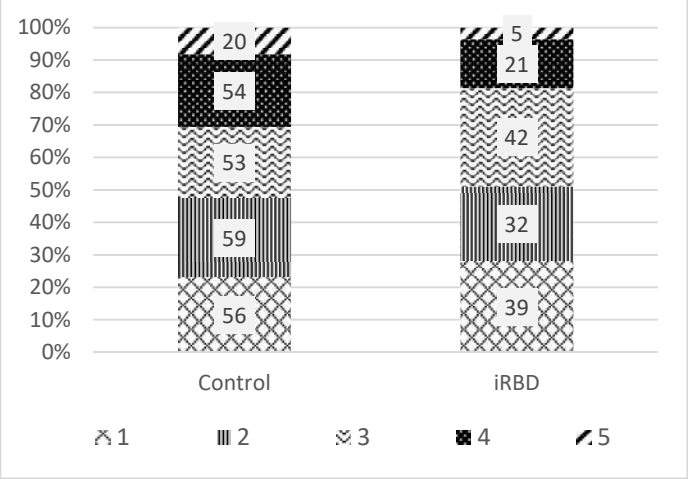
Criteria A and B must be met:
A. A history of dream enactment behaviors with injurious or potentially injurious movement associated with dream content, or behavioral manifestations occurring during REM sleep during video-PSG;
B. REM sleep without atonia (RWA), defined by an increase of tonic or phasic chin EMG activity during > 50% of each epoch of REM sleep.*
*Based on the current data, the cutoff for a diagnosis of RBD was assumed when RWA represented ≥ 18% of REM sleep time.[61]

8.3 Distribution of selected arousals/ awakening over the nocturnal sleep.

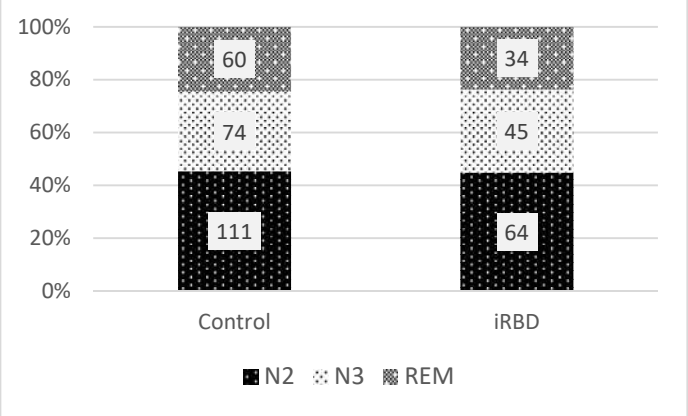
8.3.1 Percentage of selected arousals and awakening selected for spectral analysis.



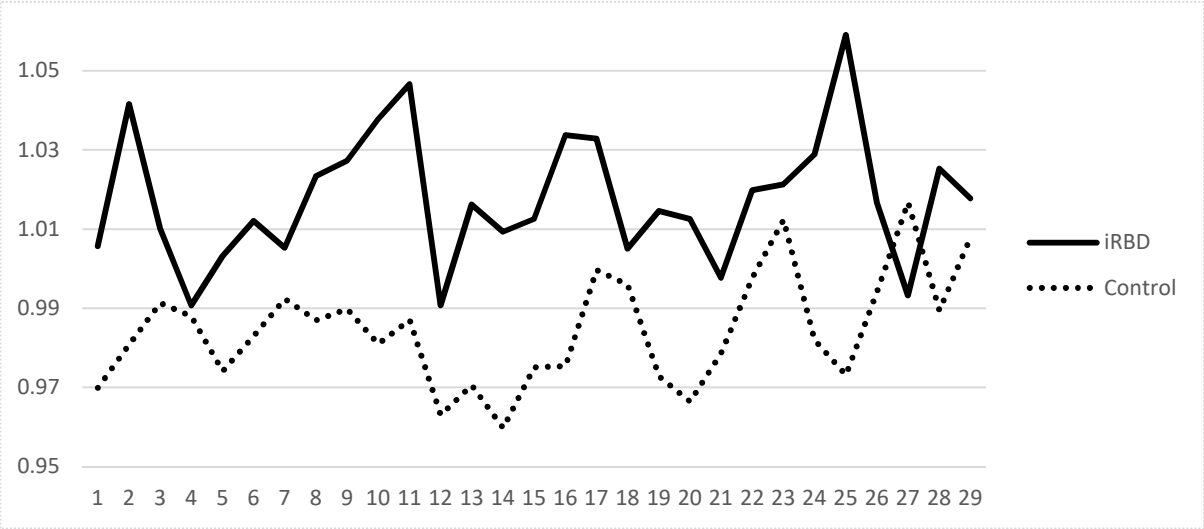
8.3.2 Distribution of selected arousals and awakening over the five sleep cycles.



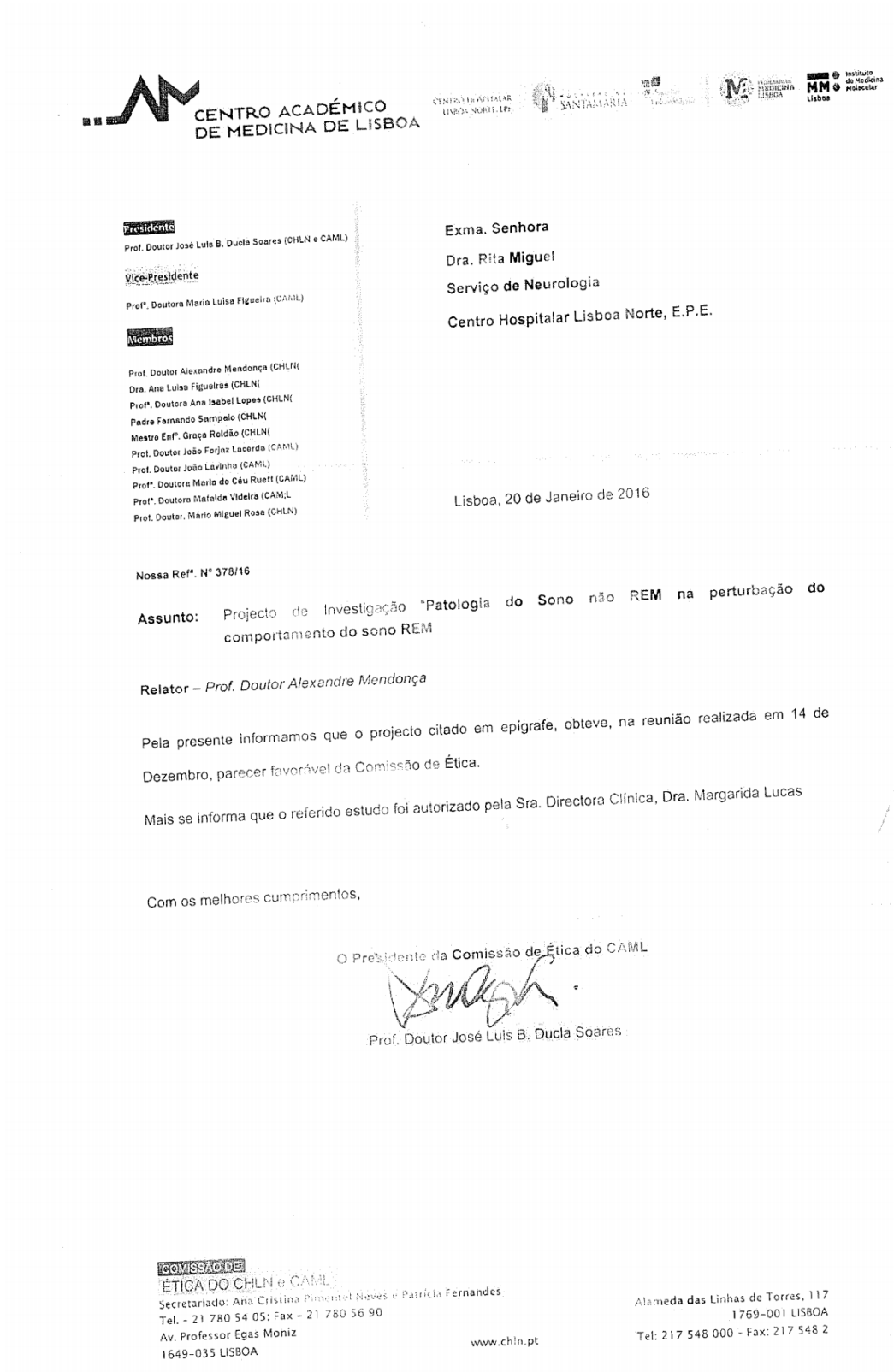
8.3.3 Distribution of selected arousals and awakening over the sleep stage (N2, N3 or REM sleep).



8.4 Mean RP ratios over 30-sec pre-arousal period in theta band during stage N2 (illustrative example).



8.5 Hospital de Santa Maria Ethics Committee approval.



CENTRO ACADÉMICO DE MEDICINA DE LISBOA

Presidente
Prof. Doutor José Luis B. Ducla Soares (CHLN e CAML)

Vice-Presidente
Profª. Doutora Maria Luisa Figueira (CAML)

Membros
Prof. Doutor Alexandre Mendonça (CHLN(
Dra. Ana Luisa Figueiras (CHLN(
Profª. Doutora Ana Isabel Lopes (CHLN(
Padre Fernando Sampaio (CHLN(
Mestre Enfª. Graça Roldão (CHLN(
Prof. Doutor João Forjaz Lacerda (CAML)
Prof. Doutor João Lavinha (CAML)
Profª. Doutora Maria do Céu Ruett (CAML)
Profª. Doutora Mafalda Videira (CAML
Prof. Doutor. Mário Miguel Rosa (CHLN)

Exma. Senhora
Dra. Rita **Miguel**
Serviço **de Neurologia**
Centro Hospitalar Lisboa Norte, E.P.E.

Lisboa, 20 de Janeiro de 2016

Nossa Refª. Nº 378/16

Assunto: Projecto de Investigação "**Patologia do Sono não REM na perturbação do comportamento do sono REM**

Relator – *Prof. Doutor Alexandre Mendonça*

Pela presente informamos que o projecto citado em epígrafe, obteve, na reunião realizada em 14 de Dezembro, parecer favorável da Comissão de Ética.

Mais se informa que o referido estudo foi autorizado pela Sra. Directora Clínica, Dra. Margarida Lucas

Com os melhores cumprimentos,

O Presidente da Comissão de Ética do CAML

Prof. Doutor José Luis B. Ducla Soares

COMISSÃO DE ÉTICA DO CHLN e CAML
Secretariado: Ana Cristina Pimentel Neves e Patrícia Fernandes
Tel. - 21 780 54 05; Fax - 21 780 56 90
Av. Professor Egas Moniz
1649-035 LISBOA

www.chln.pt

Alameda das Linhas de Torres, 117
1769-001 LISBOA
Tel: 217 548 000 - Fax: 217 548 2